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Seed coat specific weight and endosperm composition define the oil content of castor seed

Liv S. Severino[a,*], Bruna S.S. Mendes[a], Geovani S. Lima[b]

[a] Embrapa Algodão, Rua Oswaldo Cruz, 1143, CEP 58428-095 Campina Grande, PB, Brazil
[b] Universidade Federal de Campina Grande, Brazil

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ABSTRACT

Seed oil content is an important characteristic for castor (*Ricinus communis*). Studies were performed in order to detail how oil content varies among seeds and which seed characteristics are associated with the variability in oil content among genotypes. It was found that oil content is higher in large than in small seeds because of the reduced relative weight of the seed coat. The seed oil content of 40 genotypes varied between 34.6 and 56.6%. The variation in seed oil content was not associated with seed weight, but it was explained by the relative weight of the seed coat (relative weight) and by the composition of the nut (embryo + endosperm). Comparing castor seeds with 35 and 56% of oil content, the relative weight of the seed coat was reduced from 26.9 to 19.1%, and the nut oil content was increased from 50.3 to 71.3%. Reduced relative weight of the seed coat was associated with thinner seed coat.

1. Introduction

Castor (*Ricinus communis* L.) is cultivated for the oil extracted from its seed. Castor oil is used in the chemical industry for an extensive list of products, and it is historically more expensive than major vegetable oils. The oil extraction industry is very demanding on high seed oil content because this characteristic affects the efficiency of oil extraction. For that reason, increasing seed oil content is an objective of castor breeding programs (Anjani, 2010, 2014; Chandrasekaran and Liu, 2014; Severino and Auld, 2013b). In most oilseeds, including soybean (Glycine max) and *Brassica* spp., increasing seed oil content is one of the most important breeding criteria. Oil content is a complex quantitative trait that is networked with other storage and structural compounds in seed, and it is influenced by seed development and environmental conditions (Abbadi and Leckband, 2011; Brummer et al., 1997).

Most scientific reports that discuss increasing seed oil content in oilseed crops are related to genetics, genomics, gene expression, quantitative trait loci (QTL), and seed metabolism (Brummer et al., 1997; Chandrasekaran and Liu, 2014; Harwood and Guschina, 2013; Vanhercke et al., 2013; Venglat et al., 2014). Manipulating specific genes or enzymes in order to increase oil content is a complex task, considering that genomic studies in *Arabidopsis* revealed that the lipid metabolism involves at least 120 enzymatic reactions and more than 600 genes (Venglat et al., 2014). Besides the established enzymatic routes for synthesis and assembly of triacylglycerols in plant cells, the frameworks became even more complex because of recent studies that unveil new metabolic routes (Vanhercke et al., 2013).

This study adds some insights into how increased oil content could be achieved focusing on morphological characteristics and seed composition. There is large natural variability in the oil content of castor seed (Rojas-Barros et al., 2004; Velasco et al., 2015; Wang et al., 2010, 2011), and taking advantage of such variability is the best option before attempts are made for manipulating the complex enzymatic processes and specific genetic systems. This approach is valid for many oilseeds besides castor.

Crop management is very effective for increasing seed yield because castor plants are very responsive to most inputs such as fertilization and irrigation. However, castor seed oil content is little influenced by environmental conditions (Anastasi et al., 2015; Anjani, 2010; Biscaro et al., 2012; Lima et al., 2015; Patel et al., 2012; Ramanjaneyulu et al., 2013; Severino and Auld, 2013a). Thus, seed oil content depends largely on the genetic characteristics of the cultivar, rather than on crop management or environmental conditions.

This study aimed to investigate several aspects related to the oil content in castor seed in order to increase the understanding of this trait and propose strategies for its improvement. The objectives

* Corresponding author.
E-mail addresses: liv.severino@embrapa.br (L.S. Severino), bruna.mendes@embrapa.br (B.S.S. Mendes), geovanisoareslima@gmail.com (G.S. Lima).

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were to quantify the variability in seed oil content among seeds produced in the same environment and cultivar and to investigate the characteristics that explain the variability in seed oil content among genotypes.

2. Material and methods

2.1. Natural variability in a seed sample

Measurements of the relative weight of each seed component and morphological characteristics were made in order to evaluate to what extent the seed oil content is influenced by the natural variability in seed weight. If there is an association, seed oil content can be increased by selecting genotypes according to seed weight or by cropping systems that influence seed size.

The variability on morphological characteristics and oil content was assessed in a seed sample of cv. BRS Energia that was produced for commercial purpose (certified seed) under rainfed conditions and regular crop management protocols. The seeds were individually weighed and separated in groups according to the weight. The groups were composed of seeds weighing in the following ranges: <96 mg, 96–120 mg, 121–145 mg, and so on with 25-mg increments up to the class 496–520 mg. Three 5-g samples were obtained from each group. Because light seeds were less frequent, the samples in the classes <96 mg totaled less than 5 g, but the number of seeds was enough for the measurements to be made.

Data on length, width, and height were taken, and surface area and volume were calculated for each single seed. Assuming the seed' shape as an ellipsoid, the area was estimated using the equation proposed by Klamkin (1976), and the volume was estimated by the standard equation:

$$S = 4\pi\left(\frac{L^{1.61} \times H^{1.61} + L^{1.61} \times W^{1.61} + H^{1.61} \times W^{1.61}}{3}\right)^{0.62}$$

and,

$$V = \frac{4\pi \times L \times H \times W}{3}$$

in which: S = seed surface area (mm^2), L = seed length (mm), H = seed height (mm), W = seed width (mm), and V = seed volume (mm^3). The average of seed area and volume were calculated in each 5-g sample.

In this study, embryo and endosperm were not separated, and they were called nut. The following steps were made in the 5-g sample. The seeds were separated by hand in caruncle, seed coat, and nut, oven dried, and weighed. The relative weight of each seed component was calculated dividing the component's weight by the total seed weight and expressed as percentage. The oil content was measured in the nut by Soxhlet extraction with hexane for 8 h. The seed oil content, the seed coat specific weight (i.e., the seed coat thickness), and the seed density were estimated with the following equations:

$$\text{Seed oil content (\%)} = 100 \times \frac{\text{nut oilcontent} \times \text{nut weight}}{\text{mean seed weight}}$$

$$\text{Seed coat specific weight}\left(\text{mg mm}^{-2}\right) = \frac{\text{mean seed coat weight (mg)}}{\text{mean seed coat area (mm}^2\text{)}}$$

$$\text{Seed density}\left(\mu\text{g mm}^{-3}\right) = \frac{\text{mean seed weight } (\mu\text{g})}{\text{mean seed volume (mm}^3\text{)}}$$

Data was analyzed by simple and multiple linear regression. Three regression models were tested, and the option with the best fitness (R^2) was chosen: $y = a + bx$, $y = a + b/x$, or $y = a + bx + cx^2$. The significance level was $p < 0.05$. For the sake of simplicity, the linear model was chosen when the other models did not increase the R^2 by at least 5%. The regression line was omitted in the graphs when the regression model was not significant. The equations and R^2 are presented in the Figures. Multiple linear regression was used to assess the influence of two or more variables in one dependent variable. In this case, the Type III Sum of Squares calculated with the procedure GLM in the software SAS was used as the measurement of the influence of each single independent variable over the dependent variable. Six data points (out of 54 data points) of seed oil content were excluded from the analysis because they were highly divergent from the other points. Such divergence was assumed as measurement error because they were 15% lower than the estimated value (considering mean seed weight as independent variable in a regression analysis with quadratic model), while most data points diverged no more than 5% of the estimated value.

2.2. Variation among genotypes in seed oil content and other characteristics

Measurements were made in 40 castor genotypes including breeding lines and commercial cultivars. Seed samples were oven dried (65 °C, 24 h), weighed, counted, and manually separated into caruncle, seed coat, and nut. Each seed component was weighed. The nut oil content was measured by Soxhlet extraction with hexane for 8 h. The relative weight of each seed component was calculated as the component's weight divided by the total seed weight. The seed oil content was calculated from the nut oil content and the weight of all seed components (see Section 2.1). Additional measurements were made in 12 genotypes (out of the 40 genotypes). The sample was divided in three replications, and data was taken on length, height, and width of each individual seed.

The mean seed weight was calculated from the sample weight divided by seed count. The seed coat specific weight (seed coat thickness) was estimated as the seed coat weight divided by the seed coat area. The seed density was estimated as the seed weight divided by the seed volume (see Section 2.1).

Data was analyzed by regression analysis. Four models were tested, and the option with the highest R^2 was chosen. The models were $y = a + bx$, $y = a + b/x$, $y = a + bx + cx^2$, and $y = a(1 - e^{bx})$. In some cases, simple linear regression was used to assess the association of two variables. The significance level was $p < 0.05$ in all the analysis. The equations and R^2 are presented in the Figures. Multiple linear regression was used to assess the influence of selected seed characteristics in the seed oil content (see Section 2.1). The model considered the following characteristics: specific weight of the seed coat, nut oil content, relative weight of the seed coat and of the caruncle, seed weight, and seed volume. The relative weight of the nut was not considered because it is co-dependent with the relative weight of the caruncle and the seed coat.

3. Results

3.1. Variability in seed oil content and morphological characteristics in a seed sample

Even though the seeds were produced by plants with little genetic variability (certified seeds of cv. BRS Energia) and raised under the same environmental conditions, considerable variability in morphological characteristics was found. When the seeds were separated by weight; the relative weight of the caruncle was smaller in heavy than in light seeds, and the reduction was linear according to the mean seed weight (Fig. 1). Although the relative