Morphological and molecular genetic variations of some taxa of genus *Acacia* and their taxonomic significance

Usama K. Abdel-Hameed a,b,c, Hanaa H. Elenazy a and Amal M. E. Abdel-Hamid b,d

aDepartment of Biology, College of Science, Taibah University, Al Madinah, Kingdom of Saudi Arabia; bDepartment of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt; cDepartment of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt; dDepartment of Biology, College of Sciences and Arts, Taibah University, Al Ula, Kingdom of Saudi Arabia

ABSTRACT
A resemble in *Acacia* s.l. spp. morphology causes a great confusion about its identification. So, the current study’s objectives are using morphological and molecular traits for accurate identification of *Acacia* s.l. taxa, assess the discrimination of the newly proposed genera of *Vachellia* and *Senegalia* and detect the genetic variations between studied taxa. The morphological characters of 24 taxa of *Acacia* s.l. were investigated with emphasis on seeds. DNA fingerprint and the genetic relationships among the studied taxa were investigated by using 10 (RAPD) and 10 (ISSR) markers that showed a relatively equal polymorphism (about 15%). RAPD marker generated a total of 395 fragments of which 64 bands were polymorphic, while ISSR primers generated a total of 314 fragments, 46 of them were polymorphic. The generated dendrogram that based on 893 traits divided the studied taxa of *Acacia* s.l. into two major groups; *Acacia* s.s. group and *Vachellia* group that containing *Senegalia* taxa. The complementary use of morphological and molecular data gave an accurate characterization and clear the genetic variation between studied taxa of *Acacia*.

1. Introduction

Various phylogenetic studies demonstrated that *Acacia* s.l. is not monophyletic, it is currently broadly accepted to be partitioned into at least five genera *Viz. Acacia* s.s., *Vachellia*, *Senegalia*, *Acaciella* and *Mariosousa* [1]. The term *Acacia* s. s. was retained for the bulk of Australian taxa, with *Vachellia* and *Senegalia* being used in Asia and Africa [2]. Morphological characteristics were considered significant, but not enough for the characterization of *Acacia* s.l. [3]. Hence, in this study, we investigated the position of *Acacia* s.l. spp. in the new conventional arrangement, based on data generated from both morphological and molecular information from two molecular markers.

Chaudhary and Al-Jowaid [4] studied the morphological characteristics of 18 species of the genus *Acacia* s.l. in Saudi Arabia. They used several vegetative and floral characteristics to build a key to define the genus. Further, Migahid [5] described the morphology of 14 species belonging to genus *Acacia* s.l. In addition, Khan et al. [6] investigated the morphological and anatomical characteristics of eight *Acacia* s.l. species found in the Wadi Fatimah in Makkah Al-Mukarramah region. Al-Gohary and Mohamed [7] studied the morphological variations between 12 taxa of genus *Acacia* s.l. Their results completed the construction of an indented identification key at the specific and infra-specific levels.

Few authors have investigated the seed morphology of *Acacia* s. l. [8]. The seed surface sculpture patterns of nine species, one subspecies and two varieties of *Acacia* s.l. of Saudi Arabia were described [9]. The seed coat’s morphological and anatomical characteristics have been used to distinguish 11 *Acacia* s.l. species in Saudi Arabia [10] using Scanning Electron Microscopy (SEM) [11] and Light Microscopy (LM) [7].

Molecular markers have been used to identify species and populations. Because genetic markers are unaffected by environmental factors, they are useful in the conservation of plant germplasm [12,13]. Many authors have revealed the valuable data obtained from combining morphological characteristics and molecular markers for evaluating genetic diversity among...
different varieties of \textit{Acacia s.l.} as well as the role of
genetic markers in supporting the taxonomic results for
the differentiation of studied varieties [14,15]. Abdel-
Hamid [16] demonstrated that Random Amplified Poly-
morphic DNA (RAPD) was a useful tool for the identi-
fication and characterization of plants at the level of
subspecies. Mutharaian \textit{et al.} [17] used RAPD markers to
determine genetic distance and similarity among seven
\textit{Acacia s.l.} spp., which showed 326 species-specific mark-
ers with 55.82\% polymorphism to clarify the close rela-
tionships among the seven studied species.

RAPD and Inter-Simple Sequence Repeats (ISSR) have
been extensively used for plant classification
owing to their simplicity, rapidity in detecting DNA
morphism and high efficiency for analysis of genetic
diversity [18–23]. RAPD and ISSR markers were used
to study the genetic variability between five species of
\textit{Acacia}, where the RAPD marker resulted in 58
bands, with 51 unique bands and seven polymor-
phic bands, and the ISSR marker showed 75 bands of
which 42 were unique and 31 polymorphic between
studied taxa. RAPD separated \textit{Acacia ehrenbergiana}
from other investigated species, while ISSR separated
both \textit{Acacia eboica} and \textit{Acacia tortilis} from other
species [24].

The identities of \textit{Acacia s.l.} spp. in Saudi Arabia
have caused great confusion for field workers in this
country. Therefore, the goal of this research was to
use morphological characteristics, with emphasis on
seed traits for the accurate identification and system-
atic classification of 24 taxa belonging to the genus
\textit{Acacia s.l.} grown within Kingdom of Saudi Arabia and
to assess the discrimination of the newly proposed
genera of \textit{Vachellia} and \textit{Senegalia}, further objective was
to detect genetic diversity via RAPD and ISSR
markers that could provide molecular characteriza-
tion, preservation of germplasm of these important
taxa, and investigate the genetic relationships among
them.

\section{Materials and methods}

In the present study, 24 taxa of \textit{Acacia s.l.} were col-
lected from five localities in KSA representing eighteen
species, four subspecies and two varieties. The collected
plants were identified and authenticated according to
[5,25,26] (Table 1).

\subsection{Morphological traits}

The macromorphological characters of the whole plant
\textit{viz.} habit, leaf, flowers, inflorescence, pod and seeds
were extracted directly from the fresh specimens of
the available taxa. Twenty four mature seeds were
dried, cleaned, examined by Stereomicroscope and
photographed by Digital Camera 7.2 mp; six characters
of seed were studied \textit{viz.} colour, shape, texture, areola

\begin{table}[h]
\centering
\caption{The collected taxa of genus \textit{Acacia} in the current study, B.S.: A: Riyadh, B: Al-Qassem, C: Dammam, D: Al-Taif, E: Al-Bahah.}
\begin{tabular}{ll}
\hline
1. & \textit{Acacia ampliceps} Maslin, Nuytsia 1(4): 315 (1974), (IK) \\
2. & \textit{Acacia asak} Willd., Sp. Pl., ed. 4 [Willdenow] 4(2): 1077. 1806 [Apr 1806] (IK) \\
3. & \textit{Acacia coriacea} DC., Prodrumus 2 1825 (APNI) \\
4. & \textit{Acacia cuibertsonii} Luehnh., Vict. Naturalist 13: 117 (1897). (IK) \\
5. & \textit{Acacia cyclops} A.Cunn. ex G.Don, Gen. Hist. 2: 404 (1832), nom. cons. \\
6. & \textit{Acacia ehrenbergiana} Hayne, Getreue Darstell. Gew. x. t. 29. (IK) \\
7. & \textit{Acacia eboica} Schweinf., Linnaea 35: 330, t. 8, 1867 (IK) \\
8. & \textit{Acacia farnesiana} (Benth.) Brenan subsp. indica, Kew Bulletin (1957). \\
9. & \textit{Acacia johnwoodii} Benth. var. gerrardii, Trans. Linn. Soc. London 30(3): 508. 1875 [10 Apr 1875] (IK) \\
10. & \textit{Acacia nilotica} Benth. var. iraquensis, Trans. Linn. Soc. London 30(3): 508 (1875). \\
11. & \textit{Acacia gerrardii} Benth. var. najdensis Chaudhary, Acacia & other gen. \\
12. & \textit{Acacia gerrardii} Benth. (= \textit{Vachellia gerrardii} (Benth.) P. J. H. Hurter subsp. negevensis (Zohary) Ragup. & al. var. \\
13. & \textit{Acacia johnwoodii} Boulots, Kew Bull. 50(2): 327, 1995 [23 Jun 1995] (IK) \\
14. & \textit{Acacia nilotica} (Benth.) Brenan subsp. indica, Kew Bulletin (1957). \\
15. & \textit{Acacia nilotica} (Benth.) Brenan subsp. indica, Kew Bulletin (1957). \\
16. & \textit{Acacia nilotica} (Benth.) Brenan subsp. tomentosa, Kew Bulletin (1957). \\
17. & \textit{Acacia oringa} Hunde, Nordic J. Bot. 2(4): 337 (1982), (IK) \\
18. & \textit{Acacia pruinocarpa} Tindale, Conr. New South Wales Natl. Herb. 4(3) 1972 (APNI) \\
19. & \textit{Acacia salicina} Lindl., Three Expeditions into the interior of Eastern \\
20. & \textit{Acacia sclerosperma} F.Muell., Sci. Rec. 1882 (APNI) \\
21. & \textit{Acacia senegal} Willd., Sp. Pl., ed. 4 [Willdenow] 4(2): 1077. 1806 [Apr 1806] (IK) \\
22. & \textit{Acacia tortilis} Hayne subsp. radiana, Getreue Darstell. Gew. x. t. 1. (IK) \\
23. & \textit{Acacia tortilis} Hayne subsp. tortilis, Getreue Darstell. Gew. x. t. 1. (IK) \\
24. & \textit{Acacia victorinae} Benth., J. Exped. Trop. Australia [Mitchell] 333 (1848) \\
\hline
\end{tabular}
\end{table}

shape, hilum position, hilum shape. For SEM investiga-
tion, the seeds were dried and coated with golden parti-
cles and fixed to the specimen holder of Scanning Elec-
tron Microscope (SEM, Inspect S, version 3.1.2) main-

tioned, preservation of germplasm of these important
taxa, and investigate the genetic relationships among
them.

\subsection{Molecular markers}

RAPD according to [31] and Inter Simple Sequence
Repeats (ISSR) according to [32] markers were used in
this study.
2.3. DNA isolation

DNA isolation from plant tissues was carried out using DNeasy plant Mini Kit (Dneasy plant mini handbook 2016, Qiagen Inc., USA).

2.4. PCR conditions

RAPD-PCR reactions were done by using 10 arbitrary random 10-mer primers with sequences indicated in (Table 2). Reaction conditions and mixtures (25 µl total volumes) consisted of dNTPs, MgCl₂, 10X buffer, Primer, Taq DNA Polymerase and Template DNA were optimized. Amplification was carried out in a (Veriti® Thermal Cycler, Life Technologies, Applied Biosystem, NY, USA) programmed for 36 cycles as follows: 94 °C/4 min (1 cycle); 94 °C/1 min, 37 °C/1 min. 72 °C/2 min (40 cycles); 72 °C/10 min (1 cycle) and 4 °C (infinite).

ISSR-PCR reactions were done by using 10 primers with sequences indicated in (Table 2). Reaction conditions and mixtures (25 µl total volumes) consisted of dNTPs, MgCl₂, 10X buffer, Primer, Taq DNA Polymerase, BSA (bovine serum albumin) and Template DNA were optimized. Amplification was carried out in a (Veriti® Thermal Cycler, Life Technologies, Applied Biosystem, NY, USA) programmed for 36 cycles as follows: 94 °C/5 min (1 cycle); 94 °C/30 sec., 44 °C/90 sec. 72 °C/1.5 min (36 cycles); 72 °C/20 min (1 cycle) and 4 °C (infinite). For both RAPD and ISSR PCR amplification was done twice for each sample and a particular primer for giving two amplification replicates in order to saturate polymorphism within samples and ensure reproducibility of the data.

2.5. Gel electrophoresis for RAPD and ISSR markers

Agarose (12%) was used for separating products of PCR. 100 bp DNA Ladder were used (Solis BioDyne, Cat. No. 07-11-00050) its molecular sizes (MS) in bp of the 13 marker bands are: 3000, 2000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100. The run was completed for one hour at 100 V in a Bio-RadTM submarine (8 cm × 12 cm). Amplicons were visually examined with a UV transilluminator and photographed using a CCD camera (UVP, UK).

2.6. Data analysis

Unweighted Pair-Group Method using Arithmetic Averages with SAHN function due to [33] was followed to evaluate the variation of morphological characters states among the submitted taxa, each taxa was considered as operational taxonomic unit (OTU) and characters states were analyzed as binary features. In addition,
the banding patterns generated by RAPD-PCR and ISSR-PCR markers were compared to detect the differences and similarity between samples under study. Clear and distinct bands were scored as “1” for presence and “0” for absence of bands. The configuring of groups was depended on the Jaccard’s coefficient similarity values. All statistics were performed by software NTSys-PC version 2.02 by Rohlf [34].

3. Results

3.1. Morphological characteristics

The most informative and diagnostic morphological characters were: plant habit that varied between shrub in Acacia coriacea, A. cuthbertsonii, A. ehrenbergiana and A. ligulata or tree in 19 taxa. The plant length whether small in six studied taxa, medium in another six taxa or large in the remaining. The plant texture glabrous in 9 taxa, whether pubescent in one taxa A. tortilis subsp. radiana or spiny in 13 taxa (Table 3).

Leaf composition simple in 8 taxa and compound in 15 taxa. Leaf arrangement alternate in 9 taxa and opposite in 14 taxa. Lamina shape lanceolate in 5 taxa, oblong in 15 taxa and linear in 3 taxa A. coriacea, A. salicina and A. sclerosperma. Lamina apex acute in 6 taxa, obtuse in remaining 17 taxa. Lamina base decurrent in 6 taxa rounded in 16 taxa and acuminate in one taxa A. coriacea.

Inflorescence shape globular in 19 taxa or spike in 4 taxa A. asak, A. cuthbertsonii, A. cyclops and A. senegal. Inflorescence colour white in 12 taxa or yellow in 11 taxa.

Pod texture glabrous in 15 taxa, pubescent in 7 taxa and tomentose in one taxa A. nilotica subsp. tomentosa. Pod colour brown in 5 taxa, reddish brown in 4 taxa A. asak, A. farnesiana, A. gerrardii, var. gerrardii and A. origena, red in one taxa A. coriacea, greenish-red in 3 taxa A. ehrenbergiana, A. johnwoodii and A. tortilis subsp. tortilis, reddish purple in one taxa A. etbaica, yellowish brown in two taxa A. gerrardii, var. iraquensis and A. gerrardii, var. najdensis, grey in two taxa A. nilotica subsp. indica and A. nilotica subsp. tomentosa, pale brown in four taxa A. pruinocarpa, A. salicina, A. senegal and A. victoriae and golden brown in one taxa A. sclerosperma. Pod form curved in 4 taxa A. ampliceps, A. cuthbertsonii, A. gerrardii, var. najdensis and A. sclerosperma, flat in one taxa A. asak, twisted in 5 taxa and straight in remaining taxa (13). Pod dehiscence dehiscent in 11 taxa or indehiscent in remaining taxa (12). Pod apex rounded in 15 or beaked in 8 taxa.

Seed shape ovate in 7 taxa, rounded in 7 taxa, quadrate in one taxa A. gerrardii var. gerrardii, elliptic in 7 taxa, Kidney-shaped in one taxa A. senegal. Seed colour dark brown in 3 taxa A. ampliceps, A. ehrenbergiana and A. victoriae, bright brown in 3 taxa A. asak, A. cuthbertsonii and A. senegal, brown in 3 taxa A. farnesiana, A. gerrardii var. gerrardii and A. tortilis subsp. tortilis, black in 7 taxa, dark black in one taxa A. sclerosperma, greenish brown in 5 taxa and green in one taxa A. johnwoodii. Seed texture glabrous in 19 taxa or rugose in 4 taxa A. ehrenbergiana, A. etbaica, A. origena and A. senegal. Areole shape horseshoe in 6 taxa, crescent in 4 taxa A. asak, A. origena, A. sclerosperma and A. victoriae, obovate in 11 taxa and circular in two taxa A. cuthbertsonii and A. gerrardii, var. iraquensis. Hilum position terminal (15) or subterminal (8). Hilum shape slit-Like in 13 taxa, or rounded in 10 taxa (Figure 1).

Tests sculpture surface reticulate in 6 taxa, ruginata in 10 taxa, colliculate in five taxa, or alveolate in two taxa A. farnesiana and A. ligulata. Testa cell shape polygonal in 6 taxa, polyhedral in 5 taxa, irregular in 11 taxa and elongated in one taxa A. cuthbertsonii. Testa cells anticlinal wall shape wavy in 10 taxa, undulate in 9 taxa and straight in 4 taxa A. pruinocarpa, A. salicina, A. sclerosperma and A. victoriae. Testa cells anticlinal wall elevation raised in 5 taxa or depressed in 18 taxa. Testa cells periclinal wall elevation raised in 18 taxa and depressed in 5 taxa. Testa cells periclinal wall vesititure rugose in 9 taxa, tuberculate in two taxa A. asak and A. etbaica, granular in 6 taxa and smooth in 6 taxa (Figure 2). All 84 morphological character states were coded and used for computations.

| Character no. | Characters, Character states and codes |
|---------------|---------------------------------------|
| 1.            | Habit: Tree (0), Shrub (1)             |
| 2.            | Length: Small (0), Medium (1), Large (2) |
| 3.            | Texture: Glabrous (0), Pubescent (1), Spiny (2) |
| 4.            | Leaf composition: Simple (0), Compound (1) |
| 5.            | Leaf arrangement: Alternate (0), Opposite (1) |
| 6.            | Lamina shape: Lanceolate (0), Oblong (1), Linear (2) |
| 7.            | Lamina apex: Acute (0), Obtuse (1)     |
| 8.            | Lamina base: Decurrent (0), Rounded (1), Acuminata (2) |
| 9.            | Inflorescence Shape: Globular (0), Spike (1) |
| 10.           | Inflorescence colour: White (0), Yellow (1) |
| 11.           | Pod texture: Glabrous (0), Pubescent (1), Tomentose (2) |
| 12.           | Pod colour: Brown(0), Reddish brown (1), Red (2), Greenish-red(3), Reddish purple (4), Yellowish brown (5), Grey(6), Pale brown (7), Golden brown(8) |
| 13.           | Pod form: Curved (0), Flat (1), Twisted (2), Straight (3) |
| 14.           | Pod dehiscence: Dehiscent (0), Indehiscent (1) |
| 15.           | Pod apex: Rounded (0), Beaked (1)      |
| 16.           | Seed shape: Ovate(0), Rounded (1), Quadratde (2), Elliptic (3), Kidney-shaped(4) |
| 17.           | Seed colour: Dark brown(0), Bright brown (1), Brown(2) Black(3), Dark Black (4), Greenish brown (5),Green (6) |
| 18.           | Seed texture: Glabrous (0), Rugose (1) |
| 19.           | Areole shape: Horseshoe (0), Crescent (1), Obovate (2), Circular (3) |
| 20.           | Hilum position: Terminal (0), Subterminal (1) |
| 21.           | Hilum shape: Silt-Like (0), Rounded (1) |
| 22.           | Tests sculpture surface: Reticulate (0), Ruminata (1), Colliculate (2), Alveolate (3) |
| 23.           | Tests cell shape: Polygonal (0), Irregular (1), Elongated (2) |
| 24.           | Tests cell anticlinal wall shape: Wavy (0), Undulate (1), Straight (2) |
| 25.           | Tests cell anticlinal wall elevation: Raised (0), Depressed (1) |
| 26.           | Tests cell periclinal wall elevation: Raised (0), Depressed (1) |
| 27.           | Tests cell periclinal wall vesititure: Rugose (0), Tuberculate (1), Granular (2), Smooth (3) |
3.2. Molecular markers

The RAPD analysis of the 24 taxa of genus *Acacia s.l.* with the different random 10-mer primers generated a total of 395 fragments of which 64 bands were polymorphic. The molecular size of amplified fragments ranged from 122.09 bp to 2268.10 bp. The lowest number of amplified fragments was 34 bands by primer U10, while the highest amplified fragment was 47 bands by primer D18. The monomorphic bands were not detected in all samples with all primers. The total polymorphism ratio was 15.80%. The highest ratio of polymorphism generated by primer U18 (30.43%), while the primer V15 produced the lowest polymorphism (2.78%). The total number of species-specific bands scored across the 24 taxa of genus *Acacia s.l.* by RAPD marker are 64.

The highest number of species-specific bands generated from primer U18 was 14, while the lowest number of species-specific bands was 1 generated from primer V15. Analysis of the 24 taxa of genus *Acacia* with the ten ISSR primers generated a total of 314 fragments, 47 of them were polymorphic. The molecular sizes of bands varied from 198.42 bp to 1489.14 bp. The number of PCR products ranged from 26 to 37 bands. There were no monomorphic bands detected with all primers and all samples. The total polymorphism ratio was 15.41%. Forty seven molecular markers were detected as species-specific bands. The highest ratio of polymorphism generated by primer 844 (31.03%), while the primer IT1 produced the lowest polymorphism (8.57%). The total number of species-specific markers scored by
3.3. Genetic relationships by RAPD and ISSR markers

The genetic similarities among the twenty four taxa of genus *Acacia s. l.* based on Jaccard's coefficient appeared that, the highest pairwise similarity indices (0.55) resulted from RAPD marker was between *A. cuthbertsonii* and *A. sclerosperma*, also between *A. nilotica* subsp. *tomentosa* and *A. nilotica* subsp. *indica*. While the lowest similarity indices (0.10) were noticed between *A. ehrenbergiana* and *A. origena* (Appendix A). The highest pairwise similarity indices (0.62) resulted from ISSR were between *A. nilotica* subsp. *tomentosa* and *A. nilotica* subsp. *indica*. While the lowest similarity indices (0.14) were observed between *A. coriacea* and *A. gerrardii var. iraquensis* (Appendix B). The highest pairwise genetic similarity among the 24 taxa of genus *Acacia* resulted from both RAPD and ISSR markers was (0.58).
Figure 3. DNA polymorphism of the studied taxa generated by RAPD Markers.

between *A. nilotica* subsp. *tomentosa* and *A. nilotica* subsp. *indica*. The lowest pairwise similarity indices was (0.16) between *A. tortilis* subsp. *tortilis* and *A. victoriae*, also between *A. origena* and *A. coriacea*. (Appendix C).

3.4. Phenetic analysis of both morphological and molecular characteristics

The phenetic analysis of both morphological and molecular attributes generated a dendrogram (Figure 5)
that clarifies the splitting of genus *Acacia* *s.l.* into two main series; Series I included 16 taxa, *A. gerrardii* var. *najdensis* was segregated early at 0.2 taxonomic distance as a distinct identity and the remaining taxa distributed into three groups; the first one included five taxa where *A. asak* was segregated early at a taxonomic distance 0.2. *A. ehrenbergiana* and *A. johnwoodii* formed sister taxa at a taxonomic distance 0.35. *A. farnesiana* and *A. senegal* were grouped together at a taxonomic distance 0.22. The second group divided into two subgroups; one comprised *A. etbaica* and *A. origena* at taxonomic distance less than 0.3, the second subgroup contained four taxa where *A. gerrardii* var. *gerrardii* and *A. gerrardii* var. *iraquensis* the last segregated taxa at 0.32 taxonomic distance. *A. tortilis* subsp. *tortilis* and *A. tortilis* subsp. *raddiana* were grouped together in the

*Figure 4.* DNA polymorphism of the studied taxa generated by ISSR Markers.
present study. The third group contained two sister subgroups the first was *A. nilotica* subsp. *indica* and *A. priniiocarpa* at taxonomic distance 0.25. While the second was another accession of *A. nilotica* subsp. *indica* and *A. nilotica* subsp. *tomentosa* at taxonomic distance less than 0.5.

Series II included 8 taxa that distributed into two groups; the first one included four taxa where *A. coriacea* was segregated early followed by *A. ampliceps* at a taxonomic distance about 0.2, while *A. ligulata* and *A. salicina* formed sister taxa at a taxonomic distance less than 0.3. The second group contained two sister groups one of them comprised *A. cuthberstonii* and *A. sclerosperma* at a taxonomic distance less than 0.3. While the second contained *A. cyclops* and *A. victoriae* at a taxonomic distance about 0.2.

### 4. Discussion

Considering a legitimate concern for clearness, all species talked about here are alluded to by their *Acacia* s.l. names and after resolution of the interrelationships among the studied species, the name of *Acacia* s.s., *Vachellia* and *Senegalia* are imposed. The present study included 24 taxa of the former broadly circumscribed genus *Acacia* s.l. that occur in Kingdom of Saudi Arabia, either naturally or as major intruders. Genus *Acacia* s.l. should be treated as comprising at least five genera, this new classification is based on results from many morphological, genetic studies [2]. The obtained results confirmed the segregation of *Acacia* s.l. into *Senegalia* and *Vachellia* [1], where the generated dendrogram that based on the morphological and molecular attributes was divided into two main series one of them contained the taxa belonging to genus *Vachellia*, two species of *Senegalia* were nested within this group while the second group contained the taxa that belonging to *Acacia* s.s. Although the majority of *Acacia* s.s. spp. form a single series, the placement of *A. priniiocarpa* outside the main *Acacia* s.s. series and migrates to the *Vachellia* series represents a novel finding, indicating the close relationship among some taxa of *Acacia* s.s. and *Vachellia* that may be resolved by use of more morphological characters and genetic markers.

From the morphological results we can realize the benefits of using Light Microscope (LM) and SEM as diagnostic characteristics for separation and distinguishing between studied taxa of *Acacia* s.l. and the importance of seed description as a valuable taxonomic character.

The analysis of the twenty four taxa of *Acacia* s.l. with both markers RAPD and ISSR generated 709 bands, 111 from them are polymorphic bands (species-specific bands). This indicates that these markers can be used to characterize taxa used in this study [16,35–38]. However, RAPD marker detects 64 polymorphic bands that are higher than polymorphic bands scored for ISSR (47) between different taxa of *Acacia* s.l.. This may be due to the ratio of coding and non-coding sequences within the genome and differences in genome composition of different taxa used in this study [39,40]. The combined data of both markers give more balanced results for genetic variations among studied taxa [24].

The results of RAPD and ISSR polymorphism showed differences between the same taxa of *A. nilotica* subsp. *indica*, one was collected from Al-Qassim and another one collected from Al-Riyadh. The dendrogram generated by both RAPD and ISSR markers data separated them from each other, although they have the same morphological characters. This is indicating the power of these markers to distinguish between closely related samples. The explanation of the molecular genetic variability between studied taxa is dependent on the sexual reproduction, in which low levels of genetic variation as often related with sexual propagation, in addition the differences of the geographical distributions of these taxa and the effect of abiotic factors of the environment; these agreed with [24,41–45]. Also, Yadav et al. [46] demonstrated that climatic and geographical conditions have a great impact on genetic diversity of wild population of *A. nilotica* and also the high transferability of genetic resources from other related species of *A. nilotica*.

From the generated phenogram using UPGMA cluster method that was based on combined pooled data of RAPD, ISSR and morphological traits, we noticed that *A. asak* separated in a distinct entity while *A. ehrenbergiana* and *A. johnwoodii* grouped together, this is in agreement with [7], while Alaklabi [47] separated *A. ehrenbergiana* away from *A. johnwoodii* based on molecular characters investigated with nrDNA-ITS. So, the combined results of morphological and molecular attributes in this study concomitant together and solve this problems.

*Acacia farnesiana* and *A. senegal* were grouped together according to this study although, Al-Watban et al. [48] concluded that *A. farnesiana* was not closely related with *A. senegal* based on only morphological characteristics indicating that the reliance on only one tool for resolving the relationships among the studied taxa is not enough. Also, *A. cyclops* and *A. victoriae* grouped together this is not in agreement with [24] where they separate *A. victoriae* under distinct group based on two molecular markers examined with ITS and ETS, this may be due to the difference in the discrimination power of the markers that were used in both studies leading to the confirmation of that the more tools used, the more clearer the relationship.

The relationship between some taxa of *Acacia* s.l. of this study is with agreement with many previous studies. *A. etbaica* and *A. origena* found in one group this is in agreement with [49], *A. gerrardii var. gerrardii* and *A. gerrardii var. iraquensis* grouped together, this is in accordance with [50,51], *A. nilotica* subsp. *nilotica* and *A.
Figure 5. UPGAMA dendrogram based on morphological and molecular attributes of the studied taxa of genus *Acacia* s.l. Arrow heads indicating *Acacia* s.s. taxa, Asterisks indicating *Vachellia* taxa, Arrows indicating *Senegalia* taxa.

5. Conclusion

From this study, we can concluded that the comple-
mentary use of morphological and molecular data could
enable accurate identification of the studied taxa of *Acacia* s.l., in addition, the results of RAPD and ISSR markers
considered as powerful techniques for molecular char-
acterization, detecting genetic polymorphisms that can

nilotica subsp. tomentosa are in one clade [24,52] that
demonstrated this result. *A. tortilis* subsp. *tortilis* and *A.
tortilis* subsp. *raddiana* were grouped together in the pres-
ent study, this is in according with [11] and [53].
*A. coriacea* was segregated early followed by *A. ampli-
ceps*, while *A. ligulata* and *A. salicina* formed a sister taxa
this agree with [54]. *A. cuthbertsonii* and *A. sclerosperma*
grouped together this agree with [55].
discriminate between studied taxa and giving a clear view about the genetic diversity among them. The combined data gained from morphological and molecular traits succeeded for distinguishing and separated studied taxa into two major groups; *Acacia s.s.* group and *Vachellia* group that containing *Senegalia* taxa and give a valuable results that can be used for future taxonomical and genetic studies on genus *Acacia s.l.* so the segregation of *Acacia s.l* into *Senegalia* and *Vachellia* is confirmed.

**Acknowledgements**

The authors appreciate Agriculture Research Station (Riyadh, Saudi Arabia), Saudi Ministry of Environment, Water and Agriculture and seeds bank for supplying seeds of *Acacia*.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

The authors received no specific funding for this work.

**Author contributions**

U. K. A. and A. M. E. A designed the study, wrote the manuscript and analyzed the data. All authors performed experiments, validating and approved the manuscript for publication.

**Conflict of interest**

The authors declare that there is no conflict of interest at any level.

**ORCID**

Usama K. Abdel-Hameed [http://orcid.org/0000-0002-2804-4561](http://orcid.org/0000-0002-2804-4561)

Amal M. E. Abdel-Hamid [http://orcid.org/0000-0002-2013-604X](http://orcid.org/0000-0002-2013-604X)

**References**

[1] Kyalangalilwa B, Boatwright JS, Daru BH, et al. Phylogenetic position and revised classification of *Acacia s.l.* (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. Bot J Linn Soc. 2013;172(4):500–523.

[2] Maslin BR, Ho BC, Sun H, et al. Revision of *Senegalia* in China, and notes on introduced species of *Acacia*, *Acaciella*, *Senegalia* and *Vachellia* (Leguminosae: Mimosoideae). Plant Divers. 2019;41(6):353–480.

[3] Seigler DS, Ebinger JE, Miller JT. The genus *Senegalia* (Fabaceae: Mimosoideae) from the new world. Phytologia. 2006;88:38–93.

[4] Chaudhary SA, Al-Jowaid AAA. (1999). Vegetation of the kingdom of Saudi Arabia.

[5] Migahid AM. Flora of Saudi Arabia: University Libraries. 1996.

[6] Khan S, Al-Qurainy F, Nadeem M. Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. Saudi J Biol Sci. 2012;19(1):1–11.

[7] Al-Gohary IH, Mohamed AH. Seed morphology of *Acacia* in Egypt and its taxonomic significance. Int J Agric Biol. 2007;9:435–438.

[8] Mostafa EMA, Alkamali HH, Eltahir AS. Comparative anatomical study of some *Acacia* taxa seeds grown in central Sudan. Eur J Adv Res Biol Life Sci. 2017;5:36-44.

[9] Osman AK, Al-Ghamdi F, Bawadekji A. Floristic diversity and vegetation analysis of Wadi Arar: A typical desert Wadi of the Northern Border region of Saudi Arabia. Saudi J Biol Sci. 2014;21(6):554–565. doi:10.1016/j.sjbs.2014.02.001.

[10] Waly NM, Al-Zahrani HS, Felemban WF. Taxonomical studies of some *Acacia* seeds growing in Saudi Arabia. J Am Sci. 2012;8:264–275.

[11] Waly NM, Emad HM. Taxonomical studies of some *Acacia* spp. growing in Saudi Arabia. Bull Environ Pharmacol Life Sci. 2012;1:55–62.

[12] Zaman W, Ye J, Saqib S, et al. Predicting potential medicinal plants with phylogenetic topology: inspiration from the research of traditional Chinese medicine. J Ethnopharmacol. 2021;281:114515. doi:https://doi.org/10.1016/j.jep.2021.114515.

[13] Asma Ayazi TM, Zaman W, Saqib S, et al. Phylodignity and diversity of Lamiaceae based on RPS14 gene in Pakistan. Genetika. 2020;52(2):435–452.

[14] Vicente S, Mágicas C, Trindade H. Genetic diversity and differentiation of invasive *Acacia longifolia* in Portugal. Web Ecol. 2018;18(1):91–103.

[15] Pometti CL, Vilardi JC, Clal dialla AM, et al. Genetic diversity among the six varieties of *Acacia caven* (Leguminosae, Mimosoideae) evaluated at the molecular and phenotypic levels. Plant Syst. Evol. 2010;284(3–4):187–199.

[16] Abdel-Hamid AME. DNA fingerprint of *Alkanna tinctoria* subspecies in Misurata, Libya, Middle-East. J Sci Res. 2011:7-555–560.

[17] Mutharaian VN, Kamalakannan R, Mayavel A, et al. DNA polymorphisms and genetic relationship among populations of *Acacia leucophloea* using RAPD markers. J For Res. 2018;29(4):1013–1020.

[18] Alhasnawi AN, Mandal AM, Jasim HM. Using DNA fingerprinting to detect the genetic relationships in *Acacia* by inter-simple sequence repeat markers. J Pure Appl Microbiol. 2019;13(1):281–288. doi:10.22207/JPAM.13.1.30.

[19] Omondi SF. Evidence of genetic diversity and taxonomic differentiation among *Acacia Senegal*, Lamiaceae and species in Kenya based on randomly amplified polymorphic DNA molecular markers. E Afr Agri For J. 2004;50:18–40.

[20] Al-Samarai FR, Al-Kazaz AA. Molecular markers: An introduction and applications. Eur J Mol Biotechnol. 2015;9(3):118–130.

[21] Verma KS, ul Haq S, Kachhwaha S, et al. RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. 3 Biotech. 2015;9(3):288.

[22] Hosseini SK, Masoumiasl A, Dehdari M. A comparative analysis of RAPD and ISSR markers for assessing genetic diversity in Iranian populations of *Nigella sativa* L. Cell Mol Biol (Noisy-Le-Grand. 2018;64(1):52–59.

[23] Rohela GK, Jogam P, Bylla P, et al. Indirect regeneration and assessment of genetic fidelity of acclimated plantlets by SCoT, ISSR, and RAPD Markers in *Rauwolfia tetraphylla* L.: An endangered medicinal plant. Biomed Res Int. 2019;1:1-4.

[24] Sulayli AL, Moustafa MF, Eid EM. Genetic variability, antimicrobial activity and natural water-soluble vitamins
contents of five *Acacia* species growing in Jazan region, Saudi Arabia. Pakistan J Agric Sci. 2019;56:289–300.

[25] Collenette S. (1999). Wildflowers of Saudi Arabia, National Commission for Wildlife Conservation and Development (NCWCD).

[26] Chaudhary SA. Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and water. Riyadh, 2001;2:342–354.

[27] Murley MR. Seeds of the Cruciferae of northeastern North America. Am Midl Nat. 1951:1–81.

[28] Barthlott W. (1984). Microstructural features of seed surfaces.

[29] Javadi F, Yamaguchi H. RAPD and seed coat morphology variation in annual and perennial species of the genus *Cicer* L. Genet Resour Crop Evol. 2004;51(7):783–794.

[30] Salimpour F, Ebrahimiyan M, Sharifnia F. Micropophyllum morphology in selected species of salvia (Lamiaceae) in Iran. Res J Biol Sci. 2014;9:92–97.

[31] Williams JGK, Kubelik AR, Livak KJ, et al. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531–6535.

[32] Dangi RS, Lagu MD, Choudhary LB, et al. Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers. BMC Plant Biol. 2004;4(1):13.

[33] Sneath PHA, Sokal RR. (1973). Numerical taxonomy. The principles and practice of numerical classification.

[34] Rohlf FJ. Numerical taxonomy and multivariate analysis system version 2.1. New York: Exeter Publishing Setauket; 2000.

[35] Li G, Quiros CF. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet. 2001;103(2–3):455–461.

[36] Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20(2):176–183.

[37] Nagaoka T, Ogihara Y. Applicability of inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Theor Appl Genet. 1997;94(5):597–602.

[38] Abdel-Hamid AME. Micromorphological and genetic molecular variations in some taxa of asteraceae and its importance as grazing plants. Pak J Bot. 2020;52:37.

[39] Farajpour M, Ebrahimi M, Amiri R, et al. Study of genetic variation in yarrow using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Afr J Biotechnol. 2011;10:11137–11141.

[40] Patel DM, Fouqat RS, Sakure AA, et al. Detection of genetic variation in sandalwood using various DNA markers. J Biotech. 2016;61(1):55.

[41] Mustafa AM, Badr A, El-Galaly MA, et al. Genetic diversity among *Mentha* populations in Egypt as reflected by isozyme polymorphism. Int J Bot. 2005;1:188–195.

[42] Sirelkhatem R, Gaali EEL. Phylogenetic analysis in *Acacia Senegal* using AFLP molecular markers across the gum Arabic belt in Sudan. African J Biotechnol. 2009;8:4817–4823.

[43] Jindal SK, Tak A, Singh SK, et al. (2011). Molecular assessment of genetic diversity in *Acacia senegal*.

[44] Badr A, Morsy W, Abdelfattah S, et al. Genetic diversity in Artemisia monosperma and *Artemisia judaica* populations in Egypt based on morphological, karyological and molecular variations. J Med Plants Res. 2012;6:66–78.

[45] Abdel-Hamid AME. Characterization of four *salsola* species and their genetic relationship by afpl. Pak J Bot. 2016;48:1183–1187.

[46] Yadav JP, Yadav A, Kumar M, et al. Validation and transferability of simple sequence repeats (SSRs) from some species of *Acacia* genus to *Acacia nilotica* (L.). Int J Pharm Pharm Sci. 2016;8(11):95–101.

[47] Alaklabi A. Phylogenetic study of *Acacia* species using the molecular marker. Am J Plant Sci. 2015;06(19):3139.

[48] Al-Watban AA, Al-Mogren E, Doaigey AR, et al. Pollen morphology of seven wild species of *Acacia* in Saudi Arabia. African J Plant Sci. 2013;7(12):602–607.

[49] Ahmed SM, Al-Sodany YM. Preliminary authentication of some *Acacia* L. species in Taif highlands. Egypt J Bot. 2015;55(1):161–174.

[50] Hosny AM, Shawky RA, Hashim AA. Size structure and Floristic diversity of *Acacia* trees population in Taif area, Saudi Arabia. J Biodivers Endanger Species. 2018;06(01):2.

[51] Abdel-Hamid AME, Elenazy HH, Abdel-Hameed UK. DNA barcoding of some taxa of genus *Acacia* and their phylogenetic relationship. All Life. 2021;14(1):588–598. doi:10.1080/26895293.2021.1938702.

[52] Ndoye-Ndir K, Samb PI, Chevallier M-H. Genetic variability analysis of the polyploid complex of *Acacia nilotica* (L.) willd. using RAPD markers. Tropic culture. 2008;26:135–140.

[53] T.K. Khan, Mcrphological And Comparative Anatomical Studies On Some Species Of Acacia (Fabaceae) Growing In Wadi Fatma In Makkah Al-Mukaramah Region.

[54] Joseph S, Bhave M, Miller JT, et al. Rapid identification of *Acacia* species with potential salt tolerance by using nuclear ribosomal DNA markers, sustain. Agric Res. 2013;24(2):77–86.

[55] Aref IM, El-Juhany LI. Impact of sudden water stress on the growth of eight *Acacia* species. Alexandria Sci Exch. 2001;22:413–422.