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Physiological response of three wild castor bean (*Ricinus communis* L.) ecotypes exposed to different substrate moisture levels

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ABSTRACT

Objective: To analyze the morphological and physiological responses of three wild castor bean (*Ricinus communis* L.) ecotypes to four different gravimetric moisture levels.

Design/methodology/approach: The wild castor bean ecotype seeds were collected in the arid region of the State of Durango, Mexico. Three potential ecotypes were selected according to seed size and shape. A completely random greenhouse culture was established with three wild castor bean ecotypes; they were planted in substrate with four gravimetric moisture levels ($T1_{\Theta}=24\pm2\%$; $T2_{\Theta}=20\pm2\%$; $T3_{\Theta}=16\pm2\%$; $T4_{\Theta}=14\pm2\%$). The physiological measurements were carried out with LICOR's LI-6400XT portable photosynthesis system. A two-way ANOVA was conducted to obtain differences between the factors and their interactions.

Results: Ecotypes 1 and 2 had larger stems and leaves than ecotype 3. The differences in plant growth due to the effects of a 24% and 20% gravimetric moisture content were not significant ($p=0.05$). Ecotype 3 presented the highest photosynthetic rate (14.77 ± 6.14 μmol CO_2 $m^{-2}s^{-1}$); however, the differences between ecotypes were not significant. The differences were determined based mainly on substrate moisture.

Study limitations/implications: Determining the water requirements of castor bean crops allows for the optimization of water use in regions where this resource is scarce.

Findings/conclusions: Ecotype 1 seeds —which were very large, very round, and had low eccentricity— are associated with plants that have larger and wider stems and leaves. This genotype could be domesticated considering a substrate moisture content of 24% and 20%.

Key words: Intracellular carbon dioxide, Leaf vapor pressure deficit, Morphometry, Photosynthetic rate, Stomatal conductance.

Citation: Peña-Uribe, G. de J., Vázquez-Chun, C., Valdez-Cepeda, R. D., Armendáriz-Erives, S., & López-Santos, A. (2022). Physiological response of three wild castor bean (*Ricinus communis* L.)ecotypes exposed to different substrate moisture levels. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2045

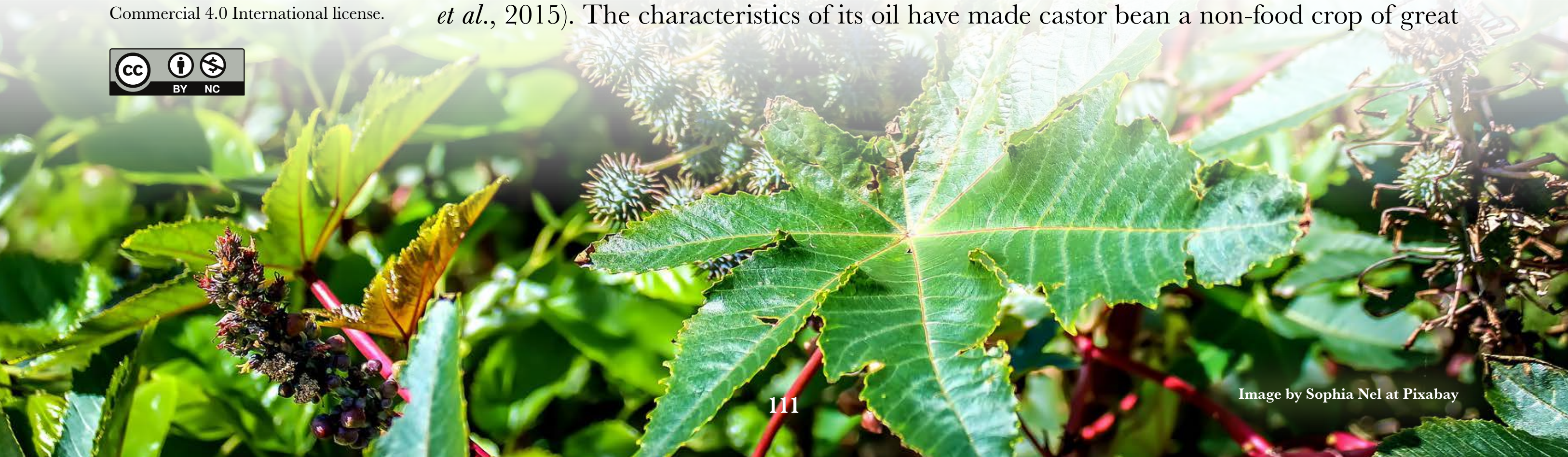
Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: May 23, 2021.
Accepted: January 3, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 111-119.

INTRODUCTION

The seeds of castor bean (*Ricinus communis* L.) (Euphorbiaceae) are used to produce castor oil, which is widely used in the chemical industry (Severino *et al.*, 2012; Lakhani *et al.*, 2015). The characteristics of its oil have made castor bean a non-food crop of great



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economic importance in the last few years (Severino *et al.*, 2012). In addition, various authors (Da Silva *et al.*, 2006; Dos Santos *et al.*, 2017; Buendía-Tamariz *et al.*, 2018) emphasize the fact that castor oil is a high-quality raw material to produce biofuel. Small-scale producers, mainly from India, China, Brazil, and Mozambique, account for 96% of the worldwide castor bean seed production (Severino and Alud, 2013). Only in 2008 did Mexico figure as the eighth castor bean producer worldwide; afterwards, production data have decreased (FAOSTAT, 2021). Although the demand for castor oil is constantly increasing, the supply is limited by the current production of castor bean, which cannot satisfy the global market's demand (Severino *et al.*, 2012).

Limited water availability is a recurring phenomenon and a main restricting factor in crop productivity in the arid tropics (Vijaya-Kumar *et al.*, 2005; Sausen and Rosa, 2010). Castor bean's tolerance to water deficit has been studied from diverse viewpoints. Different authors (Dai *et al.*, 1992; Lacerda *et al.*, 2009; Sausen and Rosa, 2010; Brito-Pinto *et al.*, 2014; Dos Santos *et al.*, 2017) agree that castor bean has high tolerance levels to water deficit and can even have a low seed yield with little available water, a situation where other species could not be cultivated (Severino *et al.*, 2012). Nevertheless, castor bean cultivation is still unknown in different regions of Mexico, which reduces its promotion and the development of crop systems (Buendía-Tamariz *et al.*, 2018).

Establishing the water requirements of castor bean cultivation allows for the optimization of water use in regions where this resource is scarce. Although castor bean is considered a species with low genetic diversity (Allan *et al.*, 2008; Foster *et al.*, 2010), it has a high phenotypic polymorphism, from very tall to very small plants (Lakhani *et al.*, 2015). Therefore, there might be significant differences in morphometric and physiological responses among castor bean ecotypes at different gravimetric moisture levels. The aim was to analyze the morphologic and physiological responses of three castor bean ecotypes collected in the arid regions of the State of Durango, Mexico, based on four soil moisture content levels. The underlying hypothesis was that ecotypes have different morphometric and physiological responses to different substrate gravimetric moisture levels.

MATERIALS AND METHODS

This research was conducted between June 2017 and May 2018 under greenhouse conditions at the Unidad Regional Universitaria de Zonas Áridas de la Universidad Autónoma Chapingo (25.893° N, 103.600° W). The climate in the region is very arid and semi-hot, with an annual average temperature between 18 °C and 22°C (BWhw). Temperatures inside the greenhouse fluctuated between 3.9 and 36.09 °C, with an overall average of 25.07±6.2 °C (Table 1).

Preparation for the experiment

The wild castor bean seeds were collected in the arid region of the State of Durango, Mexico (Table 2). Three potential seed ecotypes were selected according to size and shape (data are not shown). The first group comprised large seeds with lower eccentricity and higher roundness (Ecotype 1). The second group included medium-sized seeds, with high eccentricity, and intermediate roundness (Ecotype 2). The third group consisted of small

Table 1. Mean environmental conditions in the greenhouse during the experiment.

Year	Month	Min.T.	Max.T.	Mean T.	Hum. (%)
		°C			
2017	July	24.31	40.63	31.36	39.81
	August	23.22	43.67	32.13	40.59
	September	20.89	39.77	28.9	43.97
	October	16.7	41.1	26.51	42.73
	November	11.33	41.18	23.16	33.07
	December	8.34	29.08	16.21	54.59
2018	January	5.89	32.86	16.53	38.76
	February	13.84	38.2	23.58	44.11
	March	15.92	43.72	27.76	36.06
	April	18.36	43.1	28.65	36.02
	May	20.8	43.56	30.15	37.9

Min.T., minimum temperature; Max.T., maximum temperature; MeanT., mean temperature; and Hum., Air humidity.

seeds with highly dispersed eccentricity and roundness values (Ecotype 3). Out of each group, 36 seeds were selected. They were planted in 72-cell seedbeds with peat moss substrate. The seedbeds were irrigated daily with purified water until transplant day. The seedlings were transplanted 21 days after planting, when they had already developed two leaves.

The substrate that was used in the experiment came from soil collected in an experimental plot. According to the *World Reference Base for Soil Resources* (IUSS Working Group WRB, 2015), the soil was identified as aridic Calcisol (CLad). In addition, the soil was characterized in the laboratory following the Norma Oficial Mexicana NOM-021-RECNAT-2000 guidelines, which establish specifications for soil fertility, salinity, and classification. Bulk density was determined with the paraffin method (AS-03); soil texture

Table 2. Collection sites of castor ecotypes and their environmental characteristics.

Eco	Site	Lat.	Long.	WRB Key	RSG	Climate
E3	Gómez Palacio	25.613726	−103.4932	SNszw+LVsow/3	SOLONETZ	BWhw
E1	Guatimape	24.807201	−104.9197	SNaxszn+VRmzszp/2	SOLONETZ	BS1kw
E3	Leandro Valle	25.086666	−105.065	FLeu+KSlvcc/1	FLUVISOL	BSohw
E1	Nazareno	25.397986	−103.4201	RGsowca+CLad+VRcrca/2	REGOSOL	BWhw
E2	Nazas	25.228944	−104.113	CLlv+KSlvcc/2	CALCISOL	BWhw
E2	Nazas	25.232055	−104.1168	CLlv+KSlvcc/2	CALCISOL	BWhw
E2	Nazas	25.2306944	−104.1371	FLeu+KSlvcc/1	FLUVISOL	BWhw
E1	San Luis del Cordero	26.196666	−105.2017	CLskptp+RGskca/2R	CALCISOL	BWhw
E3	Villa Unión	23.967611	−104.0496	Flca+KSvpcn/2	FLUVISOL	BS1kw
E3	Villa Unión	23.9595	−104.0539	Flca+KSvpcn/2	FLUVISOL	BS1kw

Eco, ecotype; WRB Key, World Reference Base for Soil Resources key; and RSG, reference soil group.

was established using the Bouyoucos hydrometer method (AS-09); the AS-05 method was used to determine soil moisture; organic matter was established with the Walkley and Black method (AS-07); and electric conductivity was measured using the AS-16 method. Soil texture was identified as sandy clay loam (Table 3).

Experimental design

Twelve treatments were established based on the three castor bean ecotypes and four gravimetric moisture levels ($T1_{\theta}=24\pm2\%$; $T2_{\theta}=20\pm2\%$; $T3_{\theta}=16\pm2\%$; $T4_{\theta}=14\pm2\%$). Four repetitions were prepared, giving a total of 48 pots or experimental units. Six kilograms of dry soil were placed in each of the forty-eight 7.5-liter pots. One liter of tap water was added to each pot in order to dampen the substrate. Then a castor bean seedling was placed in each pot. All pots were labelled with the relevant ecotype, the corresponding gravimetric moisture percentage, and the repetition number. The treatments were distributed according to a completely random experimental design.

Irrigation

Water for irrigation was extracted from a deep well. Water electric conductivity was 2.85 dS m^{-1} according to the Orion Star A222 portable conductivity meter (Thermo Scientific, USA). During a 30-day acclimatization period, plant irrigation was homogenous: approximately 900 g of water were added per pot. Subsequently, pots were weighed every two days before and after irrigation, in order to estimate the quantity of water to be restored.

Morphological variables

Plants were measured every week throughout the experiment. A measuring tape was used to measure the stem height (SH) —from the substrate base to the last internode. To measure the stem width (SW), a permanent marker was first used to make a mark on the stem 1 cm above the soil surface. This mark served as a reference to measure the stem width using a Vernier caliper. Leaf length (LL) and leaf width (LW) were measured and the results were used to calculate the leaf area (LA).

Physiological variables

A LI-6400XT portable photosynthesis system (LICOR Inc., Lincoln, Nebraska, USA) was used to measure the physiological response of the three castor bean ecotype plants to the different substrate moisture levels. Once all the plants had at least one leaf with a length of over 5 cm, measurements were done every 15 days. The LI-6400XT was calibrated with

Table 3. Biophysical characteristics of aridic Calcisol collected from the experimental plot.

Sample	BD g cm^{-3}	FC	PWP	USM	OC	OM	pH	EC dS m^{-1}	N	CO_3
		%							%	
Depth (cm)										
0 a 15	1.28	27.24	14.63	12.6	0.23	0.4	8	7.72	0.1	7.3

BD, bulk density; FC, field capacity; PWP, permanent wilting point; USM, usable soil moisture; OC, organic carbon; OM, organic material; pH, hydrogen potential; EC, electric conductivity; N, nitrogen; and CO_3, carbonates.

a photon flux density within the photosynthetically active radiation spectrum of 1000 μmol $m^{-2}s^{-1}$. This device was used to estimate photosynthetic rate (*A* in μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (*gs* in mol H_2O $m^{-2}s^{-1}$), intracellular carbon dioxide concentration (*Ci* in μmol CO_2 mol^{-1}), leaf water vapor deficit (VpdL in kPa), and leaf temperature (Tleaf in °C).

Statistical analyses

Morphological and physiological variables were subjected to normality tests. Variables that presented a non-normal distribution were standardized. Two-way ANOVAs were conducted to identify the effects of the different factors on the response variables. When the results allowed the identification of significant effects of the factors, Tukey's test was applied to conduct the corresponding comparisons. Statistical analyses were conducted with the MiniTab 17 software (MiniTab Inc., USA).

RESULTS AND DISCUSSION

Morphometry

The most striking results are described in the rest of this section. Ecotype 1 and 2 plants had the tallest stems, which were especially similar when cultivated in substrates with 24% and 20% gravimetric moisture levels (Table 4). In addition, ecotype 1 plants had the widest stems when the substrate had a gravimetric moisture level of 24%. Ecotype 1 and 2 plants also had the longest and widest leaves when grown in substrates with 24% and 20% gravimetric moisture levels. Significantly, ecotype 1 plants had the largest leaf area, especially when cultivated in substrates with a gravimetric moisture content of 24%.

In general, these results are consistent with those reported by Brum *et al.* (2011), who found that castor bean seedlings grown from heavier seeds emerge faster and are taller than seedlings grown from lighter seeds. Other authors (*e.g.*, Lacerda *et al.*, 2009; Silva *et al.*, 2009; Brito-Pinto *et al.*, 2014) emphasized that, if water availability increases, the height, leaf area, and yield of castor bean plants also increases. It is worth mentioning that plants were smaller than the "Al Guarany 2002" variety (59.3 cm), according to Brito-Pinto

Table 4. Means and standard deviations of castor bean morphometric variables grouped by factors.

Factors \ Variables	Stem height (cm)	Stem width (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Ecotype					
E1	12.50 ± 3.88^{A}	0.71 ± 0.20^{A}	7.20 ± 2.07^{A}	9.24 ± 3.13^{A}	71.28 ± 37.51^{A}
E2	8.51 ± 2.57^{B}	0.58 ± 0.19^{B}	6.75 ± 2.13^{A}	8.31 ± 2.39^{AB}	60.89 ± 34.46^{A}
E3	7.46 ± 2.34^{C}	0.53 ± 0.18^{B}	5.45 ± 1.84^{B}	7.23 ± 1.99^{B}	44.08 ± 23.97^{B}
Substrate moisture					
T1⊖ 24±2%	10.91 ± 4.56^{A}	0.74 ± 0.22^{A}	7.52 ± 2.44^{A}	9.52 ± 2.48^{A}	76.63 ± 42.25^{A}
T2⊖⊖20±2%	9.93 ± 3.36^{A}	0.62 ± 0.17^{B}	6.88 ± 1.59^{A}	8.59 ± 1.75^{A}	60.87 ± 23.98^{B}
T3⊖⊖16±2%	8.74 ± 2.51^{B}	0.51 ± 0.12^{C}	5.34 ± 1.61^{B}	6.84 ± 3.33^{B}	39.30 ± 23.65^{C}
T4⊖⊖14±2%	6.63 ± 2.31^{C}	0.36 ± 0.09^{D}	4.89 ± 1.52^{B}	5.78 ± 1.17^{B}	34.99 ± 12.87^{C}

Results of Tukey's means tests are shown, capital letters represent similar groups.

et al. (2014). This could be explained by pot size: Brito-Pinto *et al.* (2014) used 30-L pots (four times larger than the ones we used in our work). However, the leaf area of our three ecotypes is larger than the leaf area of the “Al Guarany 2002” variety (35.37 cm^2). Leaf area is an important factor, because it is directly related to the leaf’s capacity to intercept light and carry out photosynthesis (Severino and Auld, 2013).

Physiology

The results for the physiological variables were as follows: only the leaves of ecotype 2 and 3 plants had a higher gs than genotype 1 (Table 5). Likewise, the lower gs is associated to the leaves of plants that were cultivated in the substrate with the lowest gravimetric moisture level (16%); their temperature and photosynthetic rate were also considerably lower than those of the leaves of plants in substrates with higher moisture content levels. However, the differences between *A*, *Ci*, *VpdL*, and *Tleaf* variable values for leaves of the three ecotypes were statistically similar. Likewise, the leaves of plants cultivated in the two lower substrate moisture levels presented higher water vapor deficits than those associated to a substrate gravimetric moisture level of 25%.

Overall, plants cultivated in pots with a higher substrate moisture level presented higher physiological activity values. This is consistent with reports by other authors (Lacerda *et al.*, 2009; Silva *et al.*, 2009; Brito-Pinto *et al.*, 2014), who mention that castor bean yields are closely related to the available moisture. According to Da Matta *et al.* (2001), values for A in plants with the highest available moisture levels are similar to those of bean (16.3 μmol CO_2 $m^{-2}s^{-1}$) and eucalyptus (14.4 μmol CO_2 $m^{-2}s^{-1}$). Moreover, Sausen and Rosa (2010) obtained maximum photosynthesis values of 15 μmol CO_2 $m^{-2}s^{-1}$ with 18% of soil moisture, while Dos Santos *et al.* (2017) recorded maximum photosynthesis values of 16.2 μmol CO_2 $m^{-2}s^{-1}$.

On the one hand, different authors (Lawlor and Tezara, 2009; Broeckx *et al.*, 2014; Dos Santos *et al.*, 2017) maintain that the decrease of *gs* is a result of stomatal closure, which is a key phenomenon and the first defense against dehydration; this might have been the

Table 5. Means and standard deviations of the physiological variables of castor bean grouped by factors.

Variables / Factors	*A*	*gs*	*Ci*	*VpdL*	*Tleaf*
Ecotype					
1	11.11±5.32	0.10±0.07^{B}	147.33±53.88	2.66±0.72	31.14±3.42
2	12.93±5.66	0.12±0.11^{AB}	127.70±149.40	2.85±0.86	32.43±3.36
3	14.77±6.14	0.16±0.12^{A}	154.90±73.00	2.84±0.97	32.81±3.32
Substrate Moisture					
T1⊖⊖24±2%	14.95±5.61^{A}	0.14±0.11^{A}	127.60±132.00	3.00±0.96^{A}	33.36±3.17^{A}
T2⊖⊖20±2%	13.22±5.21^{A}	0.14±0.11^{A}	149.38±73.50	2.68±0.81^{AB}	31.81±3.30^{B}
T3⊖⊖16±2%	7.62±4.10^{B}	0.06±0.04^{B}	161.11±57.03	2.51±0.52^{B}	29.90±3.04^{C}

A, photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$); gs, stomatal conductance (mol H_2O $m^{-2}s^{-1}$); Ci, intracelular carbon dioxide concentration (μmol CO_2 mol^{-1}); VdpL, leaf water vapor deficit (kPa); and Tleaf temperatura foliar (°C). Results of Tukey’s means tests are shown, capital letters represent similar groups.

case of the plants subjected to a substrate gravimetric moisture level of 16%. Furthermore, according to Heckenberger *et al.* (1998) and de Freitas *et al.* (2011), this decrease in stomatal conductance might also be the result of a decrease in the stomatal conductance values of castor bean plants subjected to water deficit, as a consequence of the low stomata density during leaf growth in water stress conditions.

On the other hand, the tendency of *A* to decrease and *Ci* to increase as the soil moisture availability decreases is noticeable (Table 4). The increase in *Ci* is inconsistent with reports by Ocheltree *et al.* (2014), who maintain that the reduction in the plants' stomatal conductance also entails a reduction in the CO_2 diffusion rate and the internal CO_2 concentration. This situation can reduce the efficiency of carbon fixation in plants. In light of this, the responses of plants to water deficit seem to be complex and to involve adaptive changes and genotype influence (Chaves *et al.*, 2002).

Barbour and Buckley (2007) and other authors mention that an increase in leaf water vapor deficit directly affects stomatal closure. Nevertheless, this was not observed on castor bean leaves. Oddly enough, the highest *VpdL* was observed in plants that were grown in pots with higher substrate moisture levels, which were also those with higher *gs*. This is consistent with Davies and Zang (1991), who maintain that stomatal response is frequently associated with soil water content, rather than with leaf water status. Therefore, this could mean that the plant can transpire continually without problems, which allows water to move constantly through the plant. According to several authors (McDonald *et al.*, 2002; Snyder *et al.*, 2003), this can provide more benefits for the plant, such as a better nutrient absorption from the soil.

CONCLUSIONS

Castor bean ecotype 1 has big, very round seeds with low eccentricity, which yielded plants with wide stems, with a 24% substrate gravimetric moisture level. This ecotype's plants are also associated with very long and wide leaves, when they are grown in substrates with 24% and 20% gravimetric moisture levels. Notably, castor bean ecotype 1 plants had the larger leaf area, especially when cultivated in substrates with a 24% gravimetric moisture content. In general, results indicate that this genotype could be introduced in an improvement program, considering these two substrates gravimetric moisture content conditions for its cultivation.

ACKNOWLEDGEMENTS

G.J.P.-U. is extremely grateful for the PhD grant (CVU 514128) awarded to him by the Consejo Nacional de Ciencia y Tecnología (CoNaCyT). The authors are indebted to the Universidad Autónoma Chapingo for the funding of the project "Potencialidades de la higuerilla como un cultivo alternativo en el norte de México para la adaptación y mitigación al cambio climático" (Castor bean's potential as an alternative crop in northern Mexico for climate change adaptation and mitigation) (Clave E-07-2016).

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