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Comprehensive Analysis of Castor Bean (*Ricinus communis*): Morphology, Genetics, and Chemical Composition in Riyadh, Saudi Arabia

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ABSTRACT

The castor bean plant, *Ricinus communis* L., belongs to the Euphorbiaceae family, which includes approximately 300 genera and 7,500 species. This research focuses on investigating the morphological characteristics, chemical properties, and genetic diversity of castor bean plants growing in Saudi Arabia. We used a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method to extract genomic DNA from the young leaves. To analyze genetic variation, 4 random HAP primers (HAP1, HAP2, HAP3, and HAP4) were used for RAPD-PCR analysis. A total of 32 DNA fragments were generated, of which 4 were polymorphic and 28 were monomorphic. Significant phenotypic differences were recorded between the two plant populations, and this was reflected in the genetic analysis. Chemical analysis showed a protein content of 16% in sample A and 15% in sample B, with total ash contents of 2.7% and 2.3%, respectively. Both samples exhibited similar fat contents of around 47%. The study highlights the need for further research, including DNA sequencing, to gain deeper insights into the genetic and chemical differences between these two castor bean populations.

Keywords: Morphological, Genetic diversity, Chemical composition, Castor bean

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Introduction

Castor oil, derived from *Ricinus communis* L. of the Euphorbiaceae family, is a significant non-edible oilseed cultivated primarily in drylands and semi-arid regions, especially in tropical areas. Castor plants are the source of castor oil, an essential raw material used in manufacturing a wide array of products that have many industrial, medicinal, and agricultural applications. Despite its potential, the castor plant's history of global cultivation has been limited due to the presence of the toxic protein ricin. In addition to oil, the seeds of *R. communis* have been employed across various sectors, including medicine and industry [1-4]. This plant species belongs to the Euphorbiaceae family, encompassing around 300 genera and 7,500 species, and is classified under the monotypic genus *Ricinus*. Genetic studies have shown that the family consists of 3 major lineages, which are the Phyllanthoids, Putranjivoids, and Euphorbioids, each distantly related. The castor plant shows substantial diversity in its physical characteristics, including differences in growth habits, foliage color, stem appearance, seed size, and oil content, which can make varieties appear quite distinct from one another. The plant produces a spiny, three-chambered capsule that bursts open upon ripening to release the seeds, though some varieties have capsules without spines or with softer, flexible, or non-irritating properties. Castor beans typically contain 35-55% oil content [5-8].

Ricinoleic acid, a hydroxylated fatty acid comprising 80-90% of castor oil, is widely utilized in various industries, alongside polysaccharides and numerous secondary metabolites. Recently, there has been growing interest in its

potential as biodiesel [9]. In terms of mineral composition and proximate analysis, the mean castor seed from Sokoto yields between 1 to 8 bunches, with every bunch containing 30 to 56 pods, averaging 43 pods per bunch. Proximate analysis of the seeds reveals a composition of 28% carbohydrates, 11% protein, 3.5% ash, 1.78% nitrogen, and 1.0% crude fiber [10, 11]. Advancements in biotechnology and molecular techniques have been employed to enhance castor seed productivity, increase oil content, and reduce the toxicity of ricin, thus meeting consumer demands. However, the genetic modification of castor remains a challenge due to the plant's resistance to efficient regeneration of stable, transformed plants. Additionally, the high costs associated with these biotechnological processes have limited their widespread adoption and accessibility [12-14]. Various molecular methods, such as random amplified polymorphic DNA are used to assess genetic divergence and identify differences and similarities between castor bean genotypes. RAPD has proven effective in identifying genetic variations in numerous plant species [15]. DNA barcoding, which employs a standardized gene fragment for species identification, has gained popularity in recent years as a valuable tool for biodiversity monitoring, molecular phylogeny, and evolutionary studies [16].

The combination of both matK and rbcL primers and conserved DNA sequences as barcode primers has been recognized as a reliable method for accurately identifying and distinguishing species [17]. The primary objective is to enhance seed productivity content while minimizing ricin toxicity to better serve market demands and support farmers. In particular, morphological traits are crucial phenotypic markers that play a key role in developing sustainable crops [18]. However, relying solely on geographical and morphological traits to study genetic diversity is ineffective because of the significant impact of environmental factors and plant genetic responses. In recent years, several stable and effective molecular markers have been introduced to test and analyze genetic diversity in castor beans, including SSR (single sequence repeat), AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism), and SNPs (single nucleotide polymorphism) [19, 20]. These genetic tools have enabled advancements in castor bean breeding programs, leading to the development of elite strains and new morphological varieties. Our study wants to explore the genetic diversity and chemical composition of castor bean (*R. communis*) cultivated in Saudi Arabia.

Materials and Methods

Samples of plants

Castor seeds, including hybrids and both male and female varieties, were gathered from Saudi Arabia. *R. communis* seeds with spiny capsules having three lobes were provided by Dr. Moodi Saham Alsubeie from the Biology Department at the College of Science, Imam Mohammad Ibn Saud Islamic University. The seeds were stored until further use.

Sample preparation and chemical analysis

The seeds were ground into a fine powder, and any remaining seeds were stored in sealed containers for later use.

Ash content analysis

A 2-gram sample of the seeds was placed in a petri dish and dried in the oven at 105°C for three hours. The ash content was determined by repeating the procedure in triplicate, and the percentage of residual weight was calculated as the ash content.

Protein and fat percentage determination

For fat content determination, 2 grams of the seed sample were placed in a thimble and subjected to Soxhlet extraction using petroleum ether (60-80 °C) for 5 hours. The total protein content was measured with the Kjeldahl method [21].

DNA extraction

Total genomic DNA was taken from the leaves of each castor genotype using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol. The quality and concentration of the DNA were assessed using a NanoDrop system. DNA dilutions of approximately 100 ng/µl were stored at 4 °C for subsequent PCR analysis.

RAPD PCR analysis

For RAPD-PCR, six oligonucleotide primers with an annealing temperature of either 36 °C or 38 °C were used: HAP1 (GTGATCGCAG), HAP2 (GAAACGGGTG), HAP3 (GTGATCGCAG), and HAP4 (CCGGGAATCG). PCR reactions were conducted as outlined by Williams *et al*. [22], with adjustments. The PCR reaction mixture contained 1 µl of template DNA, 1 µl of each primer (at 10 pmol/µl concentration), 17 µl of double-distilled water, and 5 µl of a PCR Pre-Mix Kit (which included 2.5 µl of I-Taq™ DNA Polymerase, 2.5 mM every deoxyribonucleotide triphosphate (dNTPs), 1× reaction buffer, and 1× gel loading buffer; iNtRON Biotechnology, INC.), making a total volume of 25 µl. The thermal cycling conditions were as follows: 35 cycles of 94 °C for 1 minute, 38 °C for 1 minute, and 72 °C for 2 minutes, with a denaturation step at 94 °C for three minutes and a final extension at 72 °C for 10 minutes.

Agarose gel electrophoresis

The verification of PCR products was done through electrophoresis on a 2% agarose gel. The gel mixture was prepared by dissolving 0.4 grams of agarose in 20 milliliters of 1× TBE buffer (Tris-borate EDTA), then heating for 1 minute and adding 2 µl of Ethidium bromide to stain the gel. To visualize the DNA fragments, 1 µl of a 100 bp DNA ladder was loaded, and the gel was submerged in 1× TBE buffer. The electrophoresis was carried out at 84 volts for 2 hours, and the bands were visualized under ultraviolet light using a gel documentation system, followed by photographic documentation. If weak bands were observed, the PCR was repeated using the same protocol, adjusting the annealing temperature to 36 °C.

Statistical analysis

The sizes of the DNA fragments were determined using Gel Analyzer 19.1 software. Only consistently amplified fragments were considered for further analysis. The protein data were processed and represented visually using Microsoft Excel 2016 for graphical presentations.

Results and Discussion

Total genomic DNA was taken from castor bean leaves using the CTAB method. The DNA samples exhibited absorbance ratios at A260/A280 that were from 1.72-1.89, with concentrations varying between 119.5 and 218.9 ng/µl.

Genotypic variations

A total of 32 DNA fragments were amplified using the primers, with 4 bands exhibiting polymorphism and 28 bands showing no variation. The polymorphic bands reflected genetic diversity between the 2 castor bean populations, as indicated by the phenotypic data recorded in the study **(Figure 1; Table 1)**. The RAPD analysis was conducted using four random primers (HAP1, HAP2, HAP3, and HAP4) to assess polymorphism in the 2 populations of *R. communis*. Among the primers tested, HAP1 and HAP3 demonstrated successful amplification in both populations, while HAP2 and HAP4 produced weaker results. This suggests that the latter primers may have a lower affinity for binding to complementary sequences in the genomic DNA of the 2 castor bean populations, possibly due to specific amplification conditions or sequence variations.

The morphological characteristics of the castor bean plants revealed a range of variations. The plant height ranged from 2.4-3.6 cm for one group and 4.5-5.9 cm for another. The seed colors varied between brown and black/brown, and the seed sizes ranged from 1.2-1.7 cm. The capsule sizes measured between 2.6 cm and 3.5 cm, and the textures of the capsules were either spiny or spineless, corresponding to the 3-lobed and 4-lobed capsules of *R. communis*, respectively **(Table 2)**.

In terms of chemical analysis, the proximate composition of the seeds from different castor bean types in Saudi Arabia was assessed. The protein content in the seeds was found to be 16% in sample A and 15% in sample B. The total ash content was 2.7% for sample A and 2.3% for sample B, while the fat content was 47% and 47.8% in the 3-lobed and 4-lobed capsule types, respectively **(Figure 2)**.

However, there are certain limitations in our study. The size of the sample was small, with only one germinated plant per variety, and the focus was primarily on varieties obtained from different sources. It would be interesting to compare these varieties with those from natural populations of *Ricinus*. Some of the material came from controlled environments like plant breeding programs, while others were sourced from botanical gardens, which may introduce genetic diversity through outbreeding. Additionally, the classification of seeds was challenging

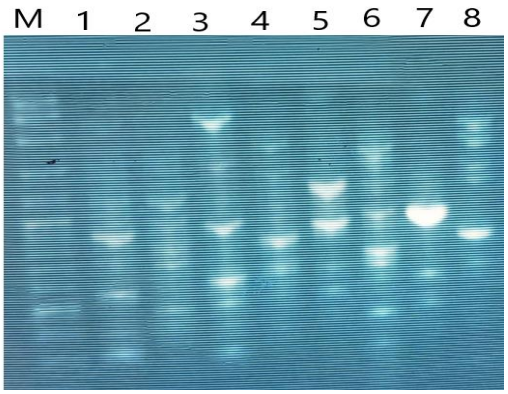
due to significant variation in seed appearance within the same variety. Due to the limited number of available samples, the seeds were grouped into two categories. The study focused mainly on visible traits, but a more comprehensive investigation of the morphology of different *Ricinus* varieties is essential for proper field identification. Polymorphisms observed in the RAPD analysis may be attributed to changes in the base sequence, such as deletions, additions, or substitutions in the priming site region [22].

Genetic variation is essential for adapting to different environments, aiding in species propagation, resource preservation, and the identification of specific genetic regions. Isolated populations typically accumulate diverse genetic traits due to environmental pressures [23]. This research utilized RAPD markers to assess the genetic diversity of castor bean plants. The high degree of polymorphism found suggests that RAPD markers are an effective way for these studies. Our study lays the groundwork for future investigations into the genetic differentiation of castor beans.

RAPD has been proven successful in identifying medicinal plants and their components [24, 25], as well as in distinguishing various ornamental plant species [26]. Several plant characteristics, such as seed, prickles, and stem color, showed notable genetic variation, likely because these traits were not selected during domestication [27, 28]. Understanding the genetic variation of castor accessions requires the analysis of both quantitative and qualitative traits [29]. In a study involving 574 accessions, 22 polymorphic EST-SSR markers were used to investigate oil content, fatty acid composition, and the plant's country of origin. Analysis methods like cluster analysis and principal component analysis consistently grouped the accessions into four distinct subpopulations [30]. The study revealed polymorphisms in EST-SSRs, with an average of 2.33 alleles per locus and allele sizes ranging between 150 and 400 bp. Dendrogram analysis divided 27 accessions into two groups, with genetic similarity coefficients ranging from 0.24-0.83. The polymorphic information content values of 0.28-0.49 indicated a moderate level of diversity in the castor bean population [31].

Table 1. Random primers showing polymorphism among castor bean (*R. communis*) growing in Riyadh Saudi Arabia

Sr. No	Primer code	Primer sequence (F/R)	No. of genotypes	Total amplified bands	Polymorphic bands	Monomorphic bands	Percent polymorphism
1	HAP1	GTGATCGCAG	2	7	1	6	14.3%
2	HAP2	GAAACGGGTG	2	7	0	7	0%
3	HAP3	GTGATCGCAG	2	8	1	7	12.5%
4	HAP4	CCGGGAATCG	2	10	2	8	20%



M = DNA Ladder 1KB, 1, 2, 3, 4 3-lobe capsule and 5, 6, 7,8 4-lobed capsule

Figure 1. Electrophoretic pattern of two types of castor bean (*R. communis*) growing in Riyadh Saudi Arabia (ethidium bromide-stained 2% agarose gel electropherogram of PCR products obtained from the RAPD analysis)

Table 2. Morphological characterization of two *R. communis* types growing in Riyadh Saudi Arabia

	***R. communis* 3-lobe capsule**	***R. communis* 4- lobed capsule**
Plant height	**2.4-3.6 cm**	**4.5-5.9 cm**
Seed color	**Brown**	**Black/Brown**

Seed size	**1.2 cm**	**1.7 cm**
Capsule size	**2.6 cm**	**3.5 cm**
Capsule texture	**Spiny**	**Spineless**
Length	**17-21 cm**	**19-23 cm**

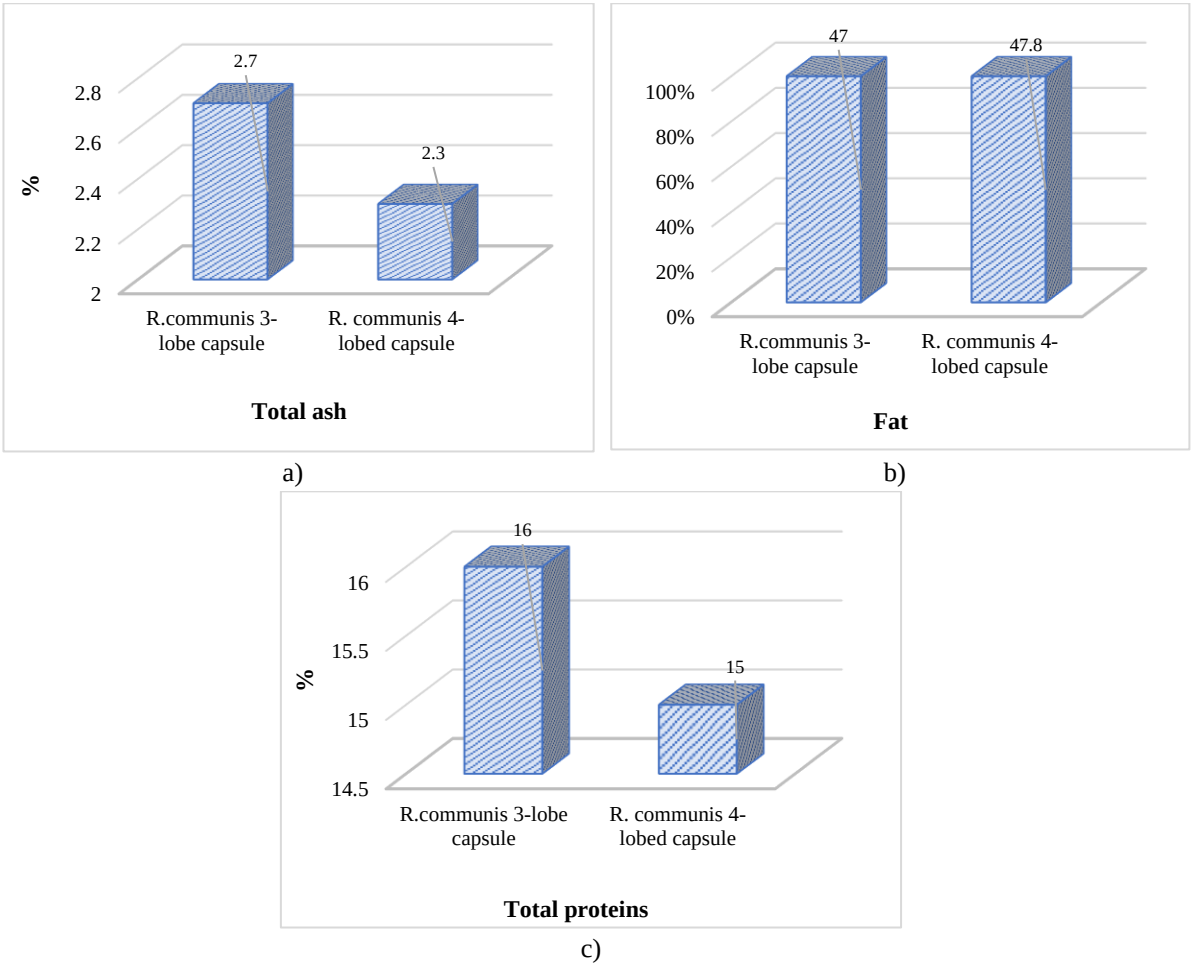


Figure 2. Chemical constituents in each 100 g of the seed of types *R. communis* growing in Riyadh Saudi Arabia

Conclusion

This study utilized 4 random HAP primers (HAP1, HAP2, HAP3, and HAP4) for RAPD analysis to assess polymorphism in two populations of castor beans (*R. communis*). Among the primers tested, HAP1 and HAP3 demonstrated successful amplification across both populations, while HAP2 and HAP4 exhibited weaker amplification, possibly due to their lower affinity for the complementary binding sites in the genomic DNA of these populations. Additionally, the primers might have specific requirements for effective amplification. The morphological and chemical characteristics of the castor bean seeds also showed notable variation, with differences observed in each 100 g seed sample. The genetic diversity revealed through marker analysis is valuable for evaluating new genotypes, which could contribute to future breeding efforts and the conservation of castor bean populations.

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Ethics Statement: None

References

1. Saadaoui E, Martín JJ, Tlili N, Cervantes E. Castor bean (*Ricinus communis* L.) diversity, seed oil and uses. Oilseed crops: yield and adaptations under environmental stress. 2017:19-33. doi:10.1002/9781119048800.ch2
2. Kanedi M, Busman H, Sutyarso S, Farisi S, Mumtazah DF. Essential oil extracted from plant tuber of nutgrass "Cyperus rotundus" effectively decreased sperm quality of mice. J Adv Pharm Educ Res. 2021;11(2):66-70.
3. Al-kadhi NA, Abass KS, Jaafar SE. Improvement effect of linseed oil on the activity of testes and physiological parameters in mice treated with Bicalutamide. J Adv Pharm Educ Res. 2020;10(3):146-54.
4. Sisca V, Jamarun N. Biodiesel production from waste cooking oil using catalyst calcium oxide derived of limestone Lintau Buo. Arch Pharm Pract. 2019;11(3):8-14.
5. Polito L, Bortolotti M, Battelli MG, Calafato G, Bolognesi A. Ricin: an ancient story for a timeless plant toxin. Toxins (Basel). 2019;11(6):324. doi:10.3390/toxins11060324
6. Yeboah A, Ying S, Lu J, Xie Y, Amoanimaa-Dede H, Boateng KG, et al. Castor oil (*Ricinus communis*): a review on the chemical composition and physicochemical properties. Food Sci Technol. 2021;41(suppl 2):399-413. doi:10.1590/fst.19620
7. Claßen-Bockhoff R, Frankenhäuser H. The 'male flower' of *Ricinus communis* (Euphorbiaceae) interpreted as a multi-flowered unit. Front Cell Dev Biol. 2020;8:313. doi:10.3389/fcell.2020.00313
8. Kaur R, Bhaskar T. Potential of castor plant (*Ricinus communis*) for production of biofuels, chemicals, and value-added products. InWaste biorefinery 2020 Jan 1 (pp. 269-310). Elsevier.
9. Swapan C, Abul-Kalam MA, Zahira Y, Abul-Kalam MM. Castor (*Ricinus communis*): an underutilized oil crop in the south East Asia. Agroecosystems – very complex environmental systems [Internet]. 2021. doi:10.5772/intechopen.92746
10. Sandoval-Salas F, Méndez-Carreto C, Ortega-Avila G, Barrales-Fernández C, Hernández-Ochoa LR, Sanchez N. A biorefinery approach to biodiesel production from castor plants. Processes. 2022;10(6):1208. doi:10.3390/pr10061208
11. Jekayinfa SO, Orisaleye JI, Pecenka R. An assessment of potential resources for biomass energy in Nigeria. Resources. 2020;9(8):92. doi:10.3390/resources908009
12. Gavrilescu M. Environmental biotechnology: achievements, opportunities and challenges. Dyn Biochem, Process Biotechnol Mol Biol. 2010;4(1):1-36.
13. Bart JC, Palmeri N, Cavallaro S. Biodiesel science and technology: from soil to oil. Elsevier; 2010.
14. Miladinovic D, Antunes D, Yildirim K, Bakhsh A, Cvejić S, Kondić-Špika A, et al. Targeted plant improvement through genome editing: from laboratory to field. Plant Cell Rep. 2021;40(6):935-51.
15. Saldaña CL, Cancan JD, Cruz W, Correa MY, Ramos M, Cuellar E, et al. Genetic diversity and population structure of capirona (*Calycophyllum spruceanum* Benth.) from the Peruvian Amazon revealed by RAPD markers. Forests. 2021;12(8):1125.
16. Acharya GC, Mohanty S, Dasgupta M, Sahu S, Singh S, Koundinya AVV, et al. Molecular phylogeny, DNA barcoding, and ITS2 secondary structure predictions in the medicinally important eryngium genotypes of east coast region of India. Genes. 2022;13(9):1678. doi:10.3390/genes13091678
17. Ismail M, Ahmad A, Nadeem M, Javed MA, Khan SH, Khawaish I, et al. Development of DNA barcodes for selected Acacia species by using rbcL and matK DNA markers. Saudi J Biol Sci. 2020;27(12):3735-42.
18. Pari L, Latterini F, Stefanoni W. Herbaceous oil crops, a review on mechanical harvesting state of the art. Agriculture. 2020;10(8):309.
19. Kim H, Lei P, Wang A, Liu S, Zhao Y, Huang F, et al. Genetic diversity of castor bean (*Ricinus communis* L.) revealed by ISSR and RAPD markers. Agronomy. 2021;11(3):457.
20. Sallam A, Alqudah AM, Dawood MF, Baenziger PS, Börner A. Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. Int J Mol Sci. 2019;20(13):3137.
21. Müller J. Dumas or Kjeldahl for reference analysis. FOSS: Hilleroed, Denmark; 2017.

22. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531-5.
23. Alqudah AM, Sallam A, Baenziger PS, Börner A. GWAS: fast-forwarding gene identification and characterization in temperate cereals: lessons from barley–a review. J Adv Res. 2020;22:119-35.
24. Shim YH, Choi JH, Park CD, Lim CJ, Cho JH, Kim HJ. Molecular differentiation of Panax species by RAPD analysis. Arch Pharm Res. 2003;26(8):601-5.
25. Yue J, Zuo Z, Huang H, Wang Y. Application of identification and evaluation techniques for ethnobotanical medicinal plant of genus Panax: a review. Crit Rev Anal Chem. 2021;51(4):373-98.
26. De Benedetti L, Burchi G, Bruna S, Mercuri A, Schiva T. Use of molecular markers to improve cut flowers longevity incarnation. InXXVI international horticultural congress: elegant science in floriculture 624. 2002 Aug 11 (pp. 343-348).
27. Temiesak P, Ponpim Y, Harada T. RAPD analysis for varietal identification in Brassica. Agric Nat Resour. 1993;27(5):37-42.
28. Silva ARD, Silva SA, Dos Santos LA, de Souza DR, Araujo GM, Dantas JLL, et al. Characterization and performance of castor bean lineages and parents at the UFRB germplasm bank. PLoS One. 2019;14(1):e0209335. doi:10.1371/journal.pone.0209335
29. DE Oliveira Neto SS, Zeffa DM, Sartori MMP, Soares DJ, Zanotto MD. Genetic variability in Brazilian castor (*Ricinus communis*) germplasm assessed by morpho-agronomic traits and gray mold reaction. An Acad Bras Cienc. 2021;93(suppl 3):e20190985. doi:10.1590/0001-3765202120190985
30. Wang ML, Dzievit M, Chen Z, Morris JB, Norris JE, Barkley NA, et al. Genetic diversity and population structure of castor (*Ricinus communis* L.) germplasm within the US collection assessed with EST-SSR markers. Genome. 2016;60(3):193-200. doi:10.1139/gen-2016-0116
31. Thatikunta R, Siva Sankar A, Sreelakshmi J, Palle G, Leela C, Durga Rani CV, et al. Utilization of in silico EST-SSR markers for diversity studies in castor (*Ricinus communis* L.). Physiol Mol Biol Plants. 2016;22(4):535-45. doi:10.1007/s12298-016-0367-x