

Morpho-molecular Association and Genetic Diversity among *Senecio* and *Conyza* species of Family Asteraceae

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Abstract

Morphological parameters and molecular attributes play an important role in studying genetic diversity. Morphological characters as leaf length and leaf width appeared in highest values in *Conyza albida* as 9.6 cm and 2.77cm respectively. *Senecio vulgaris* showed highest leaf length and diameter as 8.8 cm and 3.2 cm respectively. Molecular markers (RAPD & ISSR) produced 47 total bands with 29 polymorphic bands. Primer 2 RAPD generated three bands with molecular sizes of 870, 900 and 1100 bp characteristic for *Senecio* species only. ISSR primer HB14 produced two unique bands of molecular size 120 and 1300 bp recorded only in *Senecio asirensis*. ISSR primers have the ability to differentiate among the investigated species of *Conyza* and *Senecio* with highest polymorphism 65.38%. UPGMA dendrogram and Biplot based on molecular attributes (OPB10, HB9, HB10& HB14) and morphological characters especially plant diameter and leaf width have the clear ability to differentiate and separate among *Conyza* species and *Senecio* species into two separate groups.

Keywords: Morphological parameters; Molecular attributes; *Senecio*; *Conyza*; RAPD; ISSR.

Full length article *Corresponding Author, e-mail: haifasakit@ju.edu.sa

1. Introduction

Family Asteraceae (Compositae) is one of the most advanced vascular families in the world; it contains about 1,911 genera with 32,912 species with 43 tribes and 13 subfamilies [1]. This family is distributed in all different habitats, appeared in Antarctica, subtropics and semi-arid tropics [2-3]. The distribution in all continents, characterized these plants in the efficient flower structure with important function and help in pollination [4]. In both the highlands and the arid regions of Saudi Arabia, there are numerous wild plants. Asir, Hejaz, and the western region along the Red Sea were the areas with the most plant diversity, according to records [5]. Numerous earlier researches demonstrated that local topography and climatic impacts are primary variables influencing the level of species variety in a region [6-7]. With 150 to 210 species, including all currently recognized subgenera and sections, the Mediterranean region and south western Asia are the primary centers of biological diversity or species diversity [8]. Effective colonisation and spread of invasive alien species influenced by genetic and evolutionary processes, which are significant driving forces [9].

Due to their rapid growth and intense competition, invasive alien plants can provide a serious danger to the environment and agricultural productivity in newly introduced areas [10]. *Conyza* species that cause significant financial losses to agriculture make them important weeds [11]. About 100 plant species make up the genus *Conyza* Less., which is part of the subtribe Conyzinae and is found practically everywhere in the world. With its perturbations, the agricultural environment places selective pressure on weed populations, which inevitably leads to a shift in the plant population in the environment [12]. The species *C. canadensis* (L.) Cronquist, commonly known as horseweed, *C. bonariensis* (L.) Cronquist, commonly known as hairy fleabane, and *C. sumatrensis* (Retz.) E. Walker, commonly known as Sumatran fleabane, are a few examples of weeds that can be found in orchards, vineyards, corn, soybean, cotton, and forage crops [13]. The adaptability of introduced populations to new settings and the quick selection of individuals with higher reproductive fitness and improved phenotypic flexibility, driven by underlying genetic variation, are typically characteristics of the invasion success of introduced populations. It introduces and broadens its range; invasive populations are predicted to undergo demographic limitations that may negatively impact their genetic diversity, and the possibility for evolution [14].

Senecio L. (Compositae, Senecioneae), one of the largest genera of flowering plants, has about 1250 species. Although it is essentially universal, it is most common in America, Asia, and Africa. [15]. Due to the presence of pyrrolizidine alkaloids (PAs), many *Senecio* species are poisonous. However, certain species are beautiful, while others have antibacterial characteristics and have been used in folk medicine [16]. One of the largest genera, *Senecio* has a vast number of species; all *Senecio* species differ in their form and growth habits. They are found throughout Saudi Arabia's many geographical zones as annual, climber, perianth, succulent, semi-aquatic, stragglers, & shrubs. *Senecio asirensis*, a Saudi Arabian endemic species, is found in a small number of locations, including Jabal Fayfa, Raida, Bal

Lasmar, Jabal Samdah, Bal Jurshi, and Tannouma [17]. In order to assess role of stochastic and deterministic influences on strength & patterns of genetic variety in invading species, comparative studies of genetic diversity and population structure of invasive and native populations are needed [18].

The first stage in evaluating, describing, and categorising germplasm collections to improve their usage in plant breeding is morphological characterization. Because they provide an accessible method of evaluating genetic divergence, morphological and phenotypic characterizations have been employed to assess genetic variability [19]. The genetic homogeneity of various micro-propagated plants has been successfully assessed using a variety of PCR-based RAPD, ISSR, and SCOT molecular markers [20-21]. One of the key problems in modern biology is figuring out the genetic roots and molecular makeup of all this naturally occurring diversity [22]. Studies of genetic variety using various methods, such as DNA markers, offer valuable information for both genetic protection and productive breeding of new cultivars. DNA markers are frequently employed for phylogenetic and taxonomic investigations, or in assessing the degree of similarity and genetic distance, molecular mapping, and plant selection. They have a wide range of uses in plant molecular genetic studies [23].

Inter simple sequence repeats (ISSR) markers are straightforward to apply and reproduce. It does not require knowledge of the DNA sequence and just requires a small amount of DNA. SSR motifs are utilized to create ISSR primers, which can be applied to any plant species whose genome has an appropriate quantity and distribution of SSR motifs [24]. As a result of the longer primer distances and higher annealing temperatures compared to other arbitrary markers, Start Codon Targeted (SCoT) polymorphisms are reproducible markers that are based on the short-conserved area in plant genes surrounding the ATG translation start codon [25-27]. There are no enough researches to study the genetic diversity among different species of *Conyza* and *Senecio* species; thus this study investigated genetic diversity and polymorphism relationships between five species from each *Conyza* and *Senecio* using morphological characters and molecular markers (SCoT and ISSR). The integration of different systems can be useful for a better understanding of genetic diversity in studied species because each marker type has advantages and limitations. So, this paper gets light on the genetic tools and its role in identification and relationships among wild *Conyza* and *Senecio* in KSA for the first time.

2. Materials and Methods

2.1. Plant material

Five species of genus *Conyza* in addition to five species of genus *Senecio* species were collected from its natural habitats in KSA on spring to summer in two years 2021-2022, their scientific names were illustrated in Table 1.

2.2. Molecular study

2.2.1. DNA Extraction

Fresh leaves of the studied taxa were preserved at -20 C till use. About 0.2 gm of fresh leaves was used to extract DNA using EZ-10 Genomic DNA Kit (www.biobasic.com).

2.2.2. RAPD and ISSR analysis

Table 2 shows the sequences and annealing temperatures of five primers for RAPD and ISSR molecular markers. DNA amplification was carried out in the following manner for 40 cycles: 10 minutes at 95°C, 30 seconds at 94°C, 1 minute at annealing temperature, 2 minutes at 72°C, and one final 5minute extension cycle at 72°C. Electrophoresis on 1% agarose gels was used to separate the amplification products.

2.3. Data analysis

The mean SD of three replicates is used to represent all data. The hypothesis that genotype and salinity concentration affect the observed plant properties was tested using two-way analysis of variance (ANOVA). If there were significant differences in the means, Duncan's multiple range tests [28] were used to compare different groups. For all statistical tests, P values of 0.05 were considered statistically significant. Using PAST software, principal component analysis (PCA) was used to investigate the morphological and molecular data correlations between the *Senecio* and *Conyza* species [29]. Using the TBtools package, the heatmap was utilized to investigate the similarity and dissimilarity of examined taxa based on morphological features [30]. Cluster phylogeny was performed in NTSYSpc software version 2.1 [31] utilizing dendrogram construction with the unweight pair group method of averages (UPGMA).

3. Results and discussion

3.1. Results

3.1.1. Morphological analysis

Variation in the morphological traits among *Conyza* and *Senecio* species were presented in Table 2. For genus *Conyza*, highest plant height was recorded in *C. albida* 105 cm, where the lowest height was found in *C. stricta* 27 cm. The highest plant diameter was recorded in *C. stricta* 70.61cm followed by *C. canadensis* 65.23 cm and *C. aegyptiaca* 50.63 cm. Leaf length and width values indicated that *C. albida* has a highest value as 9.6 cm and 2.77cm, respectively. Leaf index (leaf length/leaf width) recorded in *C. bonariensis* as the highest value 14.03. Seed length showed highest value in *C. bonariensis* 1.6 mm followed by *C. albida* 1.2 mm and *C. stricta* 0.9 mm. *Conyza stricta* showed highest value of seed diameter 0.32 mm. For genus *Senecio*, *S. glaucus* have highest plant height 47.8 cm, while *S. asirensis* showed highest plant diameter 20.3 cm. *Senecio vulgaris* showed highest leaf length and diameter as 8.8 cm and 3.2 cm respectively. Leaf index showed highest value in *S. glaucus* 3.3. In addition to highest values of seed length and seed diameter were recorded in *S. glaucus* as 2.7 mm and 0.61 mm. Statistical analysis showed in Cluster analysis (UPGMA) based on morphological variations revealed that there are two groups: first group separated at 40.00 includes *C. albida* and *C. canadensis* while second group includes five species of *Senecio* and other three species of *Conyza* and also second group divided into subgroups one subgroup contains all *Senecio* species and other subgroup contain *C. aegyptiaca*, *C. bonariensis* and *C. stricta* as shown in Figure 1.

3.1.2. Molecular Analysis

For RAPD marker banding profiles were illustrated in Figure 2. Five primers generated 26 total bands with molecular size of 200- 1500 bp, from these bands 17 polymorphic bands and nine monomorphic bands. Primer 2 RAPD generated three bands with molecular sizes of 870, *Alhathloul et al., 2023*

900 and 1100 bp characteristic for *Senecio* species only. Primer RAPD 2-19 produced one band present in five species of *Senecio* with molecular size of 300 bp as characteristic band. Primers RAPD 2 and OPB10 gave the highest percentage of polymorphism 83.33 % in Table 3. For ISSR marker, banding profiles were illustrated in Figure 3. ISSR primers produced 20 total bands with size range of 400-1500 bp. Generated bands with 12 polymorphic bands and eight monomorphic bands. There are two unique bands generated from HB14 of molecular size 120 and 1300 bp recorded only in *Senecio asirensis*. Primer HB14 produced highest polymorphism 83.33 % followed by HB8 75%. RAPD marker gave highest percentage of polymorphism than ISSR marker as 65.38% and 60 % respectively as presented in Table 4. Cluster dendrogram based on molecular attributes (RAPD & ISSR) showed the studied taxa separated into two groups one group includes *Conyza* species and other group contains *Senecio* species as shown in Figure 4.

3.1.3. Data analysis

Pearson correlation among studied taxa based on morphological characters and molecular attributes showed the highest correlation among *C. canadensis* and *C. stricta* and also between *S. glaucus* and *C. albida* was 0.82 followed by the relation between *S. asirensis* and *S. glaucus* with value 0.78. The lowest correlation between *S. vulgaris* and *C. bonariensis* was -0.04 as illustrated in Figure 5. The studied *Conyza* and *Senecio* species dendrogram appeared vertically in the heatmap's results from the hierarchical cluster analysis Figure 6. There are two different groups based on the molecular and morphological characters of the studied taxa: the first outgroup contains five *Senecio* species, with two subgroups; first subgroup contains *S. asirensis* and *S. glaucus* and the rest three *Senecio* species in the second subgroup. The second outgroup consists of *Conyza* genus. This out group also classified into two subgroups. First subgroup includes *C. albida* and the second subgroup includes rest four *Conyza* species. The red color indicated a high similarity between studied taxa, while the blue color indicated a low similarity. Principle Component analysis (PCA) was performed on the morphological variables and molecular markers to illustrate the association between the studied *Conyza* and *Senecio* species. As shown in Figure 7, PCA1 is responsible for 33.1% of the variance, and the PCA2 shares with 22.1% variance. The results of the PCA showed the clear separation of genus *Conyza* on the left region; while, *Senecio* species in the right region. The first and second axes with eigenvalues 0.011 and 0.013 respectively; the small eigenvalues is indication for the stability of ordination.

length of the arrow indicated the most powerful variables in taxa ordination and classification. Also, the direction of arrow indicated to the highest correlation among taxa. PCA result showed *Senecio* species in the right part separated by the most efficient variables molecular marker (RAPD 2, OPB10) and morphological traits (leaf width, leaf length & seed width). The other group separated into the left part of PCA by morphological traits (plant diameter, plant height & leaf index) and molecular marker (HB-8 & HB-10). Cluster dendrogram based on morphological and molecular parameters showed in UPGMA divided studied taxa into two groups one group includes *Conyza* species and other group includes *Senecio* species in Figure 8.

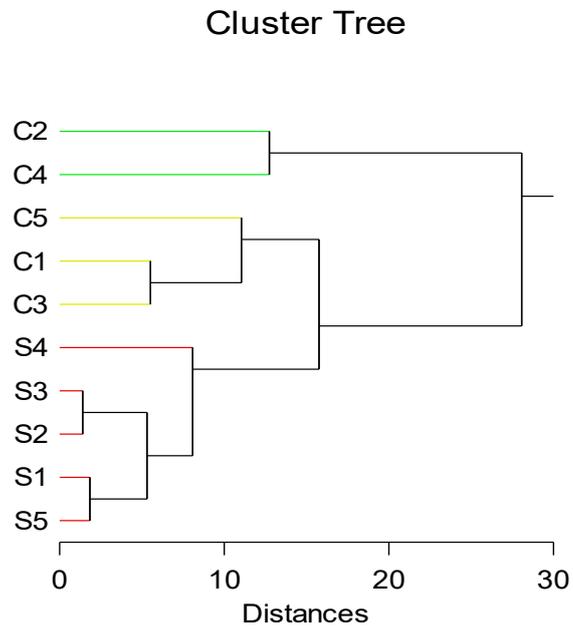


Fig. 1: Cluster UPGMA analysis showing the relationships between the studied taxa of *Conyza* and *Senecio* species using Euclidean distance Average linkage method based on morphological characters.

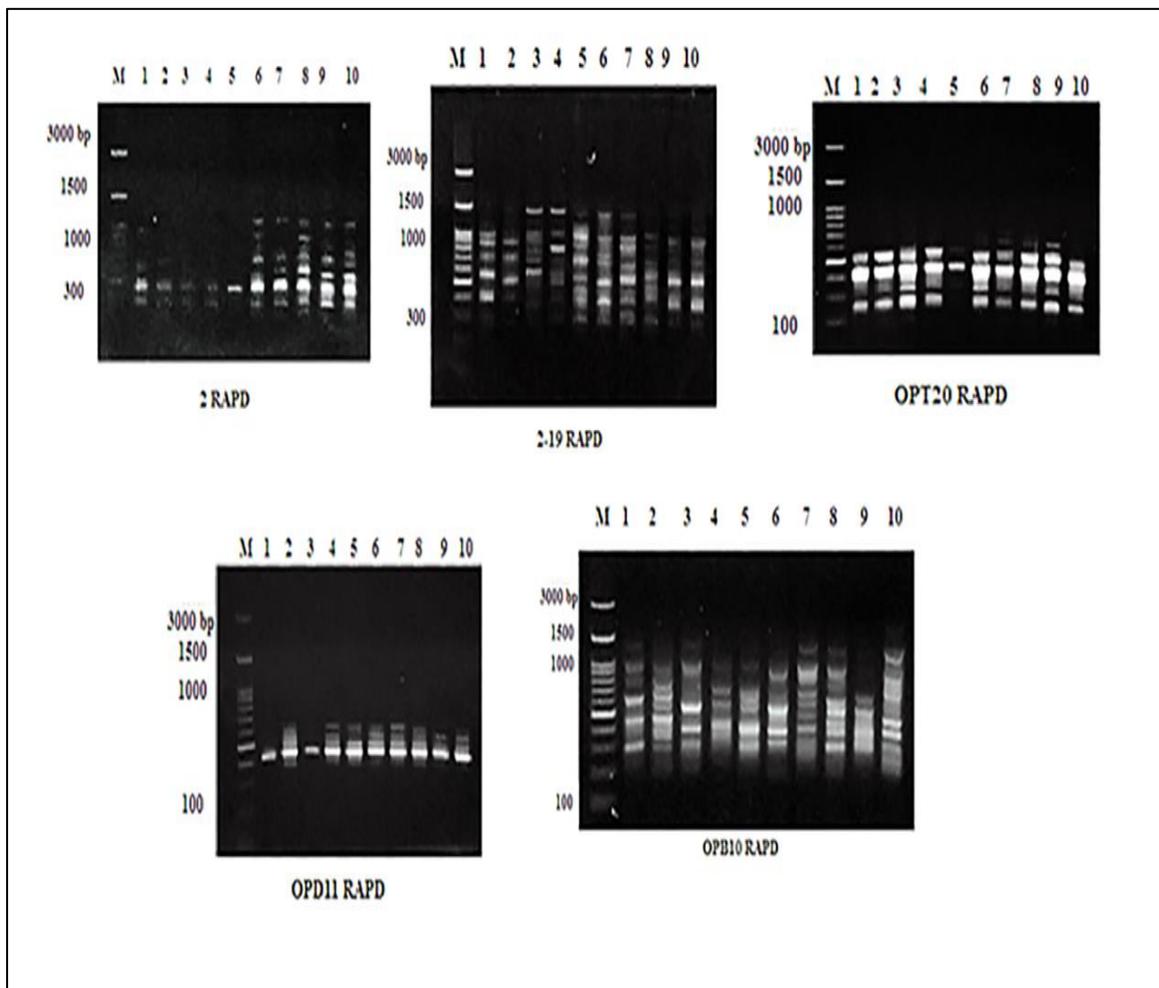


Fig. 2: Fingerprinting profiles produced by five primers of RAPD marker used to differentiate between the examined taxa of *Conyza* and *Senecio* species

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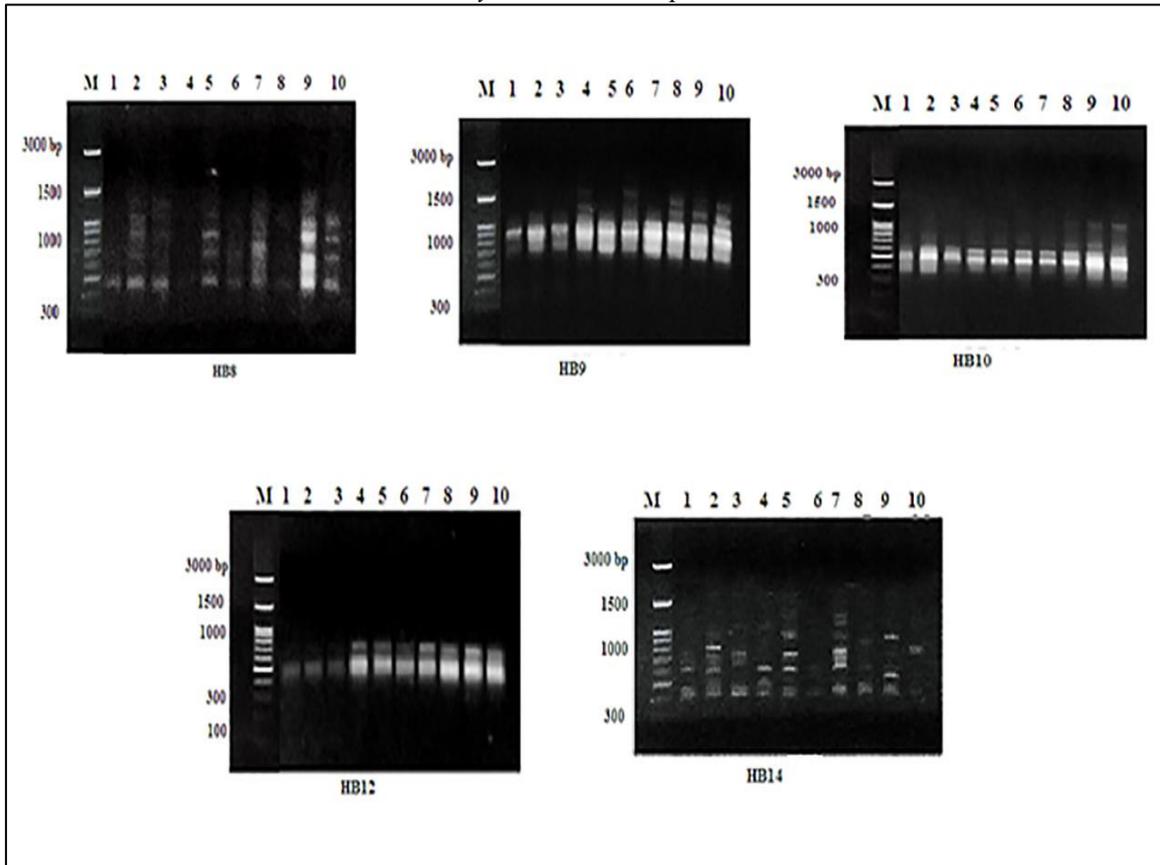


Fig. 3: Fingerprinting profiles produced by five primers of RAPD marker used to differentiate between the examined taxa of *Conyza* and *Senecio* species

Cluster Tree

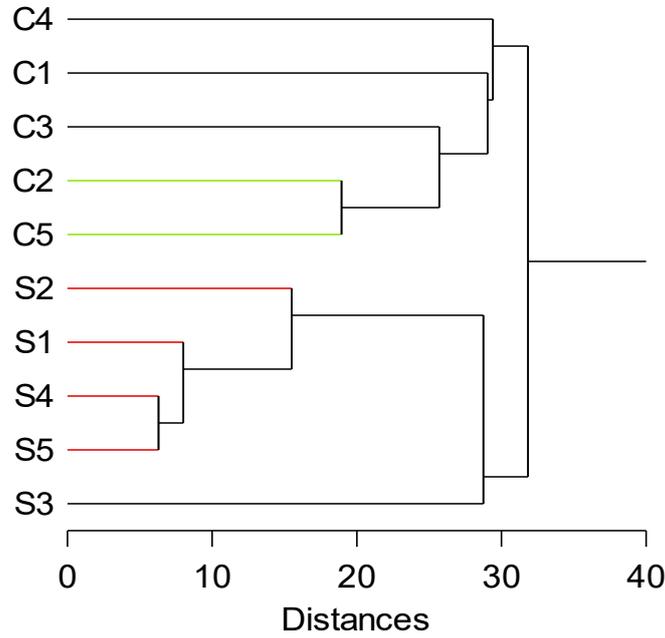


Fig. 4: Cluster UPGMA analysis showing the relationships between the studied taxa of *Conyza* and *Senecio* species using Euclidean distance Average linkage method based on molecular attributes.

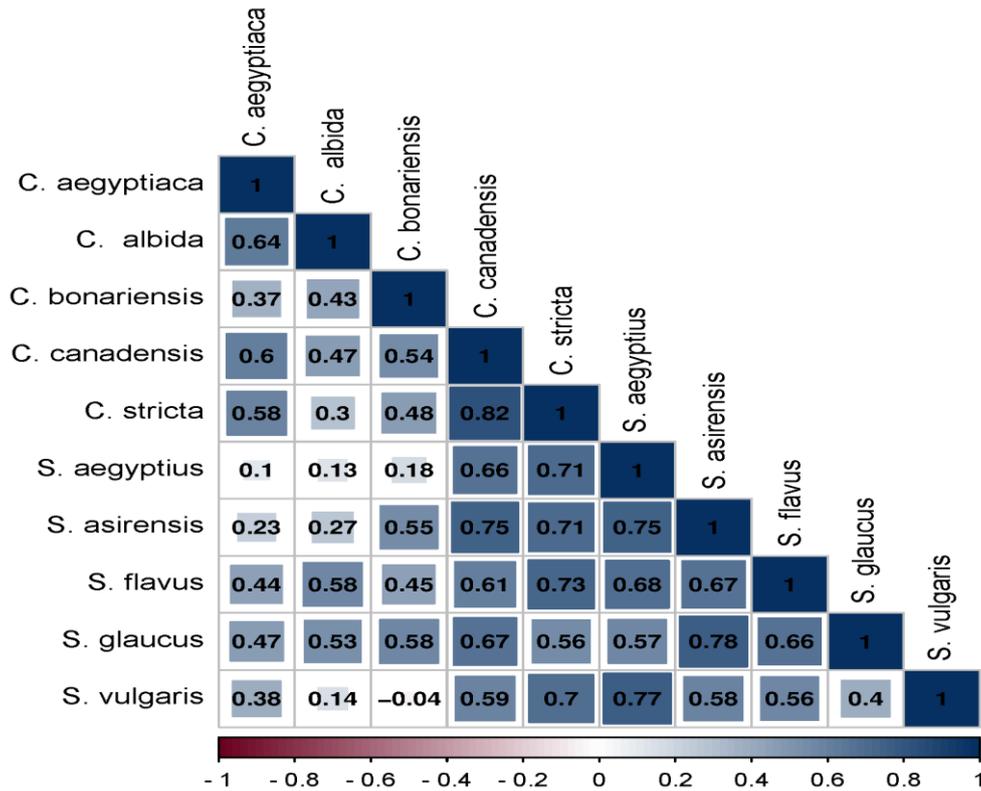


Fig. 5: Color plot correlation among the studied species of *Conyza* and *Senecio* based on morphological traits and molecular markers (RAPD & ISSR). The size of circle represents the strength of the correlation, greater the circle stronger the association

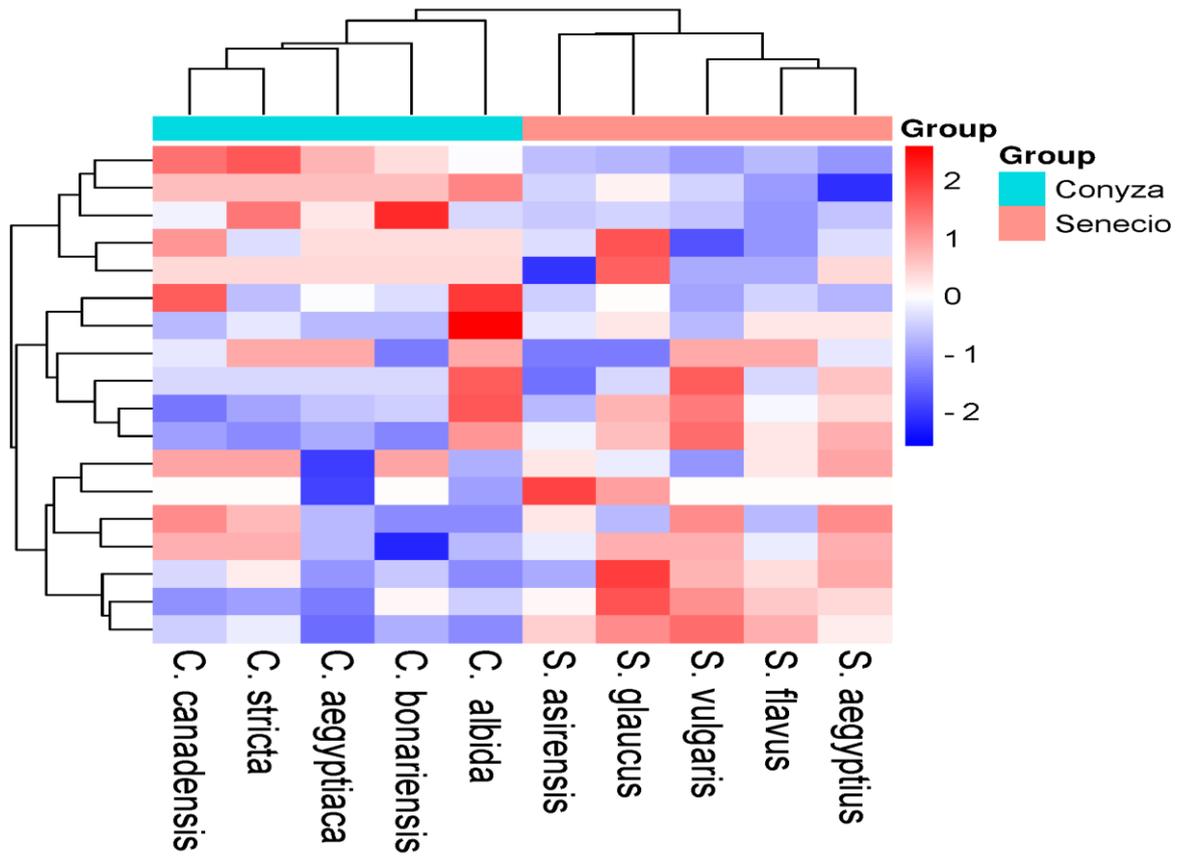


Fig. 6: Heatmap displaying the relationship between ten studied taxa in KSA and their phylogenetic relationships in relation to morphological and molecular markers. The studied taxa are indicated by the upper bar in 2 colors, whereas the color scale denotes the variable level increasing from zero (white) to 2 (deep red)

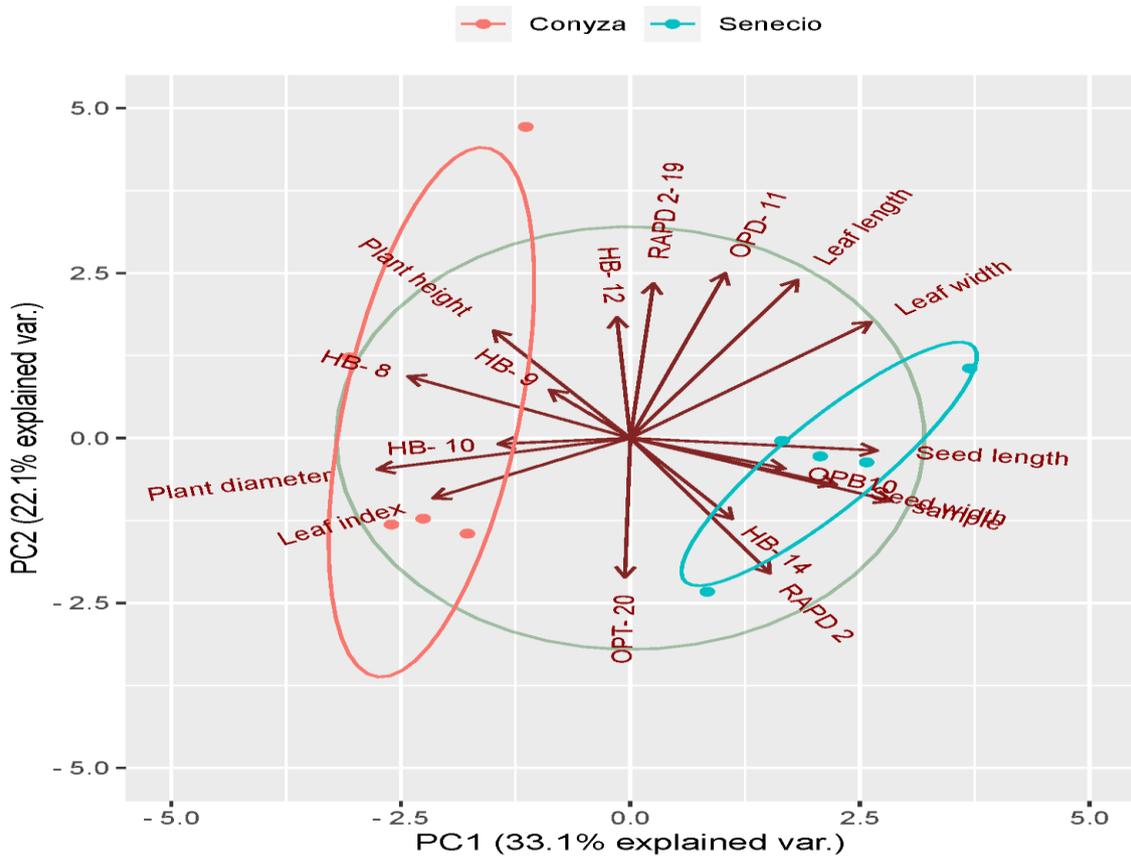


Fig. 7: Principal component analysis (PCA) of the studied taxa collected from KSA using variables and molecular markers
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Cluster Tree

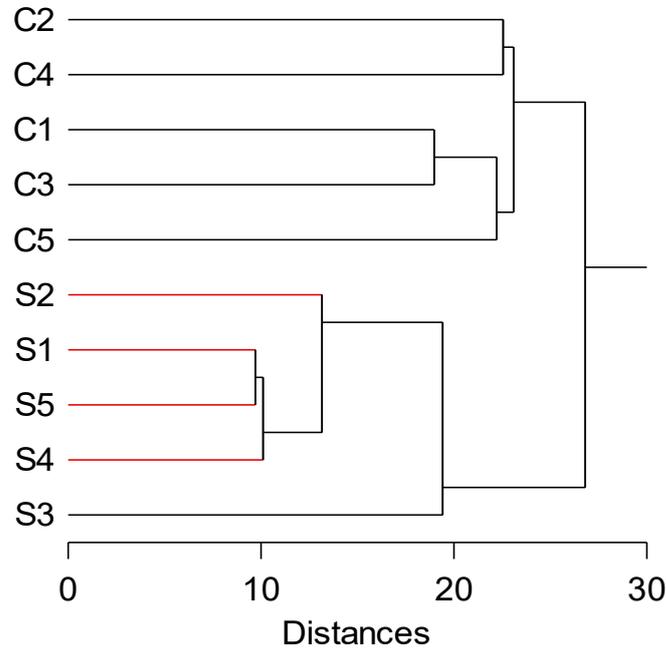


Fig. 8: Cluster UPGMA analysis showing the relationships between the studied taxa of *Conyza* and *Senecio* species using Euclidean distance Average linkage method based on morphological characters molecular attributes

Table 1: Studied taxa collected from natural habitat in KSA

No.	Studied Taxa	Code
1	<i>Conyza aegyptiaca</i> (L.) Dryand.	C1
2	<i>Conyza albida</i> Willd.ex Spreng.	C2
3	<i>Conyza bonariensis</i> (L.) Cronquist	C3
4	<i>Conyza canadensis</i> (L.) Cronquist	C4
5	<i>Conyza stricta</i> Willd.	C5
6	<i>Senecio aegyptius</i> L.	S1
7	<i>Senecio asirensis</i> Boulos & J.R.I. Wood	S2
8	<i>Senecio flavus</i> (Decne.)Sch.Bip.	S3
9	<i>Senecio glaucus</i> L.	S4
10	<i>Senecio vulgaris</i> L.	S5

Table 2. Morphological characters *Conyza* and *Senecio* studied species

No	Character	Studied Taxa									
		<i>C. aegyptiaca</i>	<i>C. albida</i>	<i>C. bonariensis</i>	<i>C. canadensis</i>	<i>C. stricta</i>	<i>S. aegyptius</i>	<i>S. asirensis</i>	<i>S. flavus</i>	<i>S. glaucus</i>	<i>S. vulgaris</i>
1	Plant height (cm)	45c±1.6	105a±2.1	37.02d±1.1	95b±2.0	27e±1.02	23.5f±0.7	31.5e±1.2	33.6d±1.4	47.8c±1.9	19.4g±0.8
2	Plant diameter (cm)	50.63b±1.6	33.87d±1.1	41.34c±1.2	65.23a±1.7	70.61a±1.7	11.34f±0.7	20.3e±0.9	18.7e±0.8	17.32e±0.6	12.6f±0.4
3	Leaf length (cm)	4.5d±0.2	9.6a±0.3	4.77d±0.2	2.8f±0.1	3.87e±0.2	6.7b±0.3	4.23d±0.2	5.66c±0.2	7.6b±0.3	8.8a±0.3
4	Leaf width (cm)	0.73d±0.02	2.77b±0.4	0.34e±0.03	0.62d±0.04	0.36e±0.04	2.5b±0.2	1.5c±0.1	1.87c±0.1	2.33b±0.3	3.2a±0.3
5	Leaf index	6.16c±0.3	3.47e±0.41	14.03a±0.2	4.51d±0.3	10.75b±0.7	2.68f±0.3	2.82f±0.2	0.72g±0.08	3.3e±0.5	2.75f±0.12
6	Seed length (mm)	0.65e±0.02	1.2c±0.1	1.6b±0.1	0.8d±0.02	0.9d±0.1	1.8b±0.1	1.6b±0.2	1.9b±0.2	2.7a±0.2	2.3a±0.2
7	Seed width (mm)	0.12e±0.01	0.1f±0.01	0.2d±0.01	0.23d±0.01	0.32c±0.01	0.43b±0.01	0.15e±0.01	0.35c±0.01	0.61a±0.01	0.41b±0.01

At $p \leq 0.05$, values with different letters indicate there are significant differences between the treatments. Data are presented as the mean of three replicates standard deviations.

Table 3: Name of molecular primers, sequence, annealing temperature and percentage of polymorphism for each primer for RAPD and ISSR markers

Markers	Primers Name	Sequence	Annealing temperature	Total bands	Polymorphic bands	% Polymorphism
RAPD	RAPD 2	5`AACGCGCAAC3`	30°C	6	5	83.33%
	RAPD 2-19	5`GCACGGCGTT3`		7	3	42.86%
	OPT-20	5`GACCAATGCC3`		4	2	50%
	OPD-11	5`AGCGCCATTG3`		3	2	66.67%
	OPB10	5`CCGTTGCCT 3`		6	5	83.33%
ISSR	HB-8	5`CACACACACACAGG3`	50°C	4	3	75%
	HB-9	5`GAGAGAGAGAGAGG3`		5	2	40%
	HB-10	5`GTGTGTGTGTGTGG3`		3	1	33.33 %
	HB-12	5`GTGTGTGTGTGTCC3`		2	1	50 %
	HB-14	5`GAGGAGGAGGC3`		6	5	83.33 %

Table 4: Number of primers, size range, total bands, polymorphic bands produced by RAPD and ISSR to differentiate between *Conyza* and *Senecio* species

Parameters		Molecular markers	
		RAPD	ISSR
Studied taxa		10	10
No. of primers		5	5
Size range (bp)		200-1500	400-1500
Total bands		26	20
Polymorphic bands		17	12
Unique bands	No. of unique bands	-	2
	Size range	-	1300
	Primers	-	HB14
% Polymorphism		65.38%	60%

3.2. Discussion

The current study examined the morphological variations between ten taxa from family Asteraceae collected from KSA. Although morphological analysis for assessing genetic diversity has many limitations, such as low polymorphism and the influence of environment on phenotypic expression, phenotypic traits were useful as a preliminary evaluation of maize genetic diversity and provided practical and critical information needed to characterize genetic resources [32-33]. The first step toward understanding plant evolution and the principles of plant divergence is species delimitation .It is, however, a difficult endeavor in plant species with recent speciation events and complex species that have faced hybridization and reticulate evolution in the past [34]. Species delimitation in the genus *Senecio* is seen to be of taxonomic and phylogenetic significance, and it can be accomplished using molecular studies [35]. Furthermore, molecular phylogenetic approaches can be used to amend morphology-based infra-generic classifications in plant taxonomy and aid in achieving a sectional classification that reflects the group's evolutionary history. This, in turn, can shed light on the speciation process in plants, particularly the genus *Senecio* [36-37]. In this investigation, morphological characteristics and molecular data from ISSR and RAPD data were used to identify the *Senecio* and *Conyza* species. Other researchers have also documented the use of multilocus molecular markers in species delimitation of various plant families [38-40].

In this study, based on phenotypic traits *Senecio aegyptius* (S1) was closed to *Senecio vulgaris* (S5); *Senecio asirensis* (S2) was closed to *Senecio flavus* (S3), while *Senecio glaucus* (S4) in separate group. For *Conyza* genus, *Conyza albida* (C2) was nearby to *Conyza Canadensis* (C4), and *Conyza aegyptiaca* (C1) was closed to *Conyza stricta* (C5). This result was similar to the result revealed by Ghahremaninejad [41] between *Senecio* species based on morphological characters. The basis for germplasm characterization is regulated by phenotypic characteristics; however, they are vulnerable to environmental challenges, limiting the number of examined features, delayed expression, and low heredity. The majority of these issues can be avoided by cytological and molecular genotyping via DNA-based screening [26]. Molecular investigations employing diverse methodologies have been applied successfully to determine genetic diversity in a variety of horticultural plants. A range of environmental factors like as life history, breeding system, seed dispersal, population size, and ecological features are thought to be primarily responsible for the level and distribution of genetic variation within and among populations in wild plant species. Furthermore, understanding genetic diversity is critical for the conservation of the most significant populations [42-43].

DNA markers are factors that are independent of environmental or local causes and have a higher amount of polymorphism [44]. RAPD marker diversity in plant species is usually equivalent to or larger than allozyme variation. In this study two molecular markers were used to estimate the genetic diversity among the studied taxa; five RAPD primers were used generated 65.38% polymorphism and 26 total

bands. This result related to the result revealed by Qari [45] who found the polymorphism 69.32 % between *Anthemis* species (Asteraceae) in KSA using 5 RAPD primers. Regarding, ISSR marker produced 20 total bands with 12 polymorphic bands and two unique bands; five ISSR primers generated 60 % polymorphism. This data showed lowest percentage of polymorphism than polymorphism generated from Yudanova et al. [46] who studied genetic diversity among rose varieties (Asteraceae) using six ISSR primers. Cluster analysis is a technique for categorizing a set of characters into groups. Genotypic clustering employs a technique known as Agglomerative Hierarchical Clustering (AHC) using Unweight Pair Group Method with Arithmetic Mean (UPGMA) [47]. Cluster analysis is statistical approach that groups things into clusters so that objects in one cluster have higher similarities than those in other clusters [48].

Euclidian distance values ranging from 0 to 1 suggest a minor dissimilarity, whereas values more than 1 imply a big dissimilarity coefficient. A low dissimilarity coefficient suggests that one or more characters have a narrow variability for each genotype [49]. UPGMA cluster analysis using phenotypic traits and molecular data clustered the studied taxa into two main groups; one group contained five species of *Conyza* genus and the second group contained the other five species from *Senecio* genus. One visual tool for clarifying the correlations and relationships between different parameters of samples under different treatments is the hierarchical cluster heatmap. The advantage of heatmaps is that they can be coupled with hierarchical clusters based on similarity or distance between them [50-51]. The heatmap cluster in this study showing the similarity between *Senecio*, and *Conyza* species. Principle component analysis is a multivariate analysis for data that is used to visualize the relationship, similarities, and differences between various plant characteristics and salinity tolerance. The highest variation interaction by morphological and physiological traits was explained by PCA [52-55]. PCA in this study showed the studied taxa are classified in to two groups based on morphological and molecular data.

4. Conclusions

The current study demonstrates how phenotypic traits and molecular analysis can be used to select wild genus from *Conyza* and *Senecio* for conservation and evolution, as well as provide a platform for the selection of specific species for broad or specific adaptations. *Senecio* and *Conyza* species showed a wide range of variability in the attributes assessed. The substantial genetic diversity discovered could be used in breeding efforts to create new cultivars while also providing important information for diversity conservation. Multivariate statistical analysis of morphological data and molecular attributes, such as principal component analysis (PCA), was able to analyze genetic diversity, define, and classify ten studied taxa. Based on morpho-molecular data, the Agglomerative Hierarchical clustering was able to find the most unrelated hybrids and populations to be utilized as parents for isolating inbred lines that when crossed would show greatest heterosis. While simply morphological or molecular studies are typically reliable in genetic variability study, we conclude that combining both approaches is frequently more reliable than each one alone.

References

- [1] S.K. Panda, L.C.N. da Silva, D. Sahal, M. Leonti. (2019). Ethnopharmacological studies for the development of drugs with special reference to Asteraceae. *Front. Pharmacol.* 10: 955.
- [2] R.I. Lewis Smith, M. Richardson. (2011). Fuegian plants in Antarctica: natural or anthropogenically assisted immigrants? *Biological Invasions.* 13(1): 1-5.
- [3] N. Roque, H.P. Bautista. (2008). Asteraceae: caracterização e morfologia floral. Salvador, Brazil: Edufba.
- [4] P. Elomaa, Y. Zhao, T. Zhang. (2018). Flower heads in Asteraceae—recruitment of conserved developmental regulators to control the flower-like inflorescence architecture. *Horticulture research.* 5: 36.
- [5] S. Collette. (1998). A checklist of botanical species in Saudi Arabia. *Int. Asclepiad Soc.* 1-8.
- [6] H. El-Kady, M. Ayyad, R. Bornkamm. (1995). Vegetation and recent land-use history in the desert of Maktala, Egypt. *Adv. Geocol.* 28: 109-123.
- [7] K. Shaltout, E. El-Halawany, M. El-Garawany. (1997). Coastal lowland vegetation of eastern Saudi Arabia. *Biodiversity & Conservation.* 6(7): 1027-1040.
- [8] K. Ömer, K. Alpaslan, B. Eyüp. (2011). Composition of the volatile oils of two *Anthemis L.* taxa from Turkey. *Naturforsch. C.* 66(11-12): 535-540.
- [9] M. van Kleunen, O. Bossdorf, W. Dawson. (2018). The ecology and evolution of alien plants. *Annual review of ecology, evolution, and systematics.* 49(1): 25-47.
- [10] P. Pyšek, J. Čuda, P. Šmilauer, H. Skálová, Z. Chumová, C. Lambertini, M. Lučanová, H. Ryšavá, P. Trávníček, K. Šemberová. (2020). Competition among native and invasive *Phragmites australis* populations: An experimental test of the effects of invasion status, genome size, and ploidy level. *Ecology and Evolution.* 10(3): 1106-1118.
- [11] M. Abdein, A.K.E. Osman. (2020). Plant diversity assessment of Wadi Al-Hilali, northern border region, Saudi Arabia. *Int. J. Bot. Stud.* 5(2): 87-95.
- [12] M. Matzrafi, T.W. Lazar, M. Sibony, B. Rubin. (2015). *Conyza* species: distribution and evolution of multiple target-site herbicide resistances. *Planta.* 242(1): 259-267.
- [13] C.A. Lazaroto, N.G. Fleck, R.A. Vidal. (2008). Biologia e ecofisiologia de buva (*Conyza bonariensis* e *Conyza canadensis*). *Ciência Rural.* 38(3): 852-860.
- [14] Q. Geng, Z. Yao, J. Yang, J. He, D. Wang, Z. Wang, H. Liu. (2015). Effect of Yangtze River on population genetic structure of the relict plant *Parrotia subaequalis* in eastern China. *Ecology and Evolution.* 5(20): 4617-4627.

- [15] J. Calvo, I. Álvarez, C. Aedo. (2015). Systematics of Senecio section *Crociseris* (Compositae, Senecioneae). *Phytotaxa*. 211(1): 1–105–1–105.
- [16] A.J. Belaunde, J.G. Sandoval, L.D. Martino, F. Senatore, V.D. Feo. (2007). Chemical composition and antibacterial activity of *Senecio nutans* essential oil. *Journal of Essential Oil Bearing Plants*. 10(4): 332-338.
- [17] S.A. Chaudhary. (2001). *Flora of the kingdom of Saudi Arabia (Vascular Plants)*. National Agriculture and Water Research Center. National Herbarium. Ministry of Agriculture and Water. Riyadh, Saudi Arabia. (1).
- [18] B.-R. Zhu, S.C. Barrett, D.-Y. Zhang, W.-J. Liao. (2017). Invasion genetics of *Senecio vulgaris*: loss of genetic diversity characterizes the invasion of a selfing annual, despite multiple introductions. *Biological Invasions*. 19(1): 255-267.
- [19] N.A. El-Tayeh, H.K. Galal, M.I. Soliman, H. Zaki. (2020). Association of morphological, ecological, and genetic diversity of *Aerva javanica* populations growing in the eastern desert of Egypt. *Agronomy*. 10(3): 402.
- [20] F. Al-Qurainy, M. Nadeem, S. Khan, S. Alansi, M. Tarroum, A.A. Al-Ameri, A.-R.Z. Gaafar, A. Alshameri. (2018). Rapid plant regeneration, validation of genetic integrity by ISSR markers and conservation of *Reseda pentagyna* an endemic plant growing in Saudi Arabia. *Saudi journal of biological sciences*. 25(1): 111-116.
- [21] L. Tikendra, T. Amom, P. Nongdam. (2019). Molecular genetic homogeneity assessment of micropropagated *Dendrobium moschatum* Sw.-A rare medicinal orchid, using RAPD and ISSR markers. *Plant Gene*. 19: 100196.
- [22] J.M. Al-Khayri, E.M. Mahdy, H.S. Taha, A.S. Eldomiaty, M.A. Abd-Elfattah, A.A.H. Abdel Latef, A.A. Rezk, W.F. Shehata, M.I. Almaghasla, T.A. Shalaby. (2022). Genetic and morphological diversity assessment of five *Kalanchoe* genotypes by SCoT, ISSR and RAPD-PCR markers. *Plants*. 11(13): 1722.
- [23] B. Kolodziej, S. Okon, A. Nucia, T. Ociepa, K. Luchowska, D. Sugier, R. Gevrenova, M. Henry. (2018). Morphological, chemical, and genetic diversity of *Gypsophila* L.(Caryophyllaceae) species and their potential use in the pharmaceutical industry. *Turkish Journal of Botany*. 42(3): 257-270.
- [24] M. Cieplak, S. Okoń, K. Werwińska. (2021). Genetic similarity of *Avena sativa* L. varieties as an example of a narrow genetic pool of contemporary cereal species. *Plants*. 10(7): 1424.
- [25] M. Abdein, H.N. Wrda, A. Osman. (2020). Genetic characterization of Genus *Tephrosia* Pers. based on Molecular markers in KSA. *International Journal of Botany Studies*. 5(2): 203-209.
- [26] M. Abdein, A.M. Imrahim, S.Y. Mohamed, S.O. Osman, S.A. Shamseldin, M.F. Maklad, M.M. Abdel-Salam, E.-S.M. Qaoud. (2022). RAPD Markers are Associated with Self-incompatibility Characteristics as Related to the Number of Seeds per Fruit of Some Mandarin and Clementine Cultivars. *Egyptian Journal of Horticulture*. 49(2): 215-230.
- [27] T. Amom, P. Nongdam. (2017). The use of molecular marker methods in plants: a review. *International Journal of Current Research and Review*. 9(17): 1-7.
- [28] D.B. Duncan. (1955). Multiple range and multiple F tests. *biometrics*. 11(1): 1-42.
- [29] Ø. Hammer, D.A. Harper. (2001). Past: paleontological statistics software package for educator and data analysis. *Palaeontologia electronica*. 4(1): 1-9.
- [30] C. Chen, H. Chen, Y. Zhang, H.R. Thomas, M.H. Frank, Y. He, R. Xia. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular plant*. 13(8): 1194-1202.
- [31] R.R. Sokal, C.D. Michener. (1958). A statistical method for evaluating systematic relationships. *Univ. Kans. Sci. Bull.* 38: 1409-1438.
- [32] M.M. Alqahtani, M.A. Abdein, O.F. Abou El-Leel. (2020). Morphological and molecular genetic assessment of some *Thymus* species. *Biosciences Biotechnology Research Asia*. 17(1): 103-113.
- [33] N. Belalia, A. Lupini, A. Djemel, A. Morsli, A. Mauceri, C. Lotti, M. Khelifi-Slaoui, L. Khelifi, F. Sunseri. (2019). Analysis of genetic diversity and population structure in Saharan maize (*Zea mays* L.) populations using phenotypic traits and SSR markers. *Genetic Resources and Crop Evolution*. 66(1): 243-257.
- [34] M. Medrano, E. López-Perea, C.M. Herrera. (2014). Population genetics methods applied to a species delimitation problem: endemic trumpet daffodils (*Narcissus* section *Pseudonarcissi*) from the southern Iberian Peninsula. *International Journal of Plant Sciences*. 175(5): 501-517.
- [35] P.B. Pelser, B. Nordenstam, J.W. Kadereit, L.E. Watson. (2007). An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. *Taxon*. 56(4): 1077-1104.
- [36] P.B. Pelser, H. de Vos, C. Theuring, T. Beuerle, K. Vrieling, T. Hartmann. (2005). Frequent gain and loss of pyrrolizidine alkaloids in the evolution of *Senecio* section *Jacobaea* (Asteraceae). *Phytochemistry*. 66(11): 1285-1295.
- [37] D. Langel, D. Ober, P.B. Pelser. (2011). The evolution of pyrrolizidine alkaloid biosynthesis and diversity in the Senecioneae. *Phytochemistry Reviews*. 10(1): 3-74.
- [38] M. Sheidai, E. Sejf, M. Nouroozi, Z. Noormohammadi. (2012). Cytogenetic and molecular diversity of *Cirsium arvense* (Asteraceae) populations in Iran. *J. Jap. Bot.* 87: 193-205.
- [39] M. Sheidai, S. Zanganeh, R. Haji-Ramezani, M. Nouroozi, Z. Noormohammadi, S. Ghsemzadeh-Baraki. (2013). Genetic diversity and population structure in four *Cirsium* (Asteraceae) species. *Biologia*. 68(3): 384-397.
- [40] R. Cicevan, A.F. Sestras, M. Plazas, M. Boscaiu, S. Vilanova, P. Gramazio, O. Vicente, J. Prohens, R.E. Sestras. (2022). Biological traits and genetic relationships amongst cultivars of three species of tagetes (Asteraceae). *Plants*. 11(6): 760.

- [41] F. Ghahremaninejad, A. Ezazi, N. Rahchamani, F. Attar. (2010). A new *Senecio* L.(Asteraceae: Senecioneae) from Northeast Iran. *Feddes Repertorium*. 121(1-2): 27-31.
- [42] S. Travis, J. Maschinski, P. Keim. (1996). An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Molecular Ecology*. 5(6): 735-745.
- [43] K. Szabo, D. Pamfil, A.S. Bădărău, M. Hârța. (2021). Assessment of genetic diversity and population structure of the endangered *Astragalus excapus* subsp. *transsilvanicus* through DNA-based molecular markers. *Plants*. 10(12): 2732.
- [44] V.H. Heywood. (2002). The conservation of genetic and chemical diversity in medicinal and aromatic plants. In *Biodiversity: biomolecular aspects of biodiversity and innovative utilization*. Springer. 13-22.
- [45] S.H.M. Qari. (2017). Detection of genetic diversity among some species of *Anthemis* L.(Asteraceae) in Saudi Arabia by using RAPD-PCR analysis. *African Journal of Plant Science*. 11(4): 92-98.
- [46] S.S. Yudanova, S.A. Plugatar, Z.K. Klimenko, V.K. Zykova, O.L. Rubtsova, O.Y. Vasilyeva. (2021). In Genetic diversity analysis of Rose varieties from Grandiflora group based on ISSR molecular markers. *BIO Web of Conferences*, 2021; EDP Sciences. 2021: 00140.
- [47] S. Mohammadi, B. Prasanna, N. Singh. (2003). Sequential path model for determining interrelationships among grain yield and related characters in maize. *Crop science*. 43(5): 1690-1697.
- [48] S. Sharma. (1995). *Applied multivariate techniques*. John Wiley & Sons, Inc. New York. ISBN: 0-47131064-6.
- [49] S.E. Mohamed, M.A. Abdein, D.M. Hikal. (2018). Molecular genetic polymorphism, morphological and the effect of peels as natural antioxidants in some squash cultivars. *Researcher*. 10(3): 97-104.
- [50] Y. Liu, X. Zhang, H. Tran, L. Shan, J. Kim, K. Childs, E.H. Ervin, T. Frazier, B. Zhao. (2015). Assessment of drought tolerance of 49 switchgrass (*Panicum virgatum*) genotypes using physiological and morphological parameters. *Biotechnology for Biofuels*. 8(1): 152.
- [51] N. Khan, A. Bano, M.A. Babar. (2019). Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum* L. *PLoS One*. 14(3): e0213040.
- [52] Q. Iqbal, M.Y. Saleem, A. Hameed, M. Asghar. (2014). Assessment of genetic divergence in tomato through agglomerative hierarchical clustering and principal component analysis. *Pakistan Journal of Botany*. 46(5): 1865-1870.
- [53] Y. Liu, X. Zhang, J. Miao, L. Huang, T. Frazier, B. Zhao. (2014). Evaluation of salinity tolerance and genetic diversity of thirty-three switchgrass (*Panicum virgatum*) populations. *BioEnergy Research*. 7(4): 1329-1342.
- [54] M.A.E.-H. Abdein. (2018). Genetic diversity between pumpkin accessions growing in the northern border region in Saudi Arabia based on biochemical and molecular parameters. *Egyptian Journal of Botany*. 58(3): 463-476.
- [55] A.K.E. Osman, M.A.E.-H. Abdein. (2019). Karyological and molecular studies between six species of *Plantago* in the Northern border region at Saudi Arabia. *Journal of Taibah University for Science*. 13(1): 297-308.