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Seedling growth of physic nut (*Jatropha curcas* L.) genotypes under different photosynthetic photon flux density

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Abstract

The light has direct effects over the plant metabolism. The response of plants to light may change according to its presence and intensity, affecting the photosynthesis process and, consequently, their growth. Due to the lack of information about levels of irradiation that allow better growth of seedlings of physic nut (*Jatropha curcas* L.), this study was conducted aiming to study the influence of different intensities of photosynthetic photon flux over the growth of seedlings of six genotypes of physic nut. The experiment followed a factorial scheme 4x4, composed by four genotypes of physic nut (CNPAE-248, CNPAE-301, CNPAE-308 and CNPAE-144) and four levels of photosynthetic photon flux (759, 438, 330 and 174 µmol m⁻² s⁻¹), arranged in completely randomized design, with four replications and the experimental plot consisting of 12 seedlings. The variables of growth and accumulation of dry matter were significantly affected by the interaction between the effect of genotypic differences and levels of photosynthetic photon flux near 330 µmol m⁻² s⁻¹ (equivalent to 50% shading). The results indicate that the cultivation of CNPAE-308 in 50% shade (330 mol m⁻² s⁻¹) resulted in an increase of 83% leaf area and 33% of vertical plant growth, compared to the unshaded. Under these conditions, it was observed an increase of 144% dry matter of shoots and 140% of total dry matter. With the results shown by this study it is clear that genotypes of physic nut can be increased up to 90% of vegetative growth when grown in 330 mol m⁻² s⁻¹ of photosynthetic photon flux density.

Keywords: Jatropha curcas, luminosity, shading, growth.

Abbreviations: NL_number of leaves, RL_root length, PH_plant height, SD_stem diameter, LA_total leaf area, DMA_dry matter of aerial part, DMR_dry matter of roots, DMT_total dry matter, GER_germination, MOI_moisture, WID_width, W1.000_weight of 1.000 seeds, , LD_larger diameter, ld_smaller diameter, PPF_photosynthetic photon flux.

Introduction

Plantations of physic nut (Jatropha curcas L.) can be formed by seeding directly on the field or using seedlings, which can be produced from seeds or rooted cuttings (Amaral et al., 2011). The use of seedlings allows a pre-selection of vigorous and uniform plants from the start, providing conditions for standardization and better initial growth of the plantations (Paulino et al., 2011). The nursery where the seedlings will be produced need provide adequate conditions for the plant growth. Environmental factors in the nursery, such as the availability of light, size of the container and composition of substrate are crucial to determinate the quality of the seedlings. Therefore, the conditions of the nursery will influence the potential of the seedling to survive in the field, grow vigorously, form a homogeny crop and keep higher yields (Ortega et al., 2006). The irradiation, among other factors, has direct influence on the plant metabolism. Thus,

informations about the adequate luminosity for seedling production is subject highlighted by researchers, especially for the agriculture in tropical regions, where the cultivation and seedling production under greenhouse conditions contribute to increase the vegetal production of many species. The response of plants to light may change according to its presence and intensity (Kendrick and Frankland, 1981), affecting their metabolism, stomatal opening, synthesis of chlorophyll and, consequently, their photosynthesis and growth (Kozlowski et al., 1991). The use of shade screens and thermic-reflector meshes modifies the environmental parameters in nurseries to promote the growth and production of plants, specifically air temperature and relative humidity (Matos et al., 2009). Shade screens represent a low-cost solution commonly used, decreasing the temperature and controlling the light intensity in nurseries, although the

scientific literature on the subject is rather scarce (Seabra et al., 2009). To establish appropriate levels of shading is important to manage the cultivation system or the nursery structure, identifying levels that promote the growth and are not harmful to the development of the species (Faria Junior et al., 2000). Therefore, to establish those levels is important to make proper use of shade screens, allowing to control the local temperature and manage the effects of irradiation, mitigating the effects of adverse conditions over the plant metabolism, particularly the photorespiration (Smith et al., 2006; Paulino et al., 2011). Another factor that must be considered for physic nut is the existence of a large genetic variability. Differential gene expression between genotypes in relation to environmental stresses have been reported, and the high genetic variability among Brazilian genotypes have been described in many studies (Laviola et al., 2010; Amaral et al., 2012; Christo et al., 2012; Martins et al., 2013; Nunes et al., 2013). Due to the lack of information about levels of irradiation that allow better growth of seedlings of physic nut, this study was conducted aiming to study the influence of different intensities of photosynthetic photon flux over the growth of seedlings of different genotypes of physic nut.

Results and Discussion

Table 2 presents the data for the photosynthetic photon flux registered in each level of shading. The average photosynthetic photon flux for PPF-1, PPF-2, PPF-3 and PPF-4 levels were 759, 438, 330 and 174 mol⁻² s⁻¹, respectively. The variables of growth and accumulation of dry matter were significantly affected by the interaction between the effect of genotypic differences and levels of photosynthetic photon flux.

Growth of genotypes under different intensities of PPF

In the study of the influence of each level of PPF over the growth of seedlings of physic nut (Table 3), it was found that; overall, the genotype CNPAE-308 presented greater vegetative growth than the other genotypes. In contrast, the genotype CNPAE 248 showed lower means for the variables related to its growth. The studies of Matos et al. (2009), regarding physiological characterization of physic nut seedlings produced at different levels of irradiance; explain, to a good extent, the possibility of success in crops cultivated in shade, although this species has evolved in unshaded conditions. However, the experiment done by these authors did not consider a possible differential expression of genes due to environmental stress provided by the limitation of the irradiation. A vigorous vegetative growth (PH, NL and LA) was observed in the seedlings of physic nut in conditions of limited irradiation, precisely near the level PPF-3, which corresponds to 330 μ mol m⁻² s⁻¹ of photosynthetic photon flux or 50% of shading. This fact is demonstrated by the adjustment of regression curves with coefficients above 0.70, within the studied range. It was not possible to adjust regression models for stem diameter and root length (Table 3). Overall, high values of leaf area under low light availability are frequently reported in many species, being explained as an adaptive strategy of plants in shaded environments to make better use of the irradiation by increasing the total surface (Campos and Uchida, 2002). Quadratic regression with maximum points were the models that better elucidated the growth of seedlings in function of the photosynthetic photon flux. The smaller growth in unshaded conditions can be explained by the prolonged

exposure to high irradiances causing harm to the seedlings. In this condition, the vegetal tissues may absorb more light than they can use, promoting photo-inhibition and degrading carotenoids. According to Santa-Barbara and Jennings (2005), high light intensities can saturate the electron transport chain in photosystem II, due the relatively slow oxidation of plastoquinone by the complex cytochrome b_6f . This excess of energy absorbed by the reaction centers can be dissipated through the photosynthetic electron transport chain, resulting in the induction of photo-inhibition and photo-bleaching of the photosystems (Strizh et al., 2005). The superiority observed in the level PPF-3 is related to the ideal balance of photons that the plant intercepts and the conditions of temperature and humidity that shading provides, promoting the growth. Kitão et al. (2000), Almeida et al. (2005) and Matos et al. (2011) also found similar results. According Radmann et al. (2001), the quantity and quality of the light absorbed by photoreceptors are extremely important to determine the growth and development of plants. Those factors cause possible changes in leaf development, changing thickness, mesophyll differentiation, leaf vascular development, cell division and stomata development (Hazarika, 2006).

Production of dry matter of genotypes of physic nut to different levels PPF

In the study of the influence of each level of PPF in dry matter accumulated in the aerial part of each genotype (Table 4), there is a significantly greater development of the seedlings of genotype CNPAE-308 in relation to the others, at both levels of irradiation studied. In treatments with less light restriction (PPF-1 and PPF-2), the genotypes CNPAE-301 and CNPAE-144 showed lower means of DMA when compared to the others, the same happened to CNPAE-248 and CNPAE-144 in the levels of higher light restriction (PPF-3 and PPF-4). Overall, a differential behavior for accumulation of DMA is observed among genotypes in each level of PPF (Table 4), which indicates the existence of differential genetic expressions to this environmental stress. This is due to the wide genetic variability among genotypes of physic nut found in Brazil, providing high phenotypic variability to genotypes subjected to different environmental stresses (Amaral et al., 2012). This genetic variability was reported by Laviola et al. (2010) and Nunes et al. (2013) studying genetic parameters of 110 genotypes of physic nut in Brazil. There was no statistical difference between the mean values of DMR of the genotypes in relation to the levels of photosynthetic photon flux studied in this experiment. As the experiment lasted 30 days, it is possible that the root development at time was still not sufficient to express variability as observed in DMA. This actually ended up influencing the outcome of DMT, causing the means to be similar between genotypes in most cases. For DMT, the seedlings of the genotypes of physic nut had similar means within each level of photosynthetic photon flux studied, except for CNPAE-301 and CNPAE-144 that showed lower accumulation of biomass in the levels PPF-1 and PPF-3, respectively (Table 4). Higher means of dry matter (DMA, DMR and DMT) were observed in the level PPF-3, corresponding to 330 μ mol m⁻² s⁻¹ of photosynthetic photon flux (50% of shading). The regression analyses for the genotypes showed better fitting to quadratic models, with coefficients above 0.77 (Table 4). The response in dry mass accumulation due the limited availability of irradiation in the environment is variable for each species, due to the different

Table 1. Characterization of seeds of Jatropha g	genotypes.
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Origin	Yield ¹	GER^2	MOI ³	$W1000^{4}$	WID^5	LD^6	ld^7
Origin	$(g plant^{-1})$	(%)		(g)	(mm)		
Minas Gerais	885	96.0	5.89	639.78	17.07	10.48	8.36
Rio G. do Sul	1200	96.0	5.92	628.62	16.84	10.33	8.25
Paraíba	400	96.0	5.97	620.41	16.95	10.40	8.28
Maranhão	550	92.0	6.05	656.26	17.20	10.89	8.23
	Rio G. do Sul Paraíba	Origin(g plant ⁻¹)Minas Gerais885Rio G. do Sul1200Paraíba400	Origin (g plant ⁻¹) (% Minas Gerais 885 96.0 Rio G. do Sul 1200 96.0 Paraíba 400 96.0	Origin (g plant ⁻¹) (%) Minas Gerais 885 96.0 5.89 Rio G. do Sul 1200 96.0 5.92 Paraíba 400 96.0 5.97	Origin (g plant ⁻¹) (%) (g) Minas Gerais 885 96.0 5.89 639.78 Rio G. do Sul 1200 96.0 5.92 628.62 Paraíba 400 96.0 5.97 620.41	Origin (g plant ⁻¹) (%) (g) Minas Gerais 885 96.0 5.89 639.78 17.07 Rio G. do Sul 1200 96.0 5.92 628.62 16.84 Paraíba 400 96.0 5.97 620.41 16.95	Origin (g plant ⁻¹) (%) (g) (mm) Minas Gerais 885 96.0 5.89 639.78 17.07 10.48 Rio G. do Sul 1200 96.0 5.92 628.62 16.84 10.33 Paraíba 400 96.0 5.97 620.41 16.95 10.40

1000 seeds; ⁵Width; ⁶Greater length; ⁷Shorter length.

Table 2. Means of intensity of photosynthetic photon flux during the experiment, Alegre-ES, Brazil, UFES, 2012.

Time	Shading (%)						
	0 (PPF-1)	30 (PPF-2)	50 (PPF-3)	80 (PPF-4)			
		$\mu mol m^{-2} s^{-1}$					
09:00	217	249	181	101			
12:00	1012	667	520	280			
15:00	520	368	289	140			
Mean*	829	438	330	174			

* Value used as independent variable in the regressions.

genetic characteristics and therefore different morphological and physiological adaptations. However, higher values of dry matter at higher levels of shading may not be a physiological adaptation, but a return to their normal growth condition, since many cultivated species are taken from naturally shaded environments and introduced into new environments under full sunlight (Fahl et al., 1994; Brant et al., 2010). In shaded environments, there is a decrease in photo-inhibition and in degradation of carotenoids, which increases the number of active photosynthetic pigments in the thylakoid membranes, consecutively promoting the photosynthesis and the accumulation of dry matter. This fact explains the better performance of the genotypes in shaded conditions. The chlorophyll *a* and carotenoids are two major pigments connected to the photosynthetic efficiency and, consequently, on to the growth and adaptation of plants to different environmental conditions (Force et al., 2003). Carotenoids play an important role in the light harvesting complex and in the protection of photosystems. Several studies have reported that these compounds protect the photosynthetic apparatus against photo-degradation by inter-conversions between molecules of xanthophyll (Ort, 2001).

Material and Methods

Description of the study and plant material

The study was conducted at the laboratory of seed analysis from the department of crop science of the Centro de Ciências Agrárias – Universidade Federal do Espírito Santo (CCA-UFES), located in the municipality of Alegre, south of Espírito Santo State, Brazil. The genotypes were originated from different regions of Brazil, supplied by the Brazilian breeding program developed by Embrapa Agroenergia; these genotypes were chosen because they are contrasting for genetic and phenotypic characteristics (Laviola et al, 2010) and the potential productivity (Table 1). The seeds were characterized regarding germination, water content, weight of 1.000 seeds, width and length (Table 1), according to the methodology proposed by Brazil (2009) and Christo et al. (2012).

Experimental design and conduct of the study

The experiment followed a factorial 4x4, composed by four genotypes of physic nut (CNPAE-248, CNPAE-301, CNPAE-308 and CNPAE-144) and four levels of photosynthetic photon flux (PPF-1, PPF-2, PPF-3 and PPF-4), arranged in completely randomized design, with four replications and the experimental plot consisting of 12 seedlings. Two seeds of physic nut were planted in containers, with 120 mL of capacity, filled with commercial substrate for seedlings. Three days after emergence, the thinning was performed to allow only one plant per container to grow. The water content of the substrate was maintained near to the field capacity by frequent irrigations. The levels of photosynthetic photon flux were applied by shading, using commercial shade screens (0%, 30%, 50% and 80% of shading) to cover the whole area over and around the seedlings. The microclimate were monitored within each experimental plot through daily readings of temperature, relative humidity and intensity of photosynthetic photon flux, collected manually at 9:00, 12:00 and 15:00 each day. The temperatures registered during the experiment ranged from 19.0 to 41.0 °C and the relative humidity was around 75%.

Evaluation of the study and calculate indices

The evaluation of the growth were performed 30 days after emergence, collecting data about number of leaves (NL), root length (RL), plant height (PH), stem diameter (SD), total leaf area (LA), dry matter of aerial part (DMA), dry matter of roots (DMR) and total dry matter (DMT). The plant height and root length were measured with graduated ruler and the the stem diameter with digital caliper. The leaf area were calculated by the methodology proposed by Severino et al. (2006), using linear dimensions of the leaves and the expression: LA= $0.84(LW)^{0.99}$, where LA is the leaf area, L is the length of the main rib and W is the width of the middle section of the leaf. The total leaf area of each plant was determined by the sum of the area of each leaf. The aerial part and roots were separated and dried in laboratory oven, with forced air circulation, at 70.0 °C until constant weight,

Constunes	PPF-1	PPF-2	PPF-3	PPF-4	Regression equation	\mathbb{R}^2
Genotypes	Plant heigh	nt – PH (mm)				
CNPAE-248	750 b	851 c	900 c	812 c	$-0.001*PPF^{2} + 0.750*PPF + 705.40$	0.83
CNPAE-301	1108 ab	1321 a	1558 a	1163 b	$-0.002*PPF^{2} + 2.240*PPF + 882.00$	0.70
CNPAE-308	1142 a	1333 a	1523 a	1249 a	$-0.002*PPF^{2} + 1.910*PPF + 966.20$	0.86
CNPAE-144	1064 ab	1206 b	1412 b	1157 b	$-0.001*PPF^{2} + 1.252*PPF + 1014.0$	0.85
	Number of	leaves – NL				
CNPAE-248	2.0 c	4.0 a	5.9 a	3.0 b	$-2E-05*PPF^{2} + 0.014*PPF + 1.132$	0.82
CNPAE-301	3.0 b	3.6 a	4.0 c	3.3 ab	$-5E-06*PPF^{2} + 0.004*PPF + 2.718$	0.76
CNPAE-308	3.5 a	3.8 a	5.0 b	3.8 a	$-2E-06*PPF^{2} + 0.001*PPF + 3.681$	0.84
CNPAE-144	3.4 a	3.8 a	4.0 c	3.5 ab	$-4E-06*PPF^2 + 0.003*PPF + 3.011$	0.80
	Stem diam	eter – SD (mr	n)			
CNPAE-248	40.0 b	49.4 b	60.9 a	47.6 a	-	
CNPAE-301	47.0 ab	49.7 b	60.7 a	49.0 a	-	
CNPAE-308	51.1 a	54.1 a	60.4 a	52.1 a	-	
CNPAE-144	48.4 ab	54.8 a	55.4 b	51.5 a	-	
	Root lengt	h - RL (mm)				
CNPAE-248	80.0 d	113.3 c	137.1 d	110.9 c	-	
CNPAE-301	115.1 b	122.7 b	150.4 b	118.3 b	-	
CNPAE-308	126.3 a	140.8 a	165.3 a	132.5 a	-	
CNPAE-144	107.6 c	120.7 b	141.7 c	117.1 b	-	
	Leaf area -	- LA (mm ²)				
CNPAE-248	1113 d	1534 c	1949 c	1531 b	$-0.002*PPF^{2} + 2.000*PPF + 1335.0$	0.93
CNPAE-301	1545 b	1768 b	2344 b	1858 a	-0.001*PPF ² + 1.370*PPF + 1773.0	0.87
CNPAE-308	1380 c	1842 a	2526 a	1504 b	$-0.005*PPF^{2} + 5.602*PPF + 847.10$	0.85
CNPAE-144	1661 a	1794 b	1983 c	1811 a	$-0.001*PPF^{2} + 0.005*PPF + 1865.4$	0.91

Table 3. Means of plant height, number of leaves, stem diameter, root length and leaf area of seedlings of genotypes of physic nut, subjected to different levels of photosynthetic photon flux – PPF (759, 438, 330 and 174 μ mol m⁻² s⁻¹)

Means followed by the same letter do not differ significantly by the Tukey test at 5% of probability. *significant by the Student's t-test at 5% of probability.

Table 4. Means of dry matter of aerial part, dry matter of roots and total dry matter of seedlings of genotypes of physic nut, subjected to different levels of photosynthetic photon flux – PPF (759, 438, 330 and 174 μ mol m⁻² s⁻¹)

Ganatunas	PPF-1	PPF-2	PPF-3	PPF-4	Regression equation	\mathbf{R}^2
Genotypes		Dry matte	er of aerial par	t – DMA (mg)		
CNPAE-248	1747 a	2502 a	3663 ab	1952 b	-0.009*PPF ² + 9.266*PPF + 876.9	0.88
CNPAE-301	1155 b	2098 b	4308 a	2191 ab	-0.009*PPF ² + 7.385*PPF + 1591.1	0.86
CNPAE-308	1662 a	2501 a	4051 a	2252 ab	$-0.009*PPF^{2} + 8.120*PPF + 1417.5$	0.84
CNPAE-144	1721 a	2050 b	3000 b	2507 a	$-0.001*PPF^{2} + 0.258*PPF + 2729.2$	0.84
		Dry matte	er or roots – D	MR (mg)		
CNPAE-248	155 a	250 a	370 a	200 a	$-0.001*PPF^{2} + 0.944*PPF + 91.5$	0.91
CNPAE-301	170 a	260 a	500 a	270 a	$-0.001*PPF^{2} + 0.785*PPF + 207.1$	0.77
CNPAE-308	183 a	280 a	400 a	210 a	$-0.001*PPF^{2} + 1.106*PPF + 78.5$	0.92
CNPAE-144	197 a	230 a	290 a	220 a	$-0.001*PPF^{2} + 0.331*PPF + 185.5$	0.87
		Total dry	matter - DM	Г (mg)		
CNPAE-248	1820 a	2730 a	3870 ab	2120 a	-0.010*PPF ² + 9.986*PPF + 944.5	0.91
CNPAE-301	1320 b	2360 a	4810 a	2470 a	$-0.010*PPF^2 + 8.190*PPF + 1806.2$	0.79
CNPAE-308	1840 a	2840 a	4400 a	2250 a	-0.012*PPF ² + 11.20*PPF + 984.3	0.87
CNPAE-144	1190 b	2280 a	3290 b	2710 a	$-0.003*PPF^{2} + 1.560*PPF + 2711.8$	0.93
Means followed by th	ne same letter do n	ot differ signific	antly by the Tuke	y test at 5% of proba	bility. *significant by the Student's t-test at 5% of probability.	

in order to determine the dry matter accumulated in each vegetal part.

Statistical analysis

The data were subjected to analysis of variance ($p \le 0.05$); and, according to the significance of the sources of variation, the means were studied using the Tukey test ($p \le 0.05$) for qualitative factors and regression analysis for quantitative factors. The regression models were chosen based on the significance of the coefficients, using the Student's t-test ($p \le 0.05$), and the coefficient of determination (R²). The analyses were made using the statistical software SISVAR (Ferreira, 2011).

Conclusion

The genotype CNPAE-308 had superior vegetative growth at both levels of irradiation studied. The best development of seedlings of jatropha occurs in shaded environments, with approximately 330 μ mol m⁻² s⁻¹ of photosynthetic photon flux, equivalent to 50% shading. The results indicate that the cultivation of CNPAE-308 in 50% shade (330 mol m⁻² s⁻¹) resulted in an increase of 83% leaf area and 33% of vertical plant growth, compared to the unshaded. Under these conditions, it was observed an increase of 144% dry matter of shoots and 140% of total dry matter.

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