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Morphological, Genetic Characterization, and Chemical Analysis of Castor Bean (*Ricinus communis*) Growing in Riyadh Saudi Arabia

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ABSTRACT

Ricinus communis L. is a flowering plant species from the Euphorbiaceae family; it contains about 300 genera and 7500 species and belongs to the monotypic genus Ricinus. This study aims to investigate the morphological, genetic diversity, and chemical analysis of castor bean (*R. communis*) growing in Riyadh Saudi Arabia. Using a modified Cetyl Trimethyl Ammonium Bromide (CTAB), total plant genomic DNA was extracted from young leaves of each genotype. Four random HAP primers (HAP1, HAP2, HAP3, and HAP4) were used for RAPD PCR analysis proximate analysis was done for chemical analysis. The primers produced 32 DNA fragments, of which 4 were polymorphic bands and 28 were not polymorphic bands polymorphism between two plants in bands was revealed in phonomertic characters that were recorded in this study. Four random HAP primers (HAP1, HAP2, HAP3, and HAP4) were used for RAPD analysis of the two castor bean (*R. communis*) populations to detect polymorphism. This study showed that the result of proximate analysis protein in the seeds was 16% in sample A and 15% in sample B, while the total ash was 2.7% in sample A and 2.3% in sample B, and fat was 47% and 47.8% in samples A and B, respectively. This study recommends that further study is needed on the two castor beans (*R. communis*) using appropriate samples from the two populations and DNA sequencing should be done for clear variation between the two types.

Keywords: Morphological, Genetic characterization, Chemical analysis, Castor bean, Riyadh, Saudi Arabia.

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INTRODUCTION

Castor oil (*Ricinus communis* L., Euphorbiaceae), an important inedible oilseed, is primarily grown in the globe's drylands and semi-arid regions, which are mainly native to the tropics [1-4]. The castor plant *R. communis* produces castor oil, which is a key chemical feedstock for a wide range of products ranging from polymers to cosmetics and has numerous applications. The presence of the toxic protein ricin has prevented its reintroduction even though castor was once widely grown around the world [5]. Furthermore, the seeds contain castor oil, which has been utilized in medicine, pharmacological, industrial, and agricultural industries [6]. *R. communis* is a flowering plant species from the Euphorbiaceae family; it contains about 300 genera and 7500 species and belongs to the monotypic genus Ricinus. In addition, molecular data have revealed that the traditional concept of Euphorbiaceae includes three major lineages, which are relatively distantly related to each other: the Phyllanthoids (genera 1–16 in this account), the Putranjivoids (genera 17 and 18), and the Euphorbioids (genera 19–75). Castor plant varies greatly in their appearance and growth. It is different in growth habit, the color of foliage, stems, seed size and color, and oil content, so varieties often bear little resemblance to one another [7, 8]. The fruit is a spiny capsule with three cells, each of which upon ripening divides into separate parts and then crumbles explosively, shattering the seeds. Some produce spineless capsules that are soft, flexible,

irritating, or non-irritating, and some castor varieties produce capsules with spines. Castor bean seeds contain approximately 35-55% oil lipids. The hydroxylated fatty acid ricinoleic acid 80-90% of total oil has industrial uses as well as polysaccharides and many secondary metabolites. And more recently its use as biodiesel has been studied [9]. Mineral composition and proximate analysis. The average castor seed in Sokoto has 1- 8 bunches, with each bunch containing several pods ranging from 30-56, with an average of 43 pods per bunch. The seed's proximate analysis was performed. It was discovered to contain 28% carbohydrate, 11% protein, 3.5% ash, 1.78% nitrogen, and 1.0 crude fiber [10, 11]. Biotechnologies and modern molecular technologies have been used to increase productivity and the amount of oil in the seeds (the main product), as well as to reduce ricin's toxic effects and meet consumers. Unfortunately, because it is recalcitrant to the efficient regeneration of stably transformed plants, the genetic transformation of castor remains challenging. In addition, the financial and technological cost of both processes resulted in their limited use and inaccessibility to all beneficiaries [12-14]. Different molecular techniques have been used to estimate the degree of genetic divergence similarities and differences between two genotypes of castor beans such as Random amplified polymorphic DNA (RAPD). RAPD is used for diversity assessment and for identifying germplasm in several plant species and has proven to be quite efficient in detecting genetic variations [15]. DNA barcoding -is a standard gene fragment1 for identification of species. It has been developing rapidly in recent years- become a useful tool for biodiversity investigation and monitoring, and molecular phylogeny and evolution [16]. Both primers (matk+rbcl) and conserved DNA sequences are used as barcode primers and would be an accurate method for the identification and differentiation of species [17]. The goal of increase the productivity and oil content of the seeds (main product), as well as reduce the toxicity of ricin, to meet the needs of the market and farmers, morphological characteristics, in particular, are important phenotypic indicators for the development of a sustainable crop [18]. However, studying genetic diversity based on morphological and geographical features alone is ineffective due to the strong influence of environmental complexity and the genetic responses of plants. Recently, many stable and efficient molecular markers have been developed and used to analyze genetic diversity in castor beans, such as SSR (Single Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism), and SNPs (Single Nucleotide Polymorphism) [19, 20], which is why genetic programs were used to improve castor beans and develop elite lines, now that some of these strains have evolved and morphological types have emerged differently. This study aims to investigate genetic diversity and chemical analysis of castor bean (*R. communis*) growing in Riyadh Saudi Arabia.

MATERIALS AND METHODS

Plant materials

The castor seed (*R. communis*) including hybrids, female, and male was collected from Riyadh Saudi Arabia; *R. communis* with spiny capsules containing 3 lobes was provided by Dr. Moodi Saham Alsubeie, Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University, keep it until used.

Sample preparation and proximate analysis

Powdered seed materials were made by using grading the remaining seeds were kept in closed containers until used.

Determination of ash content

Two grams of samples were placed into the petri dish and dried in an oven at 105 °C for three hours. Determination of ash contents was performed in triplicates and the percentage residual weight was expressed as ash content. Determination of total protein and fat percentage: 2 g samples were taken into a thimble and placed into the Soxhlet apparatus for the determination of fat content using petroleum ether (60 to 80 °C) for 5 hours. Moreover, using the Kjeldahl method, the determination of total proteins was performed [21].

DNA extraction

Using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method, total plant genomic DNA was extracted from young leaves of each genotype. Using the NanoDrop system, the

quality and quantity of the isolated DNA were determined. Dilutions of approximately ~100 ng/µl of each genotype were prepared and stored at 4 °C for further use in PCR analysis.

RAPD PCR

Six oligonucleotide primers RAPD marker with annealing temperature for each 38 °C OR 36 °C. HAP1, HAP2, HAP3, and HAP4, primer sequences (5-3) GTGATCGCAG, GAAACGGGTG, GTGATCGCAG, and CCGGGAATCG were used, respectively. PCR reactions were performed as per Williams *et al*. [22], with some modifications. The PCR reaction mixture contained 1µl template DNA, 1 µl of 10 pmol/µl of each primer, 17 µl double distilled water, 5 µl PCR Pre Mix Kit (1-Taq= 2.5 µl I-Taq™ DNA Polymerase, 5 υ/µl 2.5 mM each deoxyribonucleotide triphosphate (dNTPs), 1× reaction buffer (10×) and 1× gel loading buffer) (iNtRON Biotechnology, INC.) in the total volume of a 25 µl. The samples were subjected to 35 repeats of the following cycle: 94 °C 1 min, 38 °C for 1 min, 72 °C for 2 min with an initial denaturation of 3 minutes and a final extension of 10 minutes.

Agarose gel electrophoresis

All the above PCR products were verified by electrophoresis in 2% agarose gels (0.4 g of agarose in 20 ml 1× TBE (Tris-borate EDTA) heated for 1 min) stained with Ethidium bromide (2 µl). 1 µl of 100 bp DNA ladder was loaded into the gel. The gel has been submersed in 1 × TEB buffer and run at 84 V for 2 h. Bands were visualized under UV light at the gel documentation system and then photographed. Repetitions of PCR were performed again under the same conditions with annealing at 36 °C for those samples of weak bands.

Statistical analysis

Using Gel Analyzer 19.1, the molecular size of each fragment was estimated. Only those fragments consistently amplified were scored for analysis. Protein analysis used Excel 2016 to make graphs.

RESULTS AND DISCUSSION

Using Cetyl Trimethyl Ammonium Bromide (CTAB), total plant genomic DNA was extracted from young leaves. The absorbance ratio of DNA at A260/A280 ranged from 1.72 to 1.89 and the concentration ranged from 119.5 to 218.9 ng/ l.

Genotypic variations

Primers produced 32 DNA fragments 4 were polymorphic bands and 28 were not polymorphic bands polymorphism between two plants in bands was revealed in phonomertic characters that were recorded in this study **(Figure 1 and Table 1)**. Four random HAP primers (HAP1, HAP2, HAP3, and HAP4) were used for RAPD analysis of the two castor bean (*R. communis*) populations to detect polymorphism. Out of four random primers screened, two primers (HAP1 and HAP3) showed amplification in the two populations, while other primers (HAP2, and HAP4) show less amplification in populations. These primers may have found a low affinity to complementary binding sequences in the genomic DNA of these two castor bean populations. Moreover, these primers might have some special requirements for amplification.

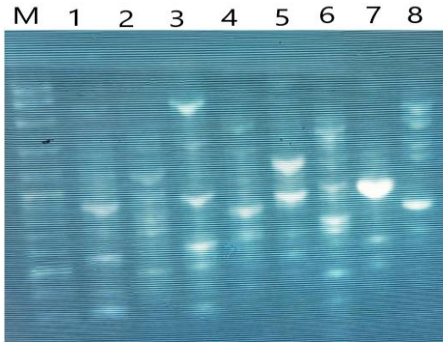
The study showed that the result of morphological characterization of plant height was 2.4-3.6 cm, 4.5-5.9 cm, seed color was brown and black/brown, seed size was 1.2 cm and 1.7 cm, capsule size was 2.6 cm and 3.5 cm, capsule texture was spiny and spineless in *R. communis* 3-lobe capsule and 4-lobed capsule, respectively **(Table 2)**. The study showed the result of chemical constituents in each 100 g of the seed of types *R. communis* growing in Riyadh Saudi Arabia. The study showed that the result of proximate analysis protein in seeds was 16% in sample A and 15% in sample B, while the total ash was 2.7 in sample A and 2.3 in sample B and fat was 47 and 47.8 in *R. communis* 3-lobe capsule and 4- lobed capsule, respectively **(Figure 2)**.

There is some distortion in this study. The sample size was limited, and each of the two varieties had only one germinated plant. This study also concentrated on the various varieties obtained from various sources. It will be fascinating to see how these varieties compare to natural Ricinus varieties. Some material was obtained from controlled sources, such as plant breeders, while others were obtained from botanical gardens. Outbreeding is a possibility, especially in botanical gardens. Furthermore, seed classification necessitates greater attention. The classification was difficult due to the wide variation in the appearance of the seeds even

within one variety. Due to a lack of additional material, the seeds were roughly divided into two groups. Finally, this research focused solely on visible characters. However, it is critical to examine the morphology of the Ricinus varieties so that anyone in the field can easily identify them. Polymorphism in the RAPD assay can occur as a result of base deletion, addition, or substitution within the priming site sequence [22]. High diversity reflects environmental adaptation, which is beneficial to its propagation, resource conservation, domestication of wild species, and screening of specific loci. Geographically isolated individuals tend to accumulate genetic variations as a result of environmental adaptations [23]. The purpose of this study is to use RAPD markers to determine the genetic diversity background of plants. The high levels of polymorphism discovered in this study indicate that RAPD markers are an appropriate tool for genetic diversity studies. This study might very well lead the way for more in-depth research into all aspects of this divergence. RAPD has been used successfully to identify medicinal plants and herbal medicinal components [24, 25]. This technique has also been reported to be useful for identifying and genotyping ornamental plants [26] and other species of plant varieties [27]. Color descriptors for the prickles, seeds, stigma, and stem showed high genetic variability, which is likely because these characteristics were not essential during the domestication process [28]. Understanding and distinguishing castor accessions required both qualitative and quantitative characteristics [29]. 574 accessions were chosen and genotyped with 22 polymorphic EST-SSR markers based on the screening results for oil content, fatty acid composition, and country of origin. Cluster analysis, population structure, and principal component analysis all produced consistent results, dividing accessions into four subpopulations [30]. Polymorphism was found in EST-SSRs. The average number of alleles detected per locus was 2.33. An allele was 150-400 bp in length. The 27 accessions were divided into two groups based on dendrogram analysis. Dendrogram genetic similarity coefficients ranged from 0.24 to 0.83. The polymorphic information content value of 0.28-0.49 revealed that the castor has a medium level of diversity [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%

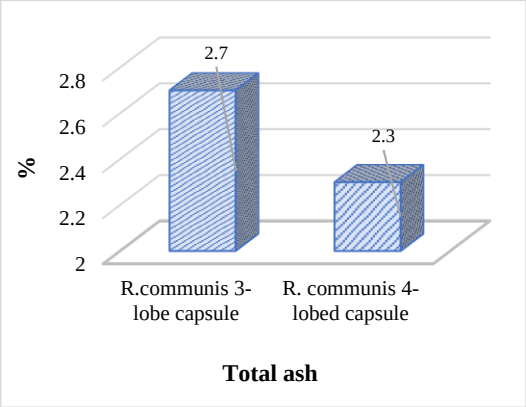


M = DNA Ladder 1KB, 1, 2, 3, 4 3-lobe capsule and 5, 6, 7,8 4-lobed capsule

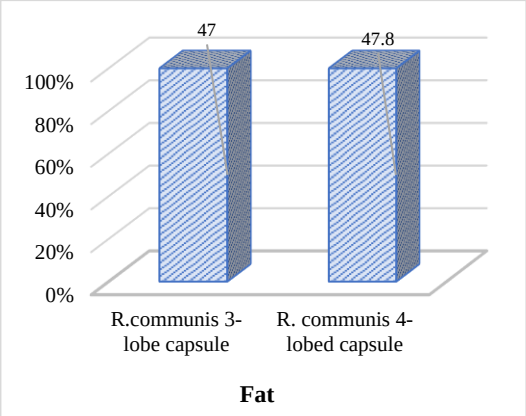
Figure 1. Electrophoretic pattern of two types of castor bean (*R. communis*) growing in Riyadh Saudi Arabia. (ethidium bromide-stained 2% agarose gel electropherogram of PCR products obtained from the RAPD analysis)

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

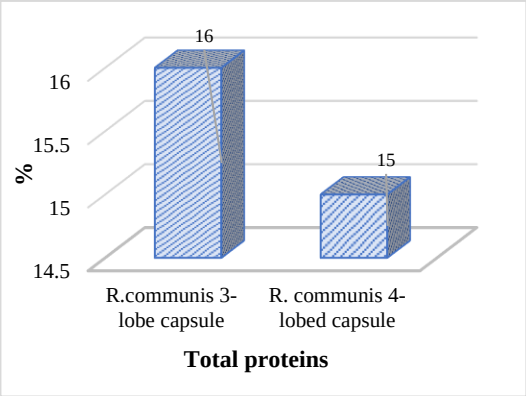
	***R. communis* 3-lobe capsule**	***R. communis* 4-lobed capsule**
Plant height	2.4-3.6cm	4.5-5.9cm
Seed color	Brown	Black / Brown
Seed size	1.2cm	1.7cm
Capsule size	2.6cm	3.5 cm
Capsule texture	Spiny	Spineless
Length	17-21 cm	19-23 cm



a)



b)



c)

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

CONCLUSION

The study concluded that Four random HAP primers (HAP1, HAP2, HAP3, and HAP4) were used for RAPD analysis of the two-castor bean (*R. communis*) populations to detect polymorphism. Out of four random primers screened, two primers (HAP1 and HAP3) showed amplification in the two populations, while other primers (HAP2, and HAP4) showed low amplification in the populations. These primers may have low affinity to complementary binding sequences in the genomic DNA of these two castor bean populations. Moreover, these primers might have some special requirements for amplification. The study showed that the result of morphological characterization varies in morphological and chemical constituents in each 100 g of the seed parameter under study. A high degree of genic variability assessed by markers would aid in the evaluation of novel genotypes for future improvement and population conservation.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: This work was approved by the Ethics Committee of Imam Mohammad Ibn Saud Islamic University (IMSIU). The data obtained from this work were analyzed according to the guidelines of ethical standards of the Declaration of Helsinki.

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