Pesquisa Agropecuária Tropical
ISSN: 1517-6398
ISSN: 1983-4063
Escola de Agronomia/UFG

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Pesquisa Agropecuária Tropical, vol. 52, e71010, 2022
Escola de Agronomia/UFG

DOI: https://doi.org/10.7440/res64.2018.03

e-ISSN 1983-4063 - *www.agro.ufg.br/pat* - Pesq. Agropec. Trop., Goiânia, v. 52, e71010, 2022

Research Article

Genetic divergence and physiological quality of dwarf castor bean lines seeds[1]

Sebastião Soares de Oliveira Neto[2], Andréia Rodrigues Ramos[2], Carlos Eduardo da Silva Oliveira[3], Edvaldo Aparecido Amaral da Silva[2], Maurício Dutra Zanotto[2]

ABSTRACT

The development of new castor bean genotypes with high grain yield and physiological seed quality is fundamental to the economic success of the crop. This study aimed to evaluate the agronomic performance and physiological quality of seeds of twelve castor bean lines, in order to determine superior genotypes. Plant height, insertion height and number of racemes, grain yield, oil content, germination percentage and vigor were evaluated. The dwarf castor bean lines H4, H5 and H11 were responsible for a grain yield above 1,400 kg ha^{-1} and are, therefore, promising for getting new hybrids. The indirect selection of dwarf castor bean lines can be performed through morphologic traits. The lines H4, H6, H9, H11 and H12 produced seeds with superior vigor and germinative quality.

KEYWORDS: *Ricinus communis* L., castor bean breeding, seed quality.

RESUMO

Divergência genética e qualidade fisiológica de sementes de linhagens de mamoneira anã

O desenvolvimento de novos genótipos de mamoneira com alta produtividade de grãos e qualidade fisiológica de sementes é fundamental para o sucesso econômico da cultura. Objetivou-se avaliar o desempenho agronômico e a qualidade fisiológica de sementes de doze linhagens de mamoneira, para determinar genótipos superiores. Foram avaliados a altura de planta, altura de inserção e número de racemos, rendimento de grãos, teor de óleo, porcentagem de germinação e vigor. As linhagens anãs de mamoneira H4, H5 e H11 foram responsáveis por produtividade de grãos acima de 1.400 kg ha^{-1} e, portanto, são promissoras para a obtenção de novos híbridos. A seleção indireta de linhagens de mamoneira anã pode ser realizada por meio de características morfológicas. As linhagens H4, H6, H9, H11 e H12 produziram sementes com vigor e qualidade germinativa superiores.

PALAVRAS-CHAVE: *Ricinus communis* L., melhoramento de mamoneira, qualidade de sementes.

INTRODUCTION

Castor bean (*Ricinus communis* L.) is an oilseed with high industrial value, due to the chemical properties of seed oil (Severino et al. 2012), being used as a lubricant in airplanes, in the manufacture of printing inks, varnishes, transparent paper and plasticizers (Tomar et al. 2017).

Brazil is one of the largest castor bean producers, occupying the third position (Savy Filho et al. 2007), and the Brazilian Savanna shows potential for expanding cultivation areas using a mechanized system (Oliveira Neto et al. 2019).

Castor bean is tolerant to biotic and abiotic stresses (Tomar et al. 2017, Khan et al. 2018) and can grow in various environmental conditions, including high temperature, drought, salinity and pest. The development of new materials which favor crop treatments and mechanized harvest, associated with a high grain yield with high physiological quality, is essential for the crop success.

Castor bean breeding programs look for traits of agronomic interest in germplasm banks to incorporate them into new cultivars (Oliveira Neto et al. 2019). Grain yield is the most studied one (Rios et al. 2013, Silva et al. 2017). The characterization of genotypes used in breeding programs provides useful information to understand and take advantage of their diversity (Cerqueira-Silva et al. 2014). Studies on this genetic diversity provide basic information

[1] Received: Nov. 29, 2021. Accepted: Mar. 08, 2022. Published: Apr. 08, 2022. DOI: 10.1590/1983-40632022v5271010.
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about parents, joining genotypes in heterotic groups, and obtaining segregating populations with greater variability in crossings (Bianchi et al. 2017).

Information on the agronomic traits of dwarf castor bean genotypes is essential for their use in breeding programs aiming to improve their performance. The production of dwarf castor bean genotypes with superior agronomic performance (high grain yield and oil content) and high physiological seed quality (germination and vigor) is ideal for the commercial success of the crop. Thus, the present study aimed to evaluate the agronomic performance, seed physiological quality and genetic divergence of twelve castor bean lines, in order to find promising genotypes.

MATERIAL AND METHODS

Twelve castor bean lines (H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11 and H12), developed by the plant breeding program of the Universidade Estadual Paulista, were evaluated in the field, in São Manuel, São Paulo State, Brazil (22º51'S, 48º26'W and altitude of 740 m). The cultivation took place between June and October 2016.

The climate classification of the area is Cwa (tropical humid, dry winter and rainy summer), with annual rainfall of 1,376 mm and average temperature of 21 ºC. Details of the experimental areas are shown in Table 1 and Figure 1. The soil is classified as Latossolo Vermelho-Amarelo Distrófico with sandy texture (Santos et al. 2018), or Dystrophic Red-Yellow Oxisol with sandy texture (USDA 2014).

The experiment was conducted in randomized blocks, with four replications. Each experimental plot consisted of 5 m in length and 4 m in width, considering as useful area of the plots only the two central lines. Sowing was carried out manually, with row spacing of 1 m and plant spacing of 0.5 m.

The sowing was manually conducted in the field in a conventional tillage system. As fertilization, 200 kg ha^{-1} of the N-P-K fertilizer (08-20-20 formulation) were applied. Weed control was performed manually. The application of fungicides and insecticides was not necessary.

The following traits were evaluated in the field: plant height; insertion height of the primary raceme; insertion height of the secondary raceme; number of racemes; stem diameter; grain yield - obtained by harvesting all the plants contained in the central lines

Table 1. Soil chemical characterization (0-20 cm depth) at the experimental area.

pH	OM	P_{resin}	K	Ca	Mg	Al	H + Al	SB	CEC	Clay	Silt	Sand	V
	g dm^{-3}	mg dm^{-3}	mmol$_c$ dm^{-3}							%			
5.0	9.0	9.0	0.2	5.0	2.0	0.0	13.0	7.0	20.0	9.5	5.0	85.5	35

H + Al: potential acidity; OM: organic matter; SB: sum of bases; CEC: cation exchange capacity; V: base saturation.

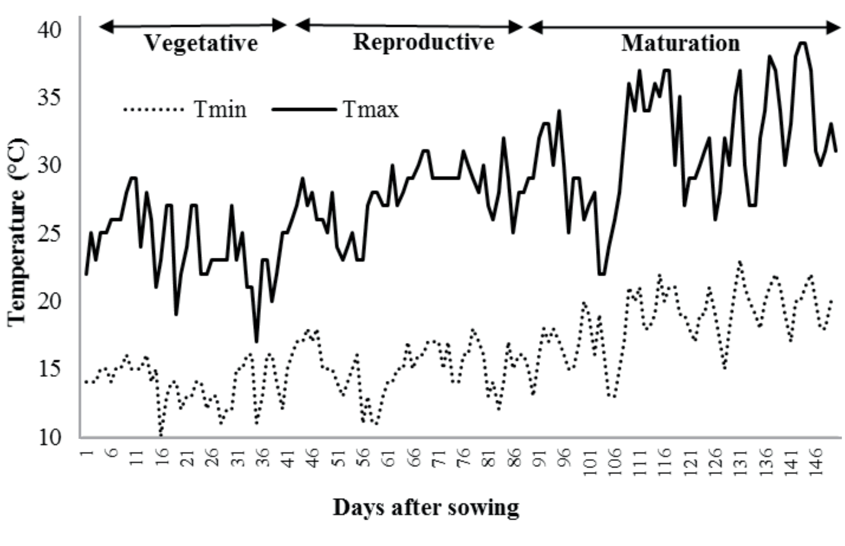

Figure 1. Minimum (Tmin) and maximum (Tmax) temperature during the castor bean cycle.

of the useful area of the plot, corrected to 13 % of humidity and subsequently extrapolated to kg ha^{-1}; and oil content (%) - determined by a SpinLock SLK 200 Magnetic Resonance Spectrometer.

After harvesting, the seeds were taken to the laboratory, where seed germination and vigor parameters were evaluated. The seed germination tests were performed according to Brasil (2009). Seed dormancy was overcome by scarifying the seeds with sandpaper. Three replications of 25 seeds were arranged in Germitest® paper rolls soaked in deionized water at 2.5 times the substrate weight. After sowing, the paper rolls were wrapped in plastic bags, tagged and kept in a germination chamber adjusted at 25 ºC, in cycles of alternate light/dark of 12 hours each. The germination percentage was determined by counting the number of normal seedlings in each replication after 7 and 14 days.

The seeds of the genotypes were produced under the same soil and climatic conditions, presented the same age, were harvested under the same maturation conditions, and presented the same time and storage conditions.

The shoot length, root length and total seedling length were measured at 7 days after sowing and expressed in centimeters. The shoot dry matter, root dry matter and total seedling dry mass were evaluated by placing the seedlings into paper bags and drying in an oven at 65 ºC, for three days, being expressed in grams.

The germination speed test was conducted together with the germination test from the daily count of the number of germinated seeds. The seeds were considered to be germinated when the primary root length was greater than or equal to 2 mm. The germination speed index (GSI) was calculated according to Maguire (1962): GSI = G1/N1 + G2/N2 + ... + Gn/Nn, where Gn is the number of seeds germinated at the nth day and N the number of days after sowing.

The agronomic and seed traits were subjected to analysis of data normality, tested by the modified Shapiro-Wilk test and then submitted to Scott-Knott analysis by the Agrostat® software. The phenotypic, genotypic and environmental correlations among the evaluated traits were estimated using variance and covariance analysis, as described by Oliveira et al. (2020). The coefficients of genotypic correlation were divided into direct and indirect effects of the evaluated traits (independent variables) on grain yield (dependent variable), using path analysis (Wright 1921). The generalized Mahalanobis distance (D2) was used to measure dissimilarity. The illustrative dendrogram of the dissimilarity pattern was prepared according to the unweighted pair group method with arithmetic mean (UPGMA), using the Genes software (Cruz 2013). The genetic divergence analysis was performed using the Tocher optimization clustering method (Rao 1952), and the Euclidean distance and Ward's minimum variance method were performed using the Action Stat Pro® software, version 3.7 for Windows (Estatcamp-Statistical Consulting, Campinas, SP, BRA). The corrplot package of the R software (R Core Team 2019) was used to evaluate the relationship among the dwarf castor bean physiological quality of seed parameters.

RESULTS AND DISCUSSION

By analyzing the agronomic traits (Table 2) of plant height, number of racemes, stem diameter and oil content, no statistical differences were observed among the genotypes by the F test. The highest averages were obtained to plant height and insertion height of the primary raceme in the H2 line (113.25 and 40.68 cm, respectively), number of racemes in H4 (4.9), stem diameter in H11 (2.33 cm) and oil content in H10 (44.8 %). The highest insertion heights of the secondary raceme were observed in the lines H2 (59.92 cm), H3 (54.80 cm), H4 (56.92 cm), H10 (56.57 cm) and H11 (55.02 cm), being approximately 10 % higher than for the other lines. The highest averages of grain yield were observed in the lines H4 (1,586 kg ha^{-1}), H11 (1,453 kg ha^{-1}) and H5 (1,403 kg ha^{-1}). The genotypes presented values twice as high as the national average (950 kg ha^{-1}) (Conab 2021). Grain yield is a trait expressed from several genes of difficult direct selection, requiring indirect selection for other important traits and thus selecting possible lines for crossing and forming highly productive hybrids (Oliveira et al. 2020).

Although no significant difference was found among the genotypes for oil content, the results are essential to knowing the oil potential of these genotypes. In Brazil, most commercialized castor bean cultivars have an oil content of 45 % (Freire 2007, Severino et al. 2006). For the lines evaluated in this study, there was a variation in oil percentage from 42.12 (H1) to 44.85 % (H10). The oil content in castor bean is a trait that brings financial benefits.

Table 2. Averages of agronomic traits for 12 castor bean lines.

Lines	PH (cm)	R1 (cm)	R2 (cm)	NR	Ø (cm)	GY (kg ha^{-1})	OC (%)
H1	108.90 a	35.52 b	52.70 b	4.65 a	1.97 a	1,015 b	42.12 a
H2	113.25 a	40.67 a	59.92 a	4.20 a	2.05 a	1,225 b	43.37 a
H3	109.30 a	35.25 b	54.80 a	4.52 a	2.12 a	1,266 b	43.57 a
H4	112.32 a	34.15 b	56.92 a	4.90 a	2.17 a	1,586 a	43.17 a
H5	103.87 a	32.72 b	51.32 b	4.30 a	2.25 a	1,403 a	43.27 a
H6	103.25 a	32.36 b	51.62 b	4.42 a	2.16 a	1,191 b	43.22 a
H7	104.52 a	32.77 b	52.32 b	4.32 a	2.13 a	1,238 b	44.55 a
H8	101.52 a	32.80 b	49.85 b	4.15 a	2.19 a	1,241 b	43.62 a
H9	97.95 a	31.87 b	50.10 b	3.87 a	2.05 a	1,185 b	43.77 a
H10	108.62 a	35.97 b	56.57 a	4.13 a	2.19 a	1,202 b	44.80 a
H11	109.12 a	33.32 b	55.02 a	4.40 a	2.33 a	1,513 a	43.62 a
H12	107.02 a	32.35 b	51.97 b	4.60 a	2.07 a	1,307 b	43.97 a
Anova	F probability						
Lines	1.55^{ns}	4.50*	2.41*	0.96^{ns}	0.96^{ns}	2.90*	1.02^{ns}
Blocks	11.54^{ns}	5.27^{ns}	7.54^{ns}	14.37^{ns}	2.32^{ns}	61.44^{ns}	0.68^{ns}
Average	106.63	34.15	53.60	4.37	2.14	1,281.35	43.59
Standard error	7.23	2.33	3.95	0.56	0.20	181.94	1.36
CV (%)	6.78	6.81	7.38	12.77	9.26	14.20	3.11

PH: plant height; R1: insertion height of the primary raceme; R2: insertion height of the secondary raceme; NR: number of racemes; Ø: stem diameter; GY: grain yield; OC: oil content; CV: coefficient of variation. * Significant at 0.05 of probability; ^{ns} not significant. Averages followed by the same letter in the column are not statistically different.

However, it does not affect the oil quality (Román-Figueroa et al. 2020).

There was no direct effect of plant height on grain yield, but there was an indirect positive effect of this trait via insertion height of the secondary raceme, and a direct negative effect of insertion height of the primary raceme on grain yield. However, an indirect positive effect via insertion height of the secondary raceme on grain yield was verified. A direct positive effect of insertion height of the secondary raceme, number of racemes and stem diameter on grain yield was observed (Figure 2). The selection of traits with direct and indirect effects on grain yield is the main way to select superior genotypes in the most diverse environmental conditions, whether stressful or not, and the path analysis provides security in this selection by breeding programs (Oliveira et al. 2020). In the selection and formation of dwarf castor bean plants, traits related to grain yield and height of racemes and plants are the most important ones, since they allow mechanized harvesting and facilitate cultivation in large areas (Zoz et al. 2021).

The coefficient of determination of the path analysis model (R^2) was equal to 0.836, featuring 83.6 % of the variation in the dependent variable in the crest model, which is explained by the variables used in the causal diagram. The coefficient of determination is an indicator for the adopted model analysis. In the case of R^2 close or equal to the unity (1), it is accepted that variations of the explanatory variables explain the variations in the dependent variable. The magnitude of the coefficient of determination is higher than that found in castor bean genotypes (Torres et al. 2015).

There was a high indirect positive effect of the oil content percentage on grain yield via insertion height of the secondary raceme and stem diameter. There was also a high indirect negative effect for the oil content percentage on grain yield via number of racemes. The dilution effect of photoassimilates may be explained by less water and nutrients in the greater number of drains (racemes) present in the plant (Figure 2). The development of several inflorescences and racemes affect the distribution of carbohydrates throughout the organs of castor bean plants, what may affect the accumulation of mass and oil content in the grains (Severino & Auld 2013). The oil content of castor bean is one of the most desirable characteristics in parents, as the higher the content, the greater the profitability of the farmer, as it is the main product generated from the cultivation of castor bean, favoring the formation of hybrids with high grain and oil yield (Oliveira Neto et al. 2019).

The direct or indirect influence of traits of interest may contribute to selecting genotypes based on specific traits, such as oil content percentage, via

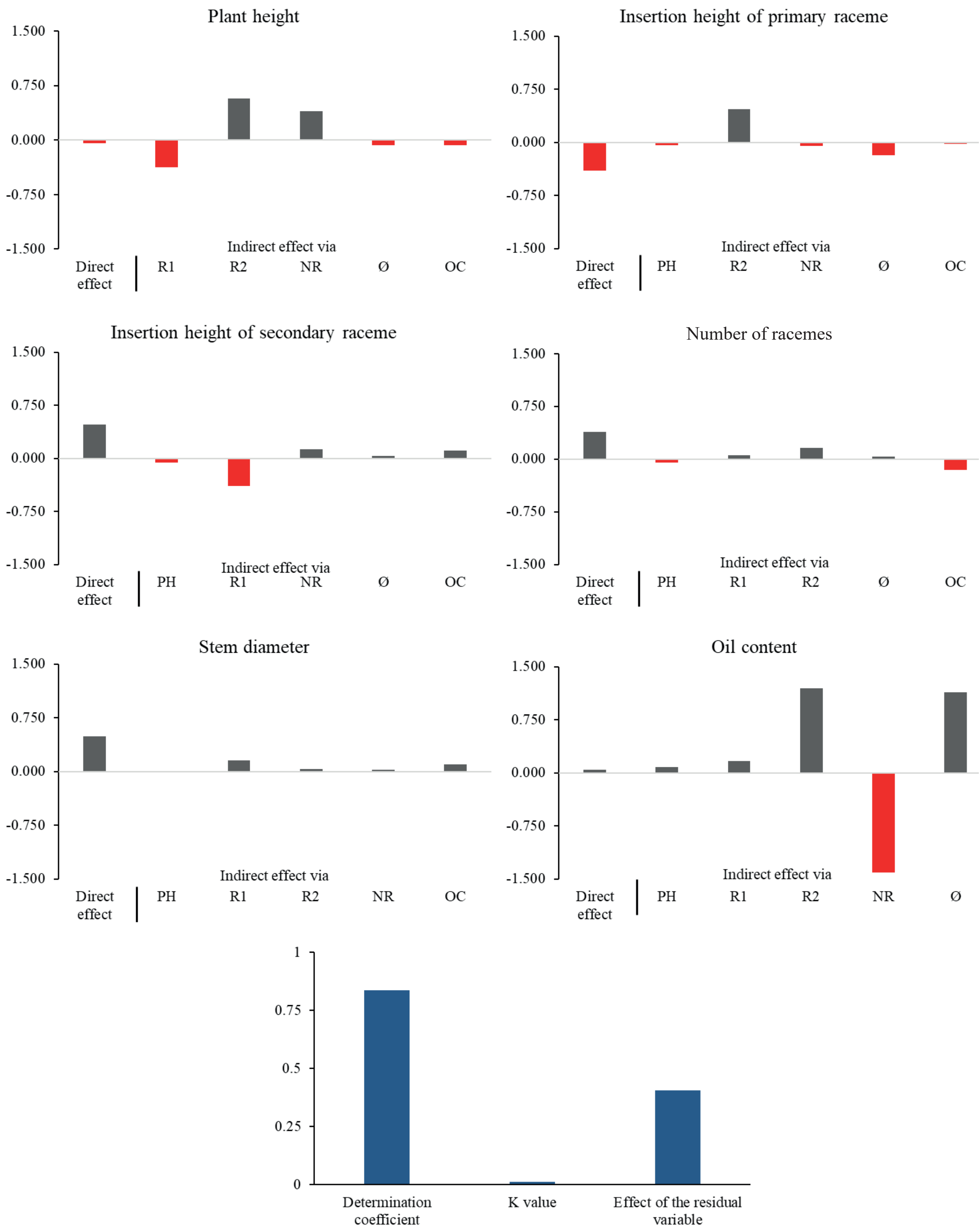

Figure 2. Estimates of the direct and indirect effects of yield components and plant traits on grain yield of dwarf castor bean lines. The columns in black explain the positive effects of the path analysis variables, the red ones the negative effects, and the blue ones represent the coefficients calculated and used in the path analysis. PH: plant height (cm); R1: insertion height of the primary raceme (cm); R2: insertion height of the secondary raceme (cm); NR: number of racemes; Ø: stem diameter (cm); GY: grain yield (kg ha^{-1}); OC: oil content (%).

the indirect effect of stem diameter and insertion height of the secondary raceme. Characteristics such as plant height, number of racemes and stem diameter were previously reported to directly affect the castor bean oil content percentage (Torres et al. 2015).

A dissimilarity among the groups was found by the UPGMA clustering method calculated by genetic distances using the Tocher method. The greatest similarity among the line groups according to the hierarchical clustering method was observed for the group 1 (red; H4, H9 and H11 lines) and group 2 (blue; H2 and H6 lines), as well as group 3 (green; H3, H8 and H7 genotypes) and group 4 (black; H1, H10, H5 and H12 lines). The greatest dissimilarity was verified between the groups 1 (H4, H9 and H11) and 4 (H1, H5, H10 and H12), being the most promising for crossings and formation of hybrids with greater genetic gain (Figure 3).

The genotypes gathered in more distant groups are considered the most promising crosses. They can increase desirable characteristics such as yield and use them as a source of genetic variability to obtain hybrids of high-yield potential (Almeida et al. 2011, Oliveira et al. 2020). The selection of genotypes should be based on the greater genetic distance and gains in the characteristics of interest, such as grain yield and oil content percentage (Torres et al. 2015). Crosses between strains provide high genetic and productive gain, as they are favored by hybrid vigor and improve the performance of future hybrids in different environments (Zoz et al. 2021). In our study, the grain yield was benefited by the oil content percentage via the stem diameter and insertion height of the secondary raceme; therefore, the productive gains by the hybrid vigor will be accompanied by the increase of the oil content percentage.

The germination percentage is important for the good establishment of the crop and influences the final yield (Marcos-Filho 2015). In castor bean, the minimum germination for commercialization of seeds in Brazil is 80 % (Brasil 2013). Among all the evaluated materials, H9 (84 %) and H12 (86.7 %) stood out. Increasing the seed vigor to improve the crop establishment and grain yield is a goal of plant breeding companies (Finch-Savage & Bassel 2016). Concerning the total seedling length, there were no statistical differences by the Scott-Knott test, being the highest average observed for H1 (10.74 cm). However, there was a significant difference for root dry mass among the formed groups, with the dwarf castor bean lines H2, H3, H5, H6, H8, H9, H10, H11 and H12 being approximately 13 % higher than the others. Also, the dwarf castor bean line H8 had a greater dry mass accumulation in the shoot than the others, being 23 % higher than the lowest averages for shoot dry mass (Table 3). The greater accumulations of seedling dry mass during germination and emergence allow for an adequate formation of the cultivation stand and even uniformity when conducting the crop treatments (Oliveira et al. 2019), in addition to favoring a better vegetative growth of plants, which results in a greater acquisition and transport of carbohydrates, sugars, photoassimilates, water and nutrients until harvest (Limede et al. 2018).

The highest means for total seedling dry mass were verified in the dwarf castor bean lines H2 (0.051 g), H3 (0.050 g), H5 (0.051 g), H6 (0.052 g), H8 (0.058 g), H9 (0.052 g), H10 (0.056 g), H11

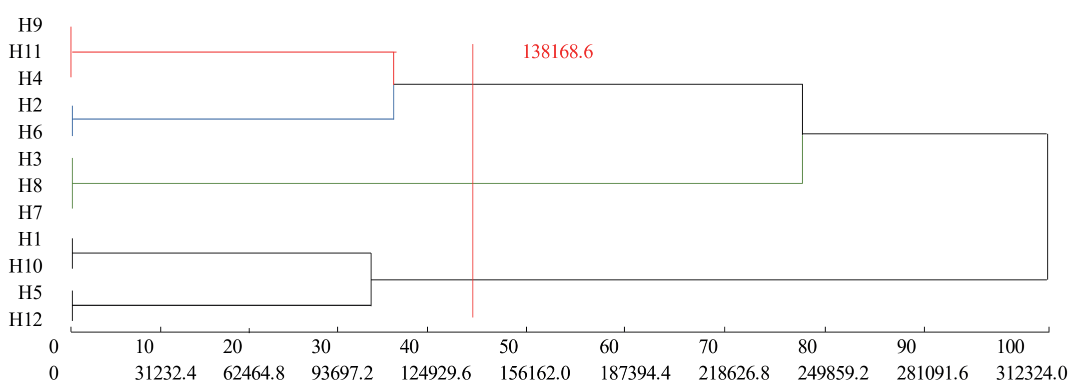

Figure 3. Dendrogram illustrating the dissimilarity pattern obtained by the unweighted pair group method with arithmetic mean (UPGMA), based on the Mahalanobis distance, in dwarf castor bean lines. Group 1: lines in red; group 2: lines in blue; group 3: lines in green; group 4: lines in black.

Table 3. Averages of seedling traits for 12 castor bean lines.

Lines	G (%)	GSI	RM (g)	SM (g)	TM (g)	RL (cm)	SL (cm)	TL (cm)
H1	64.00 b	0.232 b	0.030 b	0.015 c	0.045 b	9.50 a	1.24 a	10.74 a
H2	68.00 b	0.221 b	0.034 a	0.017 b	0.051 a	7.44 b	1.58 a	9.01 a
H3	78.66 a	0.212 b	0.037 a	0.013 c	0.050 a	7.39 b	1.04 b	8.43 a
H4	65.33 b	0.302 a	0.029 b	0.015 c	0.044 b	7.51 b	1.15 b	8.66 a
H5	61.33 b	0.223 b	0.036 a	0.015 c	0.051 a	8.91 a	1.32 a	10.23 a
H6	78.67 a	0.282 a	0.035 a	0.017 b	0.052 a	8.67 a	1.26 a	9.93 a
H7	56.00 b	0.235 b	0.030 b	0.015 c	0.045 b	7.98 b	1.31 a	9.29 a
H8	73.33 a	0.188 b	0.039 a	0.020 a	0.058 a	8.04 b	1.48 a	9.52 a
H9	84.00 a	0.260 a	0.035 a	0.018 b	0.052 a	7.39 b	1.27 a	8.65 a
H10	77.33 a	0.185 b	0.039 a	0.018 b	0.056 a	8.25 b	1.10 b	9.35 a
H11	64.00 b	0.265 a	0.036 a	0.015 c	0.051 a	7.70 b	1.13 b	8.82 a
H12	86.67 a	0.333 a	0.042 a	0.014 c	0.055 a	8.88 a	0.89 b	9.76 a
Anova	F probability							
Lines	4.39*	5.500*	4.920**	7.7800**	2.160*	2.39*	3.56**	1.840^{ns}
Blocks	1.26^{ns}	1.740^{ns}	0.170^{ns}	0.2700^{ns}	0.270^{ns}	0.09^{ns}	0.82^{ns}	0.170^{ns}
Average	71.44	0.245	0.035	0.0160	0.051	8.14	1.23	9.369
Standard error	9.25	0.038	0.002	0.0007	0.008	0.46	0.10	1.036
CV (%)	12.95	15.670	9.890	8.3200	12.350	11.28	16.63	11.060

G: seed germination; GSI: germination speed index; RM: root dry mass; SM: shoot dry mass; TM: total seedling dry mass; RL: root length; SL: shoot length; TL: total seedling length; CV: coefficient of variation. * and **: significant at 0.05 and 0.01 of probability, respectively; ^{ns} not significant. Averages followed by the same letter in the column are not statistically different.

(0.051 g) and H12 (0.055 g), which were superior to the other lines (Table 3). The use of high vigor seeds is essential to ensure a proper plant stand. Total seedling dry matter and total seedling length indicate a vigor parameter and are important for selecting genotypes with superior seed physiological quality. Thus, lines with higher total seedling dry mass values are considered more vigorous (Marcos-Filho 2015). The dwarf castor bean lines H12 (0.3339), H4 (0.3029), H6 (0.2823), H11 (0.2655) and H9 (0.2601) presented the highest averages for GSI, considereing the other lines (Table 3). The GSI is used as an indicator of seed vigor, so that high GSI values may indicate a high seed vigor and better field seedling performance (Marcos-Filho 2015).

There was a positive and significant correlation between root length and total seedling length, being also verified between shoot length and shoot dry mass, root dry mass and total seedling dry mass, shoot dry mass and total seedling dry mass; however, negative and significant correlations were found between shoot length and seed germination, shoot length and germination speed index, and also between shoot dry mass and germination speed index. This shows that germination and germination speed affect the dry mass accumulation in the shoot of castor bean seedlings (Figure 4). The correlations are important to verify the effects of seed vigor on seedling growth. However, the root growth of field crop seedlings has a greater significance for the success in establishing the stand in periods of drought, in the early growth and development (Queiroz et al. 2019).

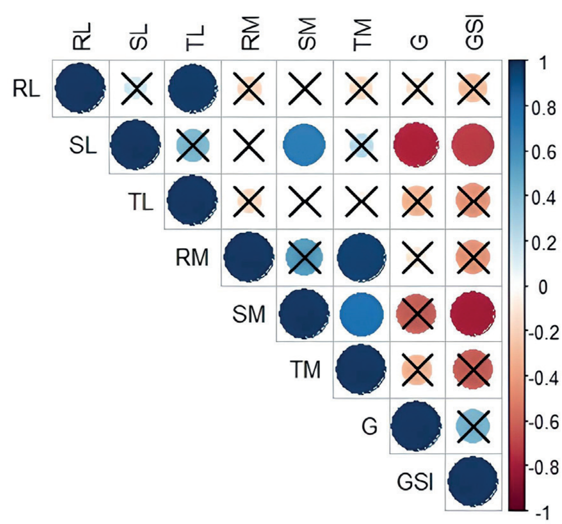

Figure 4. Heatmap of the Pearson's correlation coefficients obtained from variables analyzed in 12 dwarf castor bean lines. X indicates no significant correlation ($p < 0.05$). G: seed germination (%); GSI: germination speed index; RM: root dry mass (g); SM: shoot dry mass (g); TM: total seedling dry mass (g); RL: root length (cm); SL: shoot length (cm); TL: total seedling length (cm).

CONCLUSIONS

1. The dwarf castor bean lines H4, H5 and H11 resulted in grain yields above 1,400 kg ha^{-1} and, therefore, are promising for getting new productive hybrids;
2. The indirect selection of dwarf castor bean lines to gain grain yield can be performed through the following morphologic traits: insertion height of the secondary raceme, number of racemes and stem diameter;
3. The dwarf castor bean lines H2, H3, H5, H6, H8, H9, H10, H11 and H12 had greater gains, concerning growth and mass accumulation in the seedlings. However, the lines H4, H6, H9, H11 and H12 produced seeds with superior vigor and germinative quality.

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