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Morphology and Molecular Study of *Cassia angustifolia* Vahl. in Saudi Arabia Using RAPD Technique

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ABSTRACT

Cassia angustifolia Vahl. is native to Saudi Arabia. This shrub has been used as a laxative and even as a fungicide. The current study was designed to evaluate the morphology and molecular characterization of *Cassia angustifolia* plants from two areas in Saudi Arabia. Purified genomic DNA was subjected to PCR amplification using random primers (RAPD). The study showed that, all morphological parameters were statistically significant ($P \leq 0.05$) when comparing *Cassia angustifolia* plants from the two areas. The primers screened (APOM3, APOM9, UBC-101, UBC-104) showed amplification in all subjected samples. The sizes of the ladder DNA were 1 kb. In four primers, the 1, 2, 3, and 4 RAPD markers produced 10 bands. The observed bands ranged in size from 100 Pb to 600 Pb, with one to three bands per primer. Ten DNA fragments were polymorphic bands between both two plants were confirmed in phonomertic characters observed in this study. This suggests that *cassia angustifolia* plants from A and B are related but show morphological variation.

Keywords: *Cassia angustifolia*, Molecular, Morphology, RAPD Technique, Saudi Arabia.

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INTRODUCTION

Cassia angustifolia Vahl. which belongs to the family Fabaceae is a small, annual, or perennial, branched under-shrub grown for its medicinal value of leaves and pods which contain sennosides, rhein, aloe-amine, kaempferein, and iso-rhein in free and glycosides forms [1]. *Cassia angustifolia* is found growing in the wild in north African countries, including Ethiopia and Sudan [2]. It is native to Yemen, Somalia, and Saudi Arabia, and is now grown globally. It is used in recent and other folk medicinal systems. Its therapeutic properties help relieve constipation [3].

Medicinal plants have a long history in the Middle East and are important elements in prophetic medicine. *C. angustifolia* is found in Saudi Arabia's mountains [4]. Mistletoe is a medicinal plant with great therapeutic potential [5]. The use of polyphenol-rich plants to prevent thrombus formation is of interest and it has anti-inflammatory effect [6, 7]. Lately, a new combination therapy used metals and plants as nano-antibiotics [8]. *Cassia angustifolia* Vahl, Senna, or Sonamukhi., it is also called Tinevelly Senna. It was brought to Tirunelvelly district in Tamil Nadu from Europe in the mid-18th century, hence the name Tinnevelly Senna. The laxative properties come from sennosides A, B, C, and D found in the leaves and pods [1, 9]. Senna is a multi-habitat plant that has successfully colonized a wide range of habitats in various climates and latitudes. Eighty percent of the 350 species currently assigned to the genus are found in North America, while the rest are found in tropical Africa, Madagascar. Using DNA profiling techniques to identify genotypes has several advantages over using morphological data. DNA sequences are independent of environmental conditions and can identify plants at any stage of growth [10-12]. PCR has enabled the

development of simple and rapid DNA profiling methods [13]. Molecular markers are the best tools for genetic analysis. Most RFLP (restriction fragment length polymorphism) issues were resolved with the advent of PCR-based RAPD and SSR (microsatellites). RAPD markers allow rapid screening of the genome for genetic polymorphisms. RAPD gained popularity due to its simplicity, efficiency, ease of use, and lack of sequence information. RAPD has been used to identify and analyze genetic variation in *Cassia* species [14]. RAPD markers are a useful tool for detecting polymorphism and identifying chromosome-specific DNA fragments. RAPD reveals one allele per locus, corresponding to the amplification product. RAPD should not detect heterozygous loci. RAPD has limitations here. When a single primer generates at least one complementary, it can identify heterozygotes. This marker identifies polymorphic amplification products from each parent, species, and population loci. RAPD is a useful tool for predicting areas of maximum diversity and estimating genetic variability in natural populations. The accessions were genetically clustered into seven clusters. Identical accessions were found to be the best for hybridization and producing productive recombinants for Senna genetic improvement [15]. The current study aimed to compare *Cassia angustifolia* planted in two different areas in morphology and molecular level using the RAPD technique.

MATERIALS AND METHODS

Samples collection

The seeds of *Cassia angustifolia* were collected from Jadah western (A) and Riyadh (B) Saudi Arabia and sowed in the trial field of the Botanical Garden of Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University, and the plants were morphologically analyzed, young leaves were kept it in a clean container under -20 until used for DNA extraction.

Morphology character measurements

In this study digital Vernia Guage tool was used to measure the length and width of stems, leaves, and seeds.

DNA extraction

Samples preparation

Half of a young dry leaf was taken and cut it into small pieces then grinded using a porcelain mortar and pestle.

Extraction method

The Invitrogen™ PureLink™ DNA Kit (Thermo Fisher Scientific, USA) was used for high-quality plant DNA from a wide variety of sample types. The kit uses proven PureLink™ spin-column technology for robust yields of DNA that is ready for downstream PCR, sequencing, and other applications. Typical DNA recovery is 5–20 µg from 1 mL of the plant.

RAPD analysis and primer selection

PCR amplification of genomic DNA using random primers was performed (RAPD). 25ng of genomic DNA was amplified using four primers: OPA03, AGTCAGCCAC, OPA09, GGGTAACGCC, UBC-101, GCGGCTGGAG, UBC-104, GGGCAATGAT. The standard William *et al*., [14]. The RAPD-PCR method was used by Hamza *et al*.; Kumar [2, 16]. A 25ng template DNA (1µl), 0.5U Taq polymerase (0.5µl), 10 picomoles random primers (2µl), 100mm each dNTP(2,5µl), 50mm $MgCl_2$(1.5 µl/ml) and 1X PCR reaction buffer (2,5µl) were used to make 20 µl PCR reaction mixtures each. Double distilled water was used to make up to 20µl. Thermocycler optimized PCR mixtures. PCR had three steps: 5min initial denaturation at 94°C. Step 2: Run 40 cycles of denaturation at 94°C, annealing at 36°C, and extension at 72°C. Step 3: 7-minute final extension cycle at 72°C. To keep the product at 40°C, the PCR thermocycler was tuned. Electrophoresis was done using ethidium bromide-stained and 2% agrose gel.

Statistical analysis

The data was statistically analyzed by using excel 2010 for descriptive data as graphs. DNA analysis using ethidium bromide-stained from PCR products by gel electrophoresis.

RESULTS AND DISCUSSION

Morphological characterization

The study showed that when comparing *Cassia angustifolia* collected from the two areas, the length of plants was 78.60±0.65 cm (65- 78 cm) in seeds from the western area while in seeds from Riyadh was 55.36±0.57 cm (44- 55cm)

(Figure 1). The length of the leaf was 5.54±0.24 cm (4-6.9 cm) and 3.80±0.32 cm (2- 6.6cm) in the western area and Riyadh, respectively **(Figure 2)**. The leaf width was 1.60±0.07 cm (0.9-1.9 cm) and 0.84±0.08 cm (0.5- 1.6cm) in western area and Riyadh, respectively **(Figure 3)**. The length of the fruit long was 6.56±0.29 cm (5.6-7.5cm) in the western area and 4.00±0.25 cm (2.5- 5.2cm) in Riyadh **(Figure 4)**. The width of fruit was 2.33±0.11 cm (1.8-3cm) in the western area while in Riyadh was 1.30±0.05 cm (1- 1.5cm) **(Figure 5)**. All morphological parameters were statistically significant ($P \leq 0.05$) when comparing *Cassia angustifolia* plants from the two areas. The variation in morphology was attributed to the different environments in which samples were cultured.

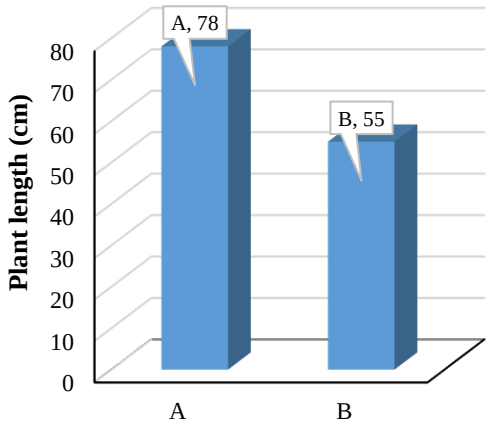


Figure 1. Length of *Cassia angustifolia* plants understudy

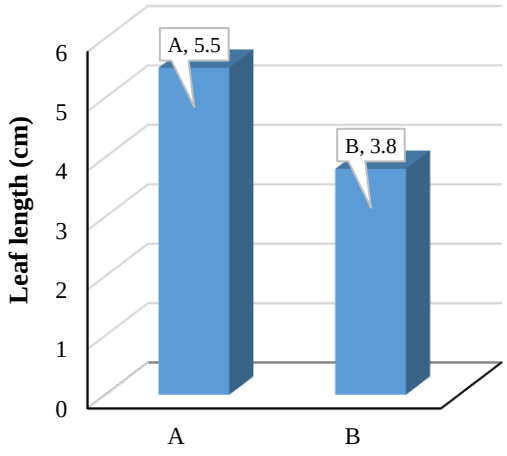


Figure 2. Length of the leaf of *Cassia angustifolia* plants understudy

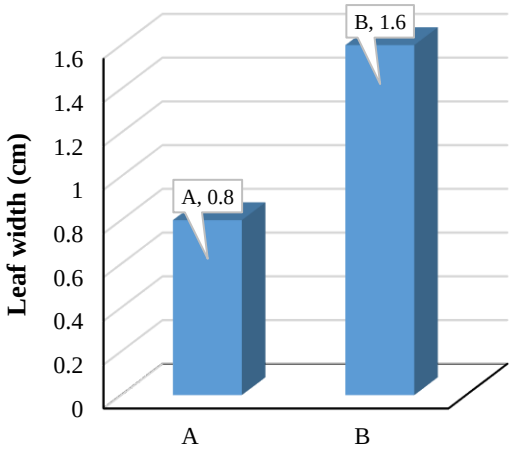


Figure 3. Leaf width of *Cassia angustifolia* plants

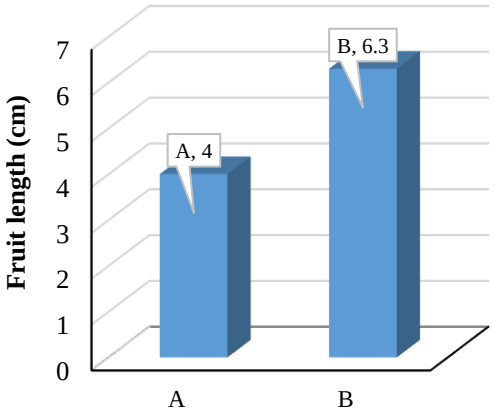


Figure 4. Length of the fruit of *Cassia angustifolia* plants understudy

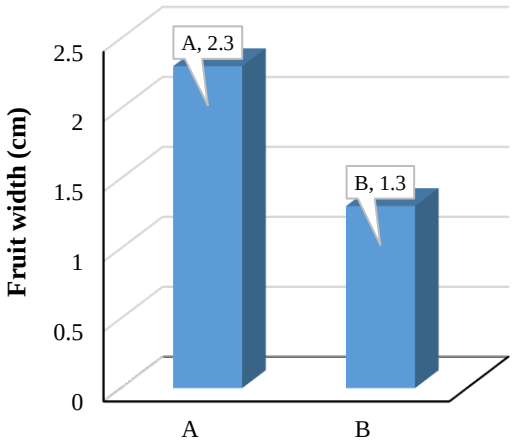
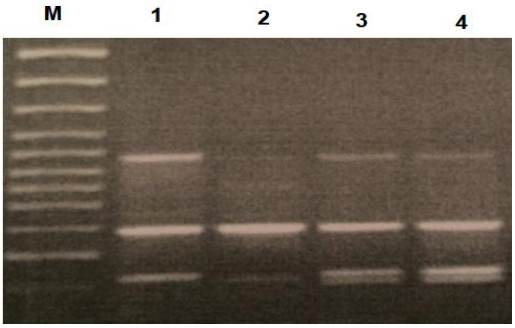


Figure 5. Width of the fruit of *Cassia angustifolia* plants

Molecular results (genetics)

Four random APOM and UBC primers (APOM3, APOM9, UBC-101, UBC-104) were used for RAPD

analysis of the two *Cassia angustifolia* populations to detect polymorphism. The primers screened, (APOM3, APOM9, UBC-101, UBC-104) showed amplification in all subjected samples. The sizes of the ladder DNA were 1 kb. The 1, 2, 3, and Four primers yielded ten RAPD bands. The observed bands ranged in size from 100 to 600 Pb, with one to three bands per primer. Ten DNA fragments were polymorphic bands between both the two plants were confirmed in phonomertic characters observed in this study **(Figure 6** and **Table 1)**.



M= DNA Ladder 1KB , 1, 2, 3 ,4 and5 = samples

Figure 6. Electrophoretic pattern of *Cassia angustifolia* populations with four random primers; APOM3, APOM9, UBC-101, and UBC-104 (ethidium bromide-stained 2% agrose gel electro photogram of PCR products obtained from the RAPD analysis)

Table 1. Primers showing polymorphism in two Saudi Arabian Cassia angustifolia populations from Jadh and Riyadh.

Sr. No	Primer code	Primer sequence (F/R)	No. of genotype	Total amplified bands	Polymorphic bands	Monomorphic ifbands	Percent Polymorphism
1	APOM 3	AGTCAGCCAC	2	3	0	3	0.00
2	APOM9	GGGTAACGCC	2	1	0	3	0.00
3	UBC-101	GCGGCTGGAG	2	3	0	1	0.00
4	UBC-104	GGGCAATGAT	2	3	0	3	0.00

Cassia is a tropical herbaceous, shrubby, and tree genus [17]. The current study found that climate and drought seasons influenced morphological parameters in Saudi Arabia. 3-7 pairs of minor leaflets and small stipules (2-3 mm). The leaflets are 2.5-4.5 cm long, 7-10 mm wide, glabrous, with an acute apex and a cuneate base. Yellow flowers (7-12) per terminal raceme. From July to September, flowers bloom. The fruit is a 4-6 cm long dehiscent pod with 10-17 seeds [18]. *Cassia* grown in different environments had less genetic variation. An important topic and challenge for plant breeding researchers is how genotype interacts with environment [19]. RAPD has been used successfully in genetic diversity among Cassia species, crop improvement, and detection of gene flow between species. RAPD is more informative, powerful, and less restrictive than RFLPs in research [20]. In *Cassia senna*, RAPD markers assessed genetic diversity (L.). The RAPD technique was used on 27 (C. senna (L.) With six primers and 31 reproducible products, we got 27 polymorphisms (7.5 fragments per primer). Three to six polymorphic bands per primer accounted for 60% of the amplification products. They had low genetic similarities. A total of 60 percent polymorphism was generated by six random primers with 10 bases each. The high polymorphism found in C. senna suggests RAPD techniques can be useful [16] Opposite to current results. Cassia's genetic variation is related to its geographic range. Species with a wide geographic range have more species variability [21-23]. This type of phylogeny is best suited for species-level phylogeny studies because it includes the homology of bands with similar migration rates. The wide adaptation of some Cassia species suggests prior nuclear DNA mutations. These traits resulted from genetic and physiological polymorphism. Molecular and morphological evidence supports Fabaceae's monophyletic status [24]. This conclusion is backed up by all phylogenetic studies using DNA sequences, as well as the degree of interrelation between groups within the family [25]. The Fabaceae are monophyletic and closely related to the *Polygalaceae*, *Surianaceae*, and *Quillajaceae* families [26]. Nodulation genes were recruited from other pathways after a polyploidy event, current evolutionary theory states [27]. Duplicated genes may be donated to nodulation pathways *via* several pathways. The pathway got arbuscular mycorrhiza and pollen tube formation genes. They co-evolved with flowering plants, laying the molecular foundation for a synergistic relationship in nodules, and are found in the root cortex and stem of Sesbaniarostrata. Frankia and rhizobial spermatophytes evolved

together (as established by the gene molecular phylogeny of rbcL, a gene coding for part of the RuBisCO enzyme in the chloroplast). Depending on the lineage, ancestral nodule formation is conserved or lost. Because of this, multiple origins for modulation are possible.

CONCLUSION

This study concluded that the two plants of *Cassia* from the western region and the Riyadh area were related but show morphological variation. The morphological variation is due to the environment. Future studies such as chemical composition are needed.

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CONFLICT OF INTEREST: None

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