BIODIVERSITY OF ARTEMISIA SPECIES FROM SAUDI ARABIA BASED ON MORPHOLOGICAL AND MOLECULAR MARKERS

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Abstract. Artemisia has a diverse range of uses, most studies have focused on the chemical nature of several species. Our study aims to evaluate the phylogenetic relationship between 7 populations of Artemisia, representing 4 species, using morphological variation and molecular polymorphism generated by 8 ISSR primers. We observed significant variations in 18 morphological characters, which was reflected in the cluster analysis of the examined accessions based on morphological variation. The phylogenetic relationships generated based on ISSR polymorphism and morphological variations indicated a close relationship between *A. judaica* and *A. abyssinica*, and between *A. monosperma* and *A. scoparia*, where each pair of species is separated in one cluster. Meanwhile, the two populations of *A. abyssinica* were highly diverse compared to the two populations of *A. judaica*. *Artemisia scoparia* species is more related to *A. monosperma* but at a low similarity level. These results were supported by morphological variations and the molecular marker individually and combined. Additionally, the tree obtained based on the Elucedine coefficient, and the population grouping based on principal component analysis (PCA) using the PAST program confirmed these results. The iMEC tool (Online Marker Efficiency Calculator) can help to analyze genetic diversity in Artemisia populations using ISSR markers.
Keywords: *ISSR markers, phylogenetic relationship, iMEC analysis, medicinal plants, Asteraceae*

Introduction

Artemisia species are a diverse group of plants that belong to the Asteraceae family. They are widely dispersed across the Northern Hemisphere, with a few species also found in the Southern Hemisphere (Kim et al., 2020). These plants can be found in arid and semi-arid habitats, with Western and Central Asia being considered their center of origin in the Northern Hemisphere (Numonov et al., 2019). The genus Artemisia is known for its high genetic diversity, with an estimated 200-500 taxa at the specific or subspecific level.

There are five species of Artemisia plants in Saudi Arabian flora, namely *A. herba-alba* Asso., *A. judaica* L., *A. monosperma* Del., *A. abyssinica* Schultz-Bip., and *A. scoparia* Waldst. et Kit (Migahid, 1996). Out of these, the final four species were chosen for a study on their genetic diversity based on their morphological variety and molecular polymorphism as indicated by ISSR markers as well as, their prevalence in the central and northern regions of the country. These plants are commonly used in traditional medicine in Saudi Arabia as well (Shanawany, 1996).

An infusion of *Artemisia judaica* flowering branches relieves gastrointestinal cramps, upset stomach, abdominal disturbances, and constipation, and is used as a stomachic, anthelmintic, expectorant, diaphoretic, analgesic, and antispasmodic in cases of intestinal colic. This species' volatile oil has antibacterial properties (Batanouny, 1999). The species *A. judaica* has been found to have various beneficial properties. One of its components, the flavone cirsimaitin, has been shown to reduce the amplitude of phasic contractions and tone of the ileum in guinea-pigs in a concentration-dependent manner in a study conducted by Abdalla and Abu Zarga (1987). Additionally, the volatile

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components of *A. judaica* have demonstrated antioxidant activity in a study conducted by El-Masry et al. (2002). Furthermore, preparations of *A. judaica* aerial parts have demonstrated anti-diabetic activity in a study conducted by Nofal et al. (2009).

According to studies by Escudero et al. (2000) and Zeng et al. (2009), *Artemisia herba* alba and *A. judaica* have shown allelopathic properties, which means they release chemicals that inhibit the growth of other plants around them. On the other hand, *A. monosperma* is commonly used in traditional medicine. Its aerial parts are used to treat coughs, colds, rheumatic pain, and stomach pain, as mentioned by Shanawany (1996).

There is a high level of genetic diversity within the genus Artemisia, particularly at the specific and subspecific levels (Shevchuk et al., 2021). Recent studies have highlighted the use of molecular markers in assessing the genetic diversity of Artemisia species. Molecular techniques, such as ISSR markers, have been employed to analyze the genetic variations among different populations of Artemisia. These markers provide valuable information about the DNA sequences and allow for a comprehensive understanding of the genetic diversity within the genus.

The combination of both morphological and molecular data has proven to be beneficial in classifying Artemisia species. The results obtained from morphological variations and molecular analyses have shown a significant correlation, indicating that clustering based on morphological parameters is associated with those derived from molecular data analysis (Kolören et al., 2016).

In recent years, DNA fingerprinting has been increasingly used to document genetic diversity and to protect rare, endangered, or overused medicinal plant species offering a promising approach. Our article aims to study the biodiversity of four Artemisia species based on analysis of their morphological traits and molecular markers collected from the southwestern region of Saudi Arabia. The species selected for the study are of medical importance and the results may contribute to conservation efforts of endangered populations of the studied species in the study area.

Materials and methods

Plant material and morphological measurements

Mature plant samples from seven accessions representing four Artemisia species (five samples for each accession) were collected from natural habitats in the Asir region in the highlands of southwestern Saudi Arabia. The names and collection locations of the collected accessions/species are given in *Table 1* and shown on a map of the study region (*Fig. 1*). *Figure 2* shows photographs of the studied species. A detailed description of the 18 morphological traits, including quantitative and qualitative traits, for each collected accession was made, and the mean ± standard deviation of each quantitative trait was calculated, and the qualitative traits were calculated based on the species description. status was recorded based on Collenette (1999) and Chaudhary (2001) are shown in *Table 2*. The seven accessions/species voucher specimens were deposited in the herbarium of the Faculty of Biological Sciences, Faculty of Sciences and Humanities, Shakra University, Al Dawadimi, Saudi Arabia.

DNA extraction and ISSR fingerprinting

DNA was extracted and purified from young leaves of mature plants of the collected samples representing all accessions/species using the Qiagen DNeasy™ Plant Minikit

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according to the manufacturer's protocol (Qiagen Inc, Valencia, CA, and USA). Eight ISSR (Inter Simple Sequence Repeats) primers were reserved for DNA fingerprinting. A total of 25 µl reaction mixture was prepared for the genomic DNA amplification reaction (12.5 µl Thermo Scientific Maxima Hot Start PCR Master Mix (2X), 0.5 µl primers, 0.5 µl template DNA, and 11.5 µl nuclease-free water-R0581). Amplification conditions were improved using a gradient thermal cycler from Biometra Uno, Germany. 20 µL of PCR product of each primer and 2 µL of loading buffer were mixed and loaded into a well of a 1.7% agarose gel. ISSR fingerprints were viewed and photographed using a Gel Works 1D Advanced Gel Documentation Device (UVP, UK) according to Alqahtani (2023). *Figure 3* shows the ISSR fingerprint profiles generated by four ISSR primers for the studied Artemisia accessions. The unique and clear ISSR band has been recorded. For data analysis, each ISSR band was considered as a single location and scored as 1 for presence and 0 for absence. The names, sequences, number of polymorphic bands, and polymorphism percentages of the eight ISSR primers are shown in *Table 3*.

Table 1. *The area, GPS coordinates, and elevation of the locations where the analyzed Artemisia species accessions were collected*

Serial	Collected accessions	Area	GPS location	Elevation (m) asl
1	*Artemisia judaica*$_{(1)}$	Abha	N 18° -14′-3.86" E 42° -32′-12.697"	2155
2	*Artemisia judaica*$_{(2)}$	Najran	N 17° -29′-25.7" E 44° -8′-12.509"	1309
3	*Artemisia scoparia*	Abha	N 18° -12′-54.303" E 42° -31′-6.211"	2237
4	*Artemisia abyssinica*$_{(1)}$	Abha (Elsoda)	N 18° -16′-18.235" E 42° - 23′- 2.406"	2784
5	*Artemisia abyssinica* $_{(2)}$	Najran	N 17° -29′-25.7" E 44° -8′-12.509"	1309
6	*Artemisia monosperma*$_{(1)}$	Jazan	N 16° -58′-25.954" E 42° -32′-36.98"	0
7	*Artemisia monosperma*$_{(2)}$	Jazan	N 16° -53′-51.698" E 42° -34′-18.765"	3

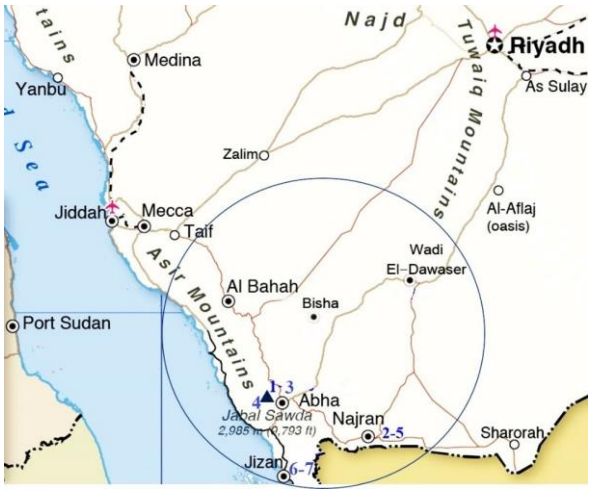

Figure 1. *Map of southwest Saudi Arabia illustrating the sites of collection of the studied Artemisia species/accessions (1–7); plotted and coded as given in Table 1*

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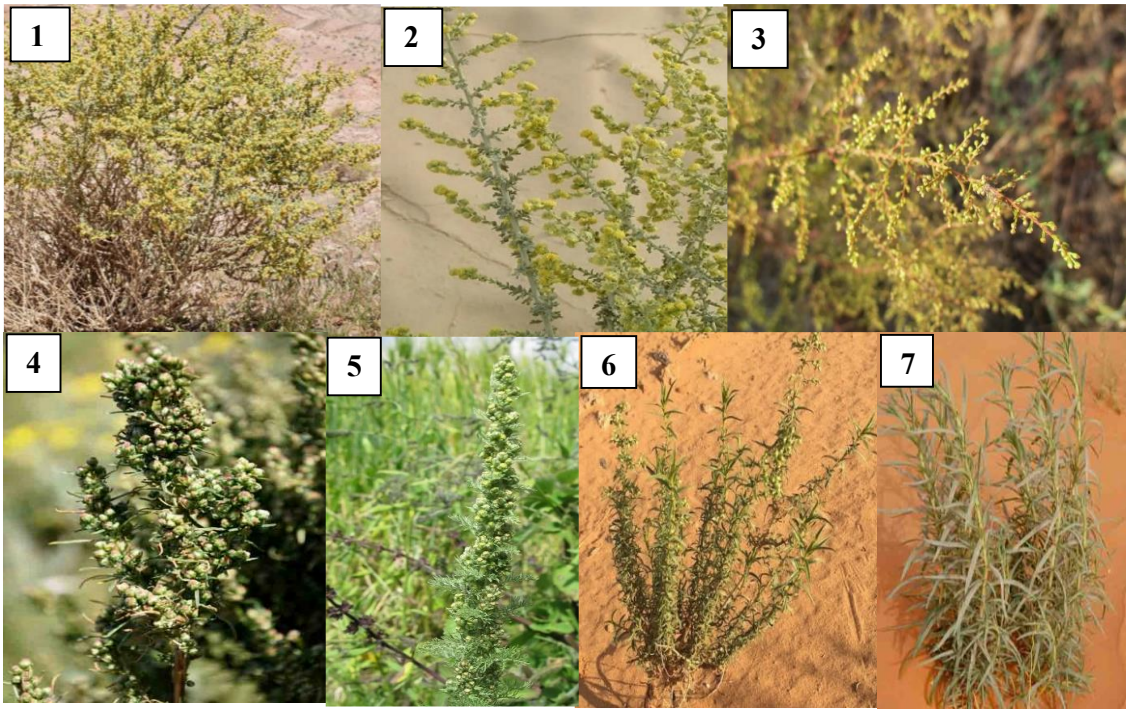

Figure 2. *Collected Artemesia species/accessions (1–7) from southwest Saudi Arabia coded as given in Table 1*

Table 2. *A list of morphological traits and their measurements and state of the examined 7 Artemisia accessions representing four species (1 –7); coded as given in Table 1*

Ser.	Character	*Artemisia* species/accession						
		***A. judaica*$_{(1)}$**	***A. judaica*$_{(2)}$**	***A. scoparia***	***A. abyssinica*$_{(1)}$**	***A. abyssinica*$_{(2)}$**	***A. monosperma*$_{(1)}$**	***A. monosperma*$_{(2)}$**
1	Plant height (cm)	$65 \pm 1.53^{***}$	$54 \pm 5.86^{**}$	37 ± 1.15	$71 \pm 7.64^{***}$	$58 \pm 4.93^{*}$	$89 \pm 3.06^{**}$	$98 \pm 4.04^{***}$
2	Plant habit	Shrub	Shrub	Herb	Herb	Herb	Shrub	Shrub
3	Stems texture	Hairy	Hairy	Smooth	Hairy	Hairy	Smooth	Smooth
4	Habit of the stem	Herbaceous	Herbaceous	Herbaceous	Herbaceous	Herbaceous	Herbaceous	Herbaceous
5	Stem color	Pale green	Pale green	Pale brown	Dark brown	Dark brown	pale green	Pale green
6	Leaves color	Pale green	Pale green	Pale green	Dark brown	Dark brown	Dark green	Dark green
7	Leaves shape	Lobed	Lobed	Lobed	Lobed	Lobed	Lobed	Lobed
8	Leaf margin	Entire	Entire	Sinuate	Entire	Entire	Sinuate	Sinuate
9	Leaf distribution	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate
10	Leaf apex	Blunt	Blunt	Blunt	Blunt	Blunt	Blunt	Blunt
11	Leaf surface	Wooly	Wooly	Smooth	Wooly	Wooly	Hairy	Hairy
12	Shape of leaf lobes	Oblong	Oblong	Liner	Liner	Liner	Oblong	Oblong
13	Number of leaf lobes	$8 \pm 0.54^{***}$	$6 \pm 0.34^{**}$	5 ± 0.39	$12 \pm 0.57^{***}$	$9 \pm 0.37^{**}$	$7 \pm 0.64^{***}$	$5 \pm 0.24^{**}$
14	Flower inflorescence	Spikes	Spikes	Spikes	Spikes	Spikes	Spikes	Spikes
15	Flower width (mm)	$5 \pm 0.24^{***}$	$4 \pm 0.32^{***}$	2 ± 0.02	$4 \pm 0.44^{***}$	$3 \pm 0.29^{**}$	$1 \pm 0.22^{***}$	$1 \pm 0.12^{***}$
16	Flower color	Yellow	Yellow	Green	Brown	Brown	Green	Green
17	Aromatic odor	Strong	Strong	Non	Strong	Strong	Weak	Weak
18	Seeds color	Yellow	Yellow	Pale yellow	Brown	Brown	Pale yellow	Pale yellow

Values expressed as means ± SD of five plant tissues in each group ** $P < 0.01$ and $^{***}P < 0.001$

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Table 3. *The number of polymorphic bands and percentage of polymorphism in the ISSR profile of 8 primers in the genome of collected Artemisia species/populations*

Serial	Primer code	Sequence (5'→3')	Number of polymorphic bands	Number of unique bands	Total number of bands	Polymorphism percentage (%)
1	HB-11	$(GT)_6CC$	14	3	17	100
2	HB-12	$(CAC)_3GC$	8	5	13	100
3	HB-13	$(GAG)_3GC$	8	1	9	100
4	HB-14	$(CTC)_3GC$	6	3	10	90
5	HB15	$(GTG)_3GC$	4	0	5	80
6	807	$(AG)_8T$	12	0	13	92.3
7	808	$(AG)_8C$	7	2	9	100
8	814	$(CT)_8TG$	5	1	10	60
Total			**69**	**15**	**86**	-

Data analysis

The morphological features were assigned codes ranging from 0 to 3 for data numerical analysis. The differences in morphological characteristics and molecular fingerprinting, either separately or in combination, were used to evaluate the connections between Artemisia accessions and species. Two software tools were used to analyze the data: NTSYS-pc (Rohlf, 2002) was used to create trees that explained the relationships between the studied Artemisia population/species and determine the degree of similarity between them using a simple matching coefficient (SM) (Sokal and Michener, 1958). In order to create a distance tree using PAST-pc Version 3.22, the evaluated accessions and species were additionally clustered based on squared Euclidean distance (Hammer et al., 2001). Additionally, a scatter diagram of the accessions under investigation was created using the Principal Component Analysis (PCA) technique in the PAST-pc. Frequently, the PCA is utilized to allocate variables to genotypes and categorize them according to their susceptibility or resilience to drought stress. In the PCA scatter plotting representation, PCA is sensitive to the original variables' relative scale (Hammer et al., 2001). Each morphological measurement was carried out in triplicate independently, and the results are shown as the mean ± standard deviation (SD). ANOVA post-hoc test analysis using Statistica 7.1 (Statsoft, 2007) was applied to the quantitative morphological characters only.

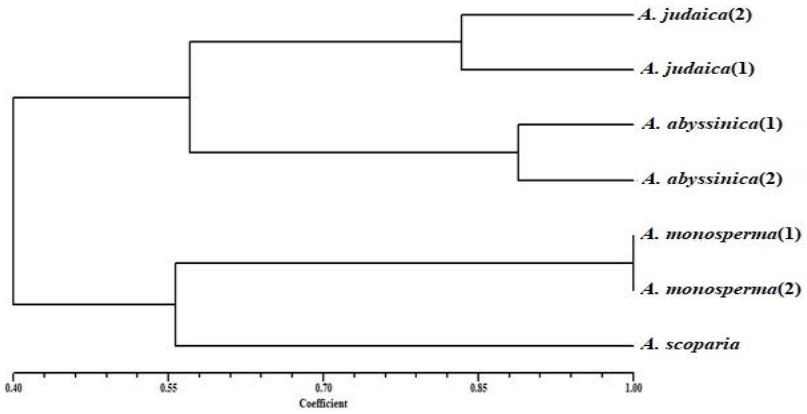

Figure 3. *UPGMA, NTSYS-pc distance tree, based on the analysis of morphological traits, computed with SM coefficient, showing the relationships among the examined Artemisia accessions*

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iMEC analysis

Eight ISSR primers were analyzed using the online iMEC software tool, using seven genotypes of Artemisia. Overview of the resulting computations is given in *Table 4*. The D parameter, or discriminating power of primer, was used to evaluate how well the primers distinguished between various Artemisia genotypes; Ahmed et al. (2019) reported this parameter. Amiryousefi et al. (2018) state that the Online Marker Efficiency Calculator (iMEC software) is a user-friendly application that calculates the following seven fundamental polymorphism indices for individual markers: resolving power (R), arithmetic mean heterozygosity (H_{avp}), discriminating power (D), heterozygosity index (H), effective multiplex ratio (E), and polymorphism information content (PIC). You may access the iMEC application at https://irscope.shinyapps.io/iMEC/.

Table 4. *Polymorphism statistics were estimated using the iMEC tool for 8 ISSR primer types using the data set from the 7 Artemisia genotypes*

Serial	Primer code	Total number of bands	H	PIC	E	Rp	H.av	MI	D
1	HB-11	17	0.4872	0.3812	7.1428	9.7142	0.00409	0.0292	0.8255
2	HB-12	13	0.4782	0.3856	5.1428	7.1428	0.00525	0.0270	0.8461
3	HB-13	9	0.4998	0.3750	4.4285	5.4285	0.00793	0.0351	0.7619
4	HB-14	10	0.4995	0.3752	5.1428	4.5714	0.00713	0.0367	0.7391
5	HB15	5	0.4310	0.4071	3.4285	1.7142	0.01231	0.0422	0.5361
6	807	13	0.4999	0.3750	6.4285	7.4285	0.00549	0.0353	0.7582
7	808	9	0.4968	0.3765	4.1428	4.5714	0.00788	0.0326	0.7921
8	814	10	0.4362	0.4048	8.1428	4.0000	0.00519	0.0422	0.5421
Average			**0.4786**	**0.3850**	**5.5001**	**5.5713**	**0.00691**	**0.0350**	**0.7251**

Heterozygosity index (H); Polymorphic information content (PIC); Effective multiplex ratio (E) Arithmetic mean of H (H.av); Marker index (MI); Discriminating power (D); Resolving power (Rp)

Results

Morphological variation among Artemisia accessions/species

Table 2 shows the results of scoring eighteen morphological traits for seven collected Artemisia accessions. The qualitative morphological traits displayed noteworthy differences among the species, whereas there were only a few dissimilarities within the species. and few differences within species, particularly in stem color, leaf color, leaf surface, stem texture, shape of leaf lobes, and flower color (*Fig. 2*). On the other hand, quantitative traits showed that accessions collected from Abha at high elevation and moderate temperatures generally have larger plant size and highest leaf lobes number compared to accessions from lower elevations in more arid areas. For instance, *A. judaica*$_{(1)}$ that was collected from Abha at an elevation of 2155 m asl had a plant height of 65 ± 1.53 cm and leaf lobes number of 8, while the same species collected from Najran at an elevation of 1309 m asl had a plant height of 54 ± 5.86 cm and leaf lobes number of 6 (*Table 2*). Similarly, *A. abyssinica*$_{(1)}$ collected from Abha region (Elsoda) at an elevation of 2784 m asl had a plant height of 71 ± 7.64 cm and leaf lobes number of 12, while the other population collected from Najran at an elevation of 1309 m asl had a plant height of 58 ± 4.93 cm and leaf lobes number of 9. The shorter species, *A. scoparia*, was collected from Abha (37 ± 1.15 cm) at 2237 m asl and leaf lobes number 5. Meanwhile, the taller species such as *A. monosperma*$_{(1)}$ and *A. monosperma*$_{(2)}$ had plant heights of 89 ± 3.06 cm and 98 ± 4.04 cm, respectively, for material collected from Jazan (*Table 2*).

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Diversity based on morphological variations

Biodiversity based on variations in morphological characters and using a simple matching coefficient, the UPGMA-NTSYS-pc phylogenetic tree (as shown in *Fig. 3*) has successfully segregated the seven carefully examined accessions into two distinct clusters. The first cluster is comprised of two accessions of *A. monosperma*, which were found to have a high similarity level with the accession of *A. scoparia*, having a lower similarity level. The second cluster consists of two species, namely *A. abyssinica* and *A. judaica*. Within this cluster, the two accessions of *A. abyssinica* were observed to cluster at a higher similarity level than the two accessions of *A. judaica*.

ISSR fingerprinting polymorphism in pteridophytes accessions

Data generated by the ISSR fingerprinting pattern of seven Artemisia accession/species were analyzed and the ISSR profiles produced by eight ISSR primers are shown in *Figure 4*. ISSR profiles produced 86 bands; 64 bands were polymorphic, seven were monomorphic, and 15 were unique. The highest number of bands (17) was produced by primer HB-11, and the lowest number (5) was produced by the two primer HB-15. The percentage of polymorphism of all primers was calculated and given in *Table 3*.

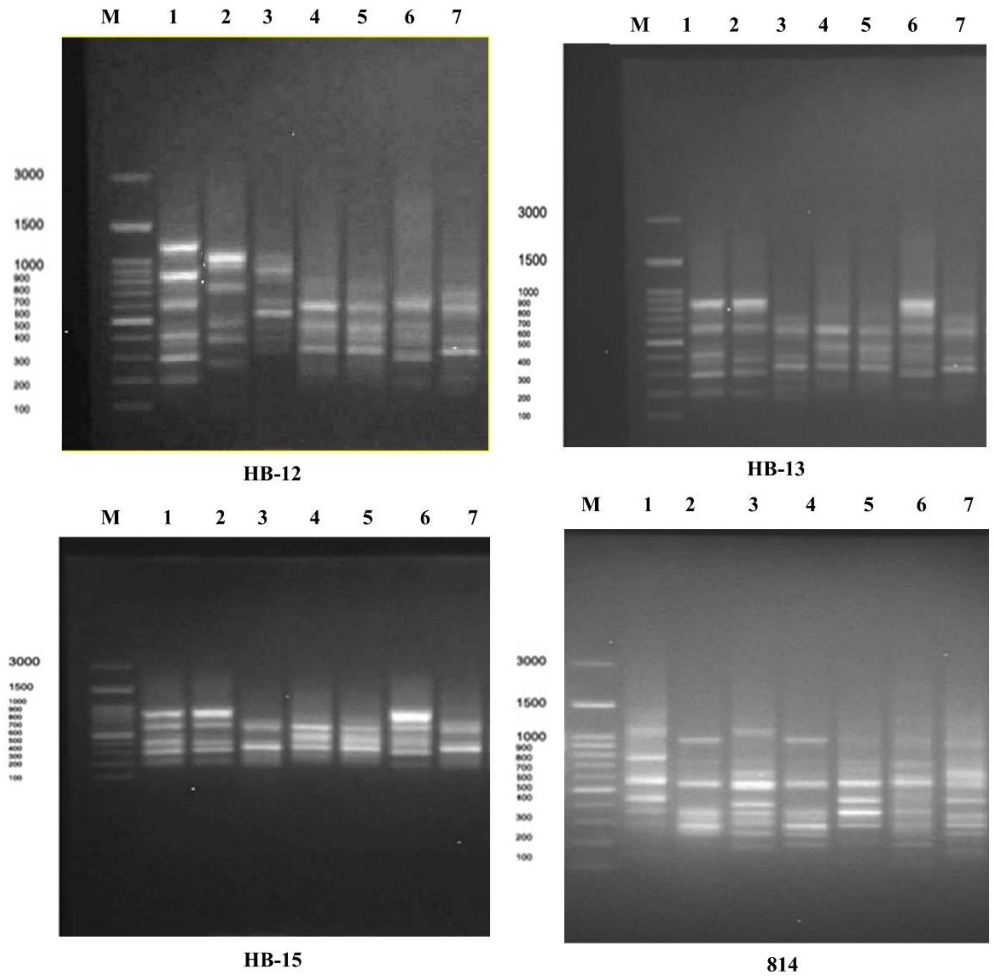

Figure 4. *ISSR fingerprinting profile produced by four ISSR primers for Artemisia accessions as coded in Table 1. *M: 100 bp marker DNA ladder*

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The iMEC software was used to determine the polymorphism indices of individual primers, which served as fundamental metrics. More detailed information regarding the fundamental measurement polymorphism indices for the primers can be found in *Table 4*. The heterozygosity index (H) had an average value of 0.4786. The polymorphism information content (PIC) for each primer had an average of 0.385. The effective multiplex ratio (E) ranged from 3.42 (recorded with the primer HB-15) to 8.14 (recorded with the primer 814), with an average of 5.5. The primer resolving power (R) varied from 1.71 (for HB-15) to 9.71 (for HB-11), with an average of 5.57. The arithmetic means H (Havp) ranged from 0.004 (for HB-11) to 0.012 (for HB-15), with an average of 0.00691. The lowest marker index (MI) value was obtained with primer HB-12, with a range of 0.0270 to 0.0422 (for HB-15) and an average of 0.0350. The primer discriminating power (D) ranged from 0.5361 (for HB-15) to 0.8461 (for HB-12), with an average of 0.7251.

Diversity based on ISSR markers

The ISSR fingerprinting analysis-based phylogenetic tree (as illustrated in *Fig. 5*) showcased that the Artemisia species and accessions can be classified into two primary groups. The first group contains *A. scoparia*, which is separated from the two accessions of *A. monosperma* at a relatively low similarity level. However, the two accessions of *A. monosperma* are clustered together at a relatively high similarity level. The second group shows a high similarity level between the two accessions of *A. judaica*, which are clustered with the *A. abyssinica*$_{(2)}$ accession in one group. However, the other accession (*A. abyssinica*$_{(1)}$) is delaminated from this group.

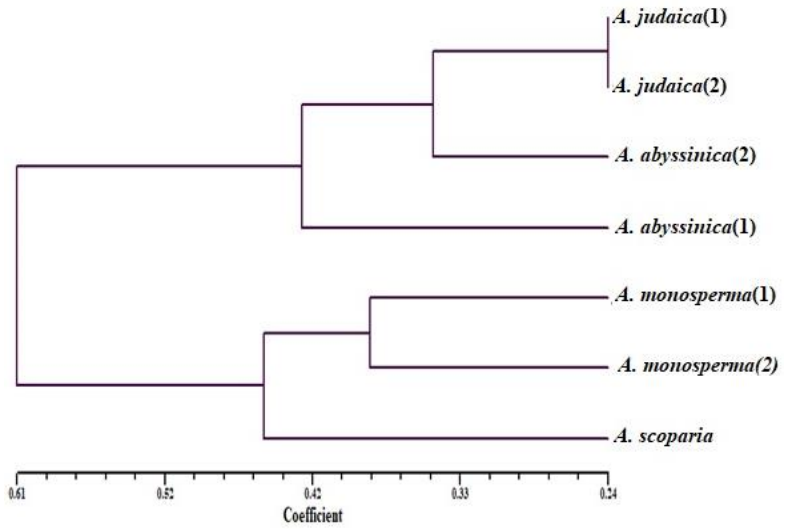

Figure 5. *UPGMA distance tree computed using the NTSYS-pc, based on the analysis of ISSR data, showing the relationships among the examined Artemisia accessions*

Relationships of species based on morphological variations in conjunction with ISSR markers

The diversity of accessions/species was assessed based on morphological variations and ISSR marker polymorphism. This analysis was done using clustering analysis based on the Euclidean equation and PCA scatter plot using PAST software. The tree, shown

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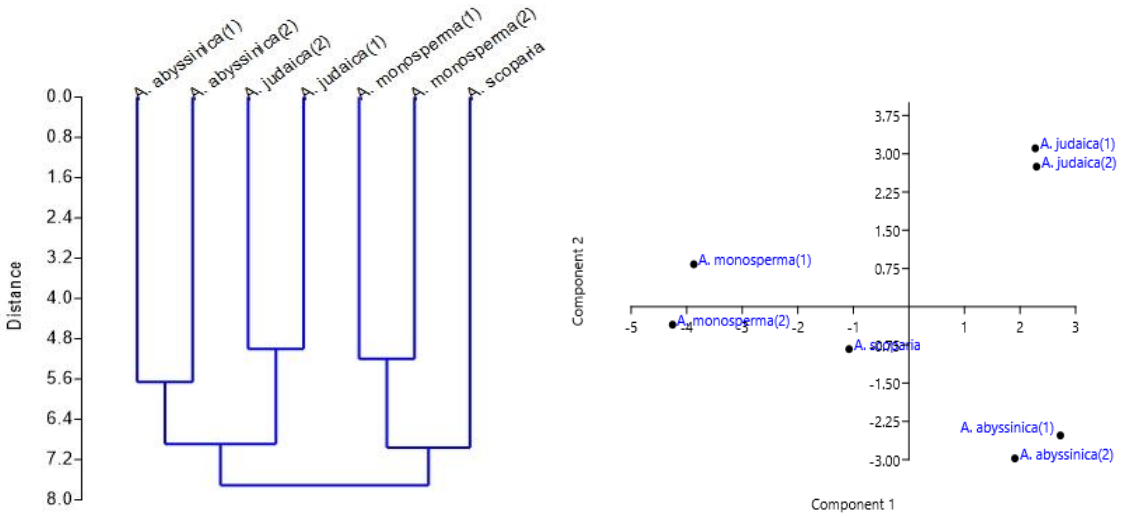
in *Figure 6a*, revealed more differentiation between the under-examined three species, while seven Artemisia accessions were divided into two groups. In the first group, there were two *A. judaica* accessions with the highest similarity level and two *A. abyssinica* accessions with relatively high similarity level. In the second group, there was a high similarity level between the two accessions of *A. monosperma*, along with *A. scoparia*, which was separated from this group at a low similarity level.

The PCA scatter plot (*Figure 6b*) showed that the seven accessions/species were clearly divided into three clusters, which were in agreement with their separation in the cluster tree. The first group included *A. judaica* accessions; the second group consisted of two accessions of *A. abyssinica*, while the third group contained the two accessions of *A. monosperma* and *A. scoparia*.



(a) **(b)**

Figure 6. *UPGMA distance tree (a) and a PCA scatter diagram of the examined species (b), constructed using the PAST-pc software showing the relationships among the examined Artemisia accessions based on the analysis of variation in the morphological traits and ISSR fingerprinting polymorphism*

Discussion

Genetic variation is crucial for effective conservation and preservation of endangered plant species, as well as for developing genetic diversity databases of plant genetic resources. Many medicinal plants in arid and semi-arid regions like Saudi Arabia face the threat of extinction due to poor regeneration under common environmental stresses such as drought and salinity, as well as overuse, grazing, and other human activities. However, there is limited research on conservation genetics for these plants. Our study aims to assess the genetic diversity of certain Artemisia species from southwest Saudi Arabia based on both morphological variations and molecular markers. A close infra-specific relationship is evident among populations representing the same species of Artemisia in the study area.

The collected *A. scoparia* species was found to be in one group with *A. monosperma* alba at a low similarity level. *A. judaica* and *A. abyssinica* were also separated as two independent groups. The morphological characters that were found differentiating the species are leaf characters (color, margin, surface, shape of leaf lobes, number of leaf

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lobes), the aromatic fragrance, Flower width, Flower color, and seed color. The variation in these qualitative characters in addition to the variation in quantitative characters differentiated *A. scoparia* and *A. monosperma* from the other two Artemisia species.

The analysis of morphological criteria reflects the differences among the populations of the four species of Artemisia in the study area. The variations among populations of the same species are mainly due to differences in the measured quantitative characteristics such as shoot length, number of leaf lobes, and Flower width.

Figure 3 shows the relationship between the examined four Artemisia species based on the morphological variations where the populations of *A. judaica* showed more variation between each other compared to those of *A. monosperma* and *A. abyssinica*. These variations may be related to the collection sites and their elevation. It has been observed that there is a wide range of morphological variations among different populations of *A. vulgaris* in Canada. Within a single population, plants show variations in leaf morphology, length, and branching habits (Barney and DiTommase, 2003). Similar morphological variations have also been noted in *A. vulgaris*, *A. roxburghiana*, and *A. absinthium* populations in Iran (Nazar and Mahmood, 2010). *A. vulgaris* displays a high level of infraspecific diversity compared to the other two species, which indicates the extreme morphological variations that occur in *A. vulgaris* populations.

In their study, Ahmed et al. (2019) and Amiryousefi et al. (2018) used molecular markers to calculate the polymorphic information content (PIC), which measures the discriminatory ability of a genetic marker. PIC and heterozygosity (H) are two indicators of the effectiveness of polymorphism as a genetic marker. For binary data, the maximum value of H and PIC is 0.5, assuming two alleles per locus. For codominant markers, these values range from 0 to 1 and are affected by the frequency and number of alleles. Our research indicates that the PIC value is a measure of a locus' discriminatory power, considering both the number of alleles and their relative frequencies. PIC values range from 0 (monomorphic) to 1 (extremely discriminative, with many alleles occurring in equal frequency). We used iMEC software to calculate the polymorphism indices for individual primers as basic measurements. *Table 4* contains more information about the primers' basic measurements and polymorphism indices.

The term PIC refers to the probability of detecting polymorphism, which occurs when a primer/primer combination is used to compare two individuals randomly selected. The probability depends on the distribution and quantity of detectable alleles. These findings are important for breeders as they provide reliable diversity sources that help them assess genetic diversity and interactions between different genotypes. The study also demonstrates that ISSR is an efficient, and convenient method to examine genetic diversity and interactions across flax genotypes. ISSR-PCR is an excellent marker for analyzing genetic variation, and it has been successfully used to establish genetic similarities in many other plants as well (Alqahtani, 2020).

The diversity based on analysis of the ISSR marker supports the separation of understudy Artemisia species/accessions based on the morphological traits but at different similarity levels. Where ISSR markers are not differentiated between the two accessions of *A. judaica* which they separated at a high similarity level in one cluster reflecting a close relationship between the two accessions of *A. judaica* which they are collected from different sites. The separation of the two accessions of *A. judaica* and the two accessions of *A. abyssinica* indicate genetical relations between the two species. These results agree with Yifru, et al. (2022) who studied the phylogenetic relationship between four species of *Artemisia* in Ethiopia based on sequencing of ITS and ETS

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regions, their results approved the presence of *A. abyssinica* and *A. judaica* in the same clade. Also, our results do not agree with Badr et al. (2012) who studied the molecular biodiversity of some accessions of Artemisia including three species *A. judaica, A. monosperma,* and *A. herba alba* based on variations in RAPD markers. Their results indicate sharp polymorphism among populations of both *A. judaica* and *A. monosperma* which they separated as two independent groups. In our study, the separation of *A. judaica* and *A. monosperma* species in two different clusters is not supported by Badr et al. (2012).

Also, our results are congruent with the taxonomic delimitation of *A. monosperma* in subgenus Dracunculus and of *A. judaica* in subgenus Artemisia (Torrell and Valle`s, 2001; Pellicer et al., 2011). The presence of *A. monosperma* and *A. scoparia* species in one cluster confirms the relationship between the two species. This result agrees with Pellicer et al. (2011) who studied the phylogenetic relationship between a large sample of Artemisia species based on sequencing of ribosomal and chloroplast DNA. Their results indicate a taxonomic relationship between *A. monosperma* and *A. scoparia.* These results are also confirmed by the tree generated based on the analysis of morphological and ISSR data (*Fig. 5)*.

The PCA scatter plot, which was generated using the Elucedine coefficient based on the analysis of morphological trait variations and ISSR fingerprinting polymorphism (*Fig. 6B*), confirmed the grouping of the collected Artemisia species and accessions.

Conclusion

The phylogenetic relationship based on molecular ISSR profiles and morphological variations indicates the taxonomic relationship between the examined four artemisia species *A. judaica, A. abyssinica, A. monosperma,* and *A. scoparia.* The results clearly indicate that *A. abyssinica* is more polymorphic than the other three species. This may be attributed to the larger distribution area of this species in the flora of Saudi Arabia. Geographic and local ecological variations may be regarded to have played a role in the genetic diversity of the examined populations of Artemisia species in the study area of Saudi Arabia. The results showed a close relationship between *A. judaica, A. abyssinica,* and between *A. monosperma,* and *A. scoparia*.

Our findings support the use of ISSR markers as a quick, easy, and affordable method for examining genetic diversity. Also, the separation of the above species clusters justifies further research on closely related species as important genetic resources for potential economic and medicinal uses of Artemisia species based on their affinity.

Conflict of interests. The author declares that he does not have any conflict of interests.

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