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Molecular diversity in some medicinal members of Asteraceae

Ragab I. Abd El-Fattah*, Hediat M.Salama, H.A. Hussein and Reham Ayman

Botany Department, Faculty of Science, ZagazigUniversity, Zagazig, Egypt
***Corresponding author** E-mail: ragabtima@yahoo.com

ABSTRACT **: Identification and characterization of the plants is very important to protection and economical use. This work used the RAPD method for show the molecular characteristics of six species of Asteraceae. The study was carried out from the April 2021 to July 2021. five primers were used to amplify the DNA from the studied species. The obtained results of the RAPD profiles ranged from 100 to 1500bp. About 40 bands were cleared for the studied six species. The number of bands per species varied from 2 to 12 for primer. The highest similarities value was obtained at Sonchus oleraceus and Senecio desfontainei (88%) and the lowest obtained at Bidens pilosa and Cichorium pumilum (61%). The RAPD-PCR dendrogram classified the studied species of into definite clusters according the primer. Primer c separated Cichorium pumilum and Senecio desfontainei in the first clusters as a genetically different about the others in the second cluster, meanwhile primer d separated Urospermum picroides and Cichorium pumilum in definite clusters than others. From this study, it can conclude that, the RAPD can differentiate between the six Aster species, through rapid method to economical use as well as identification and conservation.**

KEYWORDS: Asteraceae members; Molecular fingerprinting; Medicinal plants; RAPD; Sharkia Province.

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I. INTRODUCTION

Asteraceae is from the largest families that inhabits almost every environment (Mohammed, 2020). It comes from the Aster, characteristic flower heads composed of many small flowers protected by bracts. Zhang *et al.*, (2014) showed that many Asteraceae species are stresses tolerate as thermal, salinity, drought, radiation and high wind.

Many plant species are vulnerable to disappear and not represented in the seed bank so the preservation of wild plant species are urgently needed (Hegazy *et al.*, 2009). The preservation of plants is beginning with demonstration and identification than classification. The morphological features such as shape, color, texture, and odor are used for identification and classification but these methods are not sufficient to discrimination and authentication (Zhang *et al*., 2007). Joshi *et al.*, (2004) showed also that chromatographic techniques and marker compounds are also limited due to variable sources and chemical complexity.

Molecular techniques represented important tools in the genetic analysis of population structure and **plant taxonomy** (Tharachand *et al*., 2012). Kalpana *et al*., (2004) cleared that DNA markers **documented** because the genetic data is unique for each species and is independent variable as age, physiological conditions, and environmental factors. Meanwhile Weder, (2002) reveled that among many molecular techniques, random amplified polymorphic DNA (RAPD) is suitable and not require information about the DNA sequence to be

amplified. RAPD as molecular markers for plant breeding, genetic relationships, taxonomic and systematic analyses of plants were common due to its procedural simplicity (Bartish *et al*., 2000; Ranade *et al.*, 2001). Moreover genetic diversity in various plant species are estimated by used RAPD (Wang *et al*., 2005; Lu *et al*., 2006; Liu *et al*., 2007; Zheng *et al.*, 2008). The advantages of the RAPD include (i) **appropriate** for anonymous genomes, (ii) applicable to only limited quantities of DNA (iii) low expense (Bartish *et al*., 2000 ; Ranade *et al.*, 2001). RAPD were used for assesses of genetic diversity in many plant species (Wang *et al*., 2005; Lu *et al*., 2006; Liu *et al*., 2007; Zheng *et al*., 2008).

In this study, RAPD technique is carried for rapid characterization of six plant species of Asteraceae at Sharkia Province with 5 primers to show genetic variation and relationships between the species, as well as the application of the obtained data for t**he degree of convergence on the scale of plant taxonomy.**

II. Material and Methods

Plant samples

The shoots (with intact leaves) from 6 species belong Asteraceae, were collected from different regions of Sharkia Province (Table 1). The samples were individually placed in sealable polythene bags, transported to laboratory, and then kept frozen until DNA extraction.

Table (1): A detailed description of species based on RAPD banding pattern

Species	Locality	Nature and permanence	Common name	Habitat	Phenology
Sonchusoleraceus	Zagazig , Dyarb	Annual herb	Sowthistle	Silty clay	Fruiting
Bidenspilosa	Zagazig , Dyarb	Annual herb	Spanish Needles	Silty clay	Fruiting
Seneciodesfontainei	Zagazig , Belbis	Annual herb	Groundsel	Sandy loam	Fruiting
Lactucaserriola	Zagazig ,	Annual herb	Prickly lettuce	Silty clay loam	Flowering
Urospermumpicroides	Zagazig	Annual herb	Prickly goldenfleece	Silty clay loam	Flowering
Cichoriumpumilum	Dyarb	Annual herb	Wild endive	Silty clay	Flowering

Genomic DNA isolation

Genomic DNA was isolated from leaf samples in a sterile mortar containing liquid nitrogen and thoroughly crushed with a sterile pestle for DNA extraction. An anion exchange chromatography-based DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) was used for genomic DNA isolation and purification. The purity and quantity of isolated DNA were assessed spectrophotometrically (GeneQuant-1300; GE Healthcare, Buckinghamshire, UK).

RAPD-PCR analysis

RAPD analysis beads (GE Healthcare, Buckinghamshire, UK) were used for RAPD-PCR analysis (**Roehrdanz and Flanders, 1993**). Five primers (GE Healthcare) were used in this study, its names and sequence in Table (2).

Table (2): primers names and sequences

No.	Name	Sequence
a	C1	TTCGAGCCAG
b	N8	ACCTCAGCTC
c	B12	CCTTGACGCA
d	H5	AGTCGTCCCC
e	P8	GGAGCCCAG

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components:
   - Dream Taq Green PCR Master Mix (2X) 12.5 µl
   - primer 2 µl
   - Template DNA(50ng/1µl) 1 µl
   - Water, nuclease-free 9.5 µl
   - Total volume 25 µl
3. Gently vortex the samples and spin down.
4. Place the reactions in a thermal cycler. Perform PCR using the recommended thermal cycling conditions as shown in Table(3):

Table (3): PCR recommended thermal cycling conditions

Step	Temperature,°C	Time	Number of cycles
Initial denaturation	92	5 min	1
Denaturation	92	30 sec	40
Annealing	35	1 min	
ramp up to 72º C		5 min	
Extension	72	2 min	
Final Extension	72	10 min	1

Gel images obtained using Proxima C16 Phi+ (Isogen Life Science) UV transilluminator and Opticom (version 3.2.5; OptiGo) imaging system.

Gel image analysis of the RAPD bands of the different taxa using different RAPD primers done using an Amersham 100-bp ladder (GE Healthcare) and the Total Lab TL100 1D software (version 2008.01).

Data analysis

RAPD-PCR-amplified fragments scored as present (1) or absent (0). The bands scored that only cleared (Collard and Mackill, 2009). The comparisons between accessions, on the bases of the proportion of common bands resulted by the primers used using the Jaccard's similarity coefficient with the help of the StatistiXL program (version 1.7).

III.RESULTS AND DISCUSSIONS

The RAPD banding patterns of the 6 plant species are shown in Figure 1. RAPD-PCR was able to amplify the DNA from six Aster species by using 5 primers (a- e). The RAPD profiles generated amplified products ranging from 100 to 1500bp. A total of 40 bands were observed for all samples of species using 5 primers. The number of well-defined and major bands per plant species for a single primer ranged from 6 to 12. The maximum number of well-defined or major bands was at primer d (12 bands) and the minimum number at primer a (6 bands) (Figure 1).Primer c produced distinct banding patterns for all the plant species tested.

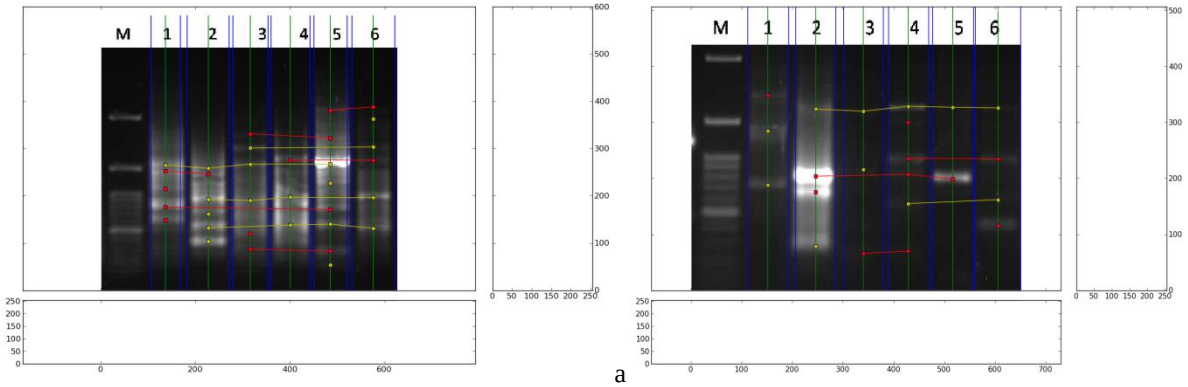
The (d) primer (H5) scored five monomorphic amplicons out of 12 TAF, meanwhile each of B12 (c) and P8 (e) primers scored only one monomorphic amplicons out of 7 TAF (Tables, 4 and 5). Primer C1 (a) scored two monomorphic amplicons out of 6 TAF and primer N8 (b) scoredthree monomorphic amplicons out of 8 TAF.

Polymorphic information content (PIC):

The percentage of polymorphism per primer for six plant species of Asteraceae were ranging from 58% at primer d (H5) to 86% at each of c (B12) and e (P8) primers (Table, 5). Unique bands recorded the highest value (3) at H5 (d) primer meanwhile the lowest value (1) at a, c and e primers. Moreover highest Non-unique bands (5) were at c and e primers. *Urospermum picroides* gave two positive markers (M^+) by using two primers namely C1 (a) at AF06 and B12 (c) at AF06, meanwhile *Cichorium pumilum* gave two positive markers (M^+) by using N8 (b) at AF13 and AF14. Negative markers (M^-) were detected by *Cichorium pumilum at* amplicons AF03, moreover *Urospermum picroides* gave negative markers by using N8 (b) primer at AF10&AF11.

The dendrogram (Fig.2), of RAPD-PCR product profiles for 6 plant species by using 5 (a, b, c, d, e) primers classified the 6 plant species of Asteraceae into clusters. The clusters different according the primer used. Primersc and d classified the species into two main clusters. Primer c separated *Cichorium pumilum* and *Senecio desfontainei* in the first clusters as a genetically dissimilar species with all other ones in the second cluster, meanwhile primer d separated *Urospermum picroides* and *Cichorium pumilum* in the first clusters with all other ones in the second cluster.Primers b and e classified the species into three clusters, all of them nearest *Sonchus oleraceus* and *Urospermum picroides* than other species, moreover primer a classified the studied Asteraceae species into four clusters and separated each of *Sonchus oleraceus* and *Senecio desfontainei* separate cluster.

The similarity matrix values for 6 plant species of Asteraceae by using 5 primers were ranging from ranged from 61 to 88% (Table 6). The highest similarity was observed between *Sonchus oleraceus* and *Senecio desfontainei* (88%) and the lowest between *Bidens pilosa* and *Cichorium pumilum* (61%).



a

b

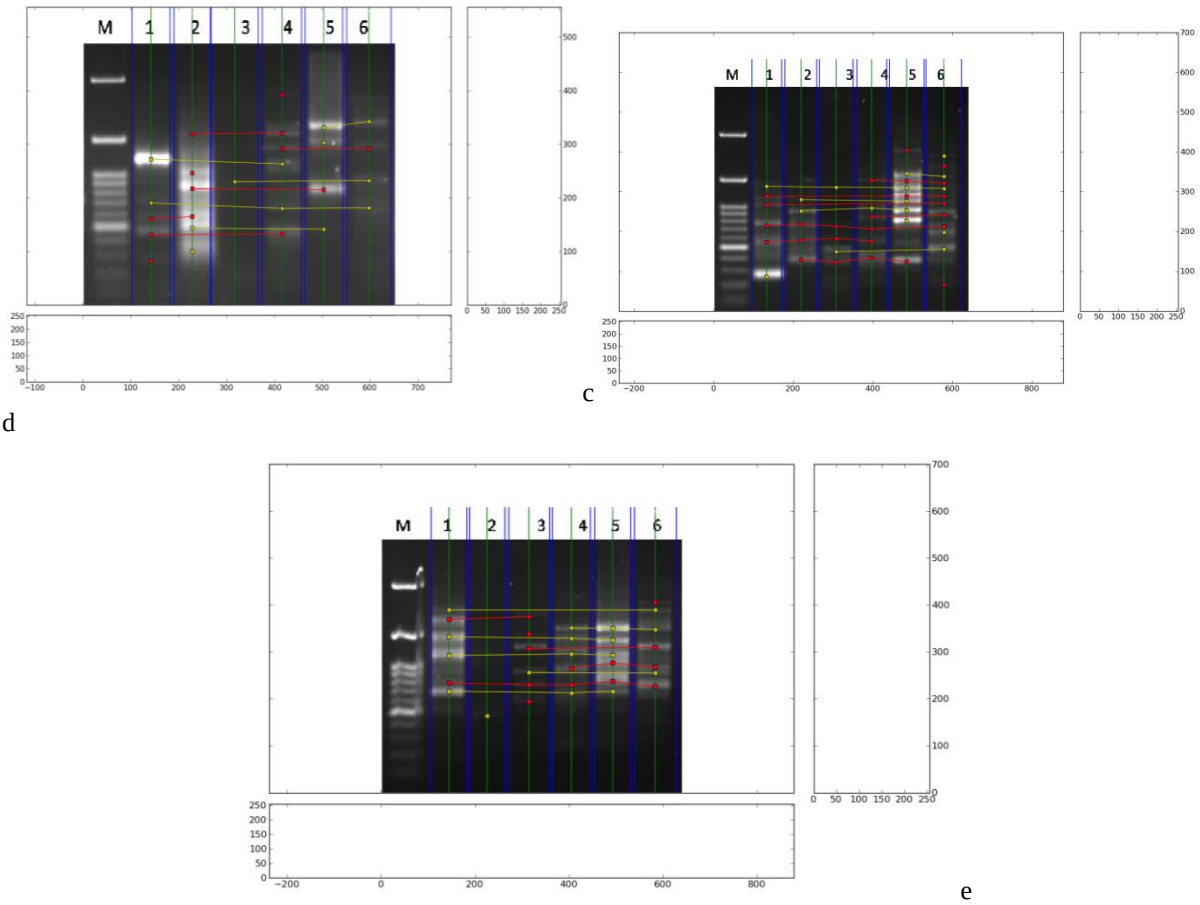


Fig. (1): RAPD-PCR product profiles of 6 plant species. Lane M = 100-bp molecular weight marker; lane1 = ***Sonchus oleraceus***; lane2 = ***Bidens pilosa*** lane3 = ***Senecio desfontainei*** lane4 = ***Lactuca serriola*** lane5 = *Urospermum picroides* lane***6=Cichorium pumilum*** a, b, c, d, e= RAPD primers. Arrows indicate the 800-bp position of the molecular weight marker.

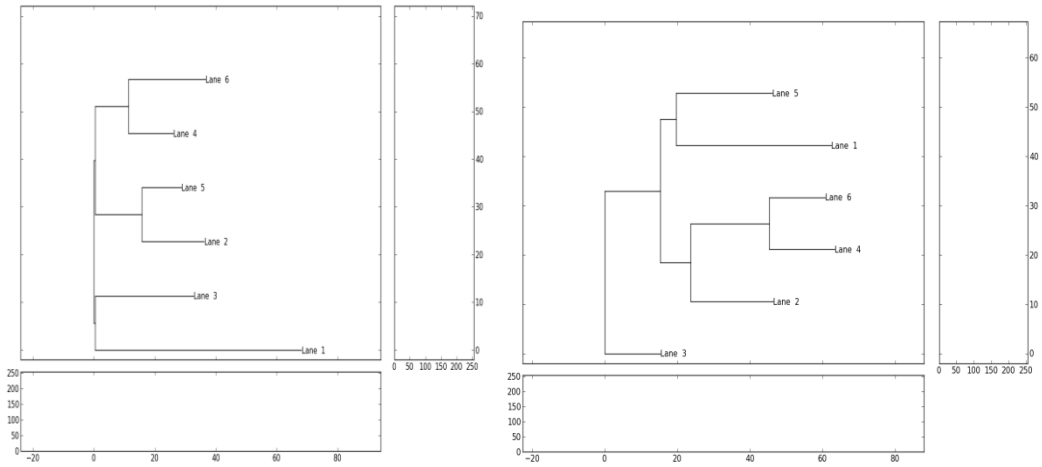
Table (4): RAPD-PCR product in six plant species belongAsteraceaeusing five primers

Amplicon	**Mol. S (bp.)**	**1**	**2**	**3**	**4**	**5**	**6**	**MM***
C1 (a)								
AF01	1500	1	1	1	1	1	1	
AF02	1200	1	1	1	1	1	1	
AF03	1000	1	1	1	1	0	1	M^-
AF04	900	0	1	0	1	0	1	
AF05	800	0	0	0	1	0	1	
AF06	700	0	0	0	1	0	0	M^+

N8 (b)								
AF07	1500	1	1	1	1	1	1	
AF08	1200	1	1	1	1	1	1	
AF09	1000	1	1	1	1	1	1	
AF10	900	1	1	1	0	1	1	M^-
AF11	800	1	1	1	0	1	1	M^-
AF12	700	0	1	1	0	1	1	
AF13	600	0	0	0	0	1	0	M^+
AF14	500	0	0	0	0	1	0	M^+
B12 (c)								
AF15	1500	1	1	1	1	1	1	
AF16	1200	1	1	0	1	1	1	M^-
AF17	1000	1	1	0	1	1	1	M^-
AF18	900	1	1	0	1	0	1	
AF19	800	1	1	0	1	0	0	
AF20	700	0	1	0	1	0	0	
AF21	600	0	0	0	1	0	0	M^+
H5 (d)								
AF22	1500	1	1	1	1	1	1	
AF23	1200	1	1	1	1	1	1	
AF24	1000	1	1	1	1	1	1	
AF25	900	1	1	1	1	1	1	
AF26	800	1	1	1	1	1	1	
AF27	700	0	0	0	1	1	1	
AF28	600	0	0	0	0	1	1	
AF29	500	0	0	0	0	1	1	
AF30	400	0	0	0	0	1	1	

AF31	300	0	0	0	0	0	1	M $^{+}$
AF32	200	0	0	0	0	0	1	M $^{+}$
AF33	100	0	0	0	0	0	1	M $^{+}$
P8 (e)								
AF34	1500	1	1	1	1	1	1	
AF35	1200	1	0	1	1	1	1	M $^{-}$
AF36	1000	1	0	1	1	1	1	M $^{-}$
AF37	900	1	0	1	1	1	1	M $^{-}$
AF38	800	1	0	1	1	1	1	M $^{-}$
AF39	700	1	0	1	1	1	1	M $^{-}$
AF40	600	0	0	0	0	0	1	M^{+}

1 = ***Sonchus oleraceus***; 2 = ***Bidens pilosa*** 3 = ***Senecio desfontainei*** 4 = ***Lactuca serriola*** 5 = *Urospermum picroides* ***6=Cichorium pumilum,*** *MM: Molecular marker M^{+}: Positive marker M^{-}: Negative marker



a b

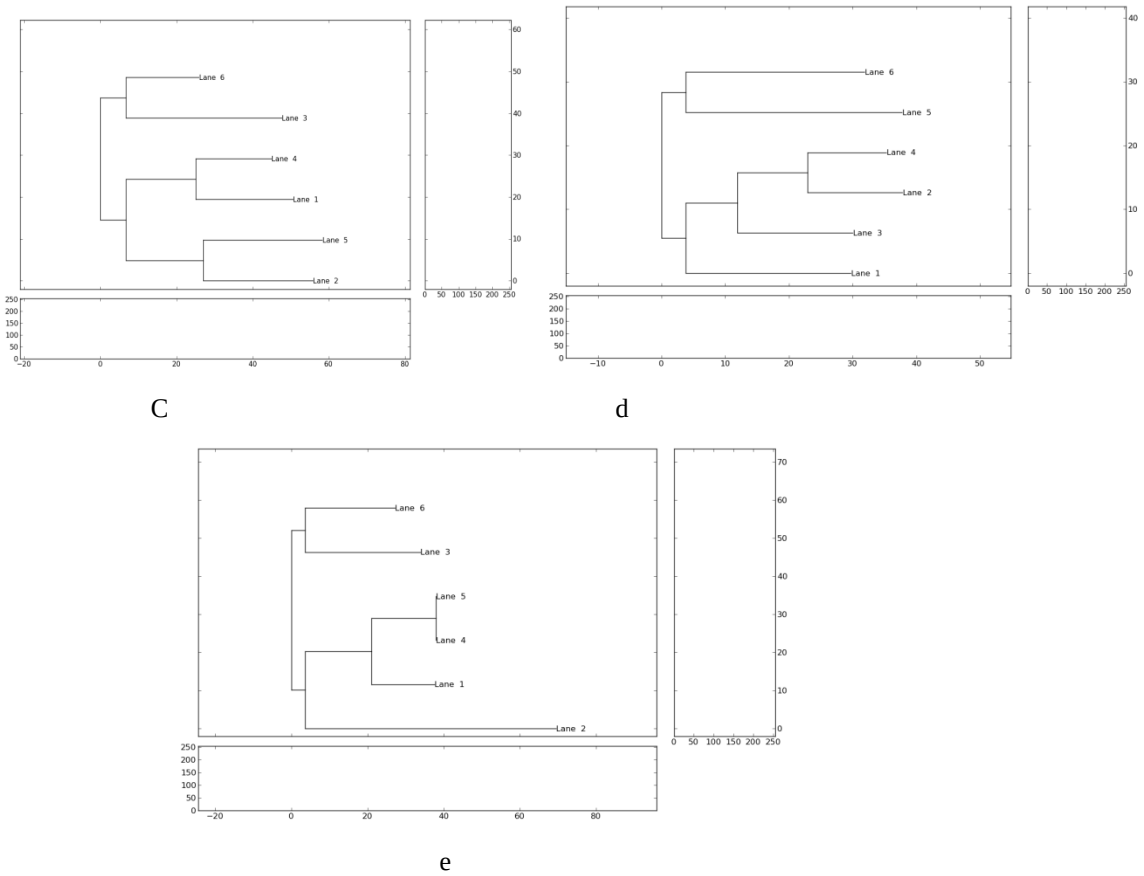


Fig. (2): The Dendrogram of RAPD-PCR product profiles for 6 plant species by using 5 primers(a, b, c, d, e), lane1 = ***Sonchus oleraceus***; lane2 = ***Bidens pilosa*** lane3 = ***Senecio desfontainei*** lane4 = ***Lactuca serriola*** lane5 = *Urospermum picroides* lane ***6=Cichorium pumilum***.

Table (5): Polymorphism per primer among the 6 plant species of Asteraceaeusing RAPD-PCR

Bands / Primers	MB	PB		TAF	P%
		UB	NB		
C1 (a)	2	1	3	6	67
N8 (b)	3	2	3	8	63
B12 (c)	1	1	5	7	86
H5 (d)	5	3	4	12	58
P8 (e)	1	1	5	7	86

MB= Monomorphic bands, PB = Polymorphic bands, UB=Unique bands, NB= Non- unique bands, TAF= Total number of amplified fragments, P%= Polymorphism percentage

Table (6): Similarity matrix (%)for 6 plant species of Asteraceae based on RAPD banding pattern

Species	1	2	3	4	5	6
1. *Sonchus oleraceus*	-		.			
2. *Bidens pilosa*	82	-				
3. *Senecio desfontainei*	88	74	-			
4. *Lactuca serriola*	84	76	73	-		
5. *Urospermum picroides*	80	68	81	71	-	
6. *Cichorium pumilum*	68	61	65	67	73	-

The present work was done for establishing a phylogenetic relationship between six species of Asteraceae collected from different locations from Sharkia province by using molecular marker. Molecular characterization depends on the quality of DNA successful isolated. Problems are reported for the isolation of plant DNA. DNA isolated contains colored materials as polysaccharides and phenolic compounds (Vanijajiva *et al.*, 2005). To allow the isolation of DNA from the plants studied, use of DNeasy plant Mini Kit, found suitable for RAPD-PCR amplification. RAPD has many advantages as rapidity, simplicity and not need any prior genetic information, source or age about the species (Welsh and McClelland, 1990; Micheli *et al.*, 1994). Shinde *et al*., (2007) showed that the advantageous of RAPD for the identification at little DNA exists in the dried material and also because sequence data are difficult to obtain. So RAPD has been successfully utilized for the identification of many medicinal plants (Tochika-Komatsu *et al.*, 2001; Um *et al*., 2001) and herbal medicinal constituents (Shinde *et al*., 2007). The significant similarity between species DNA sequences in the amplified region in sometime distinguishes plant species (Choo and Blenis , 2009)

The crucial factor for obtaining amplified products is PCR conditions, especially for plants (Jones *et al*., 1997). The overall temperature profiles (especially the annealing temperature) inside the PCR tubes are identical, RAPD fragments are then likely to be reproducible (Skroch and Nienhuis, 1995). This study represented that protocol worked well for the 6 plant species studied. The product obtained for the PCR analysis distinguish the 6 plant species even with the use of any primer from a to e. However, the combination of 5 primers cleared better resolution (pair wise similarity level ranged from 0 to 0.7). The obtained results are in agreements with other findings, which indicated the combination of primers provides better resolution (Vanijajiva *et al*., 2005).

The obtained results showed that the number of amplicons per primer and size of the amplified fragments varied between different species. Only one primer (H5, d)) scored five monomorphic amplicons out of 12 TAF, meanwhile two primers B12 (c) and P8 (e) scored one monomorphic amplicons out of 7 TAF. B12 (c) and P8 (e) produced the highest polymorphism scored five polymorphic amplicons out of seven. *Cichorium pumilum* gave the lowest similarity value across the other studied Aster species by using the five RAPD-PCR primers; meanwhile *Sonchus oleraceus* gave the highest value. *Cichorium pumilum* is one of the annual wild plants growing in Egypt, grows as one of the undesired weeds infecting Egyptian fields as well as other fields in some of the Mediterranean countries (Gervillaet *et al*., 2019 ; El-Shafey and AbdElgawad, 2020).*Sonchus oleraceus* is found almost everywhere in the world, edible and have medicinal value due to richer in vitamins A, D and E. It is broad-based most commonly occur in the temperate regions and tropical mountains (Puri *et al*., 2018).

The RAPD markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of the studied six species. The molecular variation, in combination with agronomic and morphological characteristics of species, may useful in the degree of tolerant. The data reported by each of (Pasqualone *et al*.. 2000 ; Carvalho *et al.*, 2009 ; Najaphy *et al.*, 2012) were in this line.

The Polymorphic information content (PIC) indicated that discrimination capacity is very important steps to evaluate the germplasm banks where many species need to be characterized and identified. The PIC per primer ranged between 58 to 86%. The obtained data is in same line with (Suresh *et al*., 2013), who showed that PIC values varied in faba bean genotypes by developing 55 novel polymorphic cDNA –SSR markers. (Zamanianfard *et al.*, 2015) stated that Polymorphism information content index (PIC) is a good efficiency of the used primers in

discrimination of the individuals. Moreover the low PIC data by some ISSR markers due to studied low number of ISSR loci. Many workers (Ebrahimi *et al.*, 2010; Pirseyedi *et al.*, 2010; Soriano *et al.*, 2011) recorded similar results for different plant species.

To explore the genetic relationship among the studied species of wild populations of *Asteraceae*, cluster dendrogram was constructed from combined data of molecular markers. The dendrogram separated the studied species into distinct clusters. The clusters different according the primer used. Primers c and d classified the species into two main clusters meanwhile primer c separated *Cichorium pumilum* and *Senecio desfontainei* in defiant clusters as a genetically dissimilar species. Primer d separated *Urospermum picroides* and *Cichorium pumilum* in the one cluster than all other species in the second cluster. Primers b and e classified the species into three clusters. The obtained data nearest *Sonchus oleraceus* and *Urospermum picroides* than other species, moreover primer a classified the studied Asteraceae species into four clusters and separated each of *Sonchus oleraceus* and *Senecio desfontainei* separate cluster. (Khaled *et al.,* 2017) showed the beneficial application of more than one marker to assess the degree of diversity and relatedness of samples, especially those growing in different sites. Moreover (Sharma *et al*., 2016) assess genetic divergence between 16 accessions of *Stevia rebaudiana* to evaluate the efficiency of RAPD marker for show the genetic diversity. The analysis of the data clustered the genotypes, based on their geographic locations. However, (Idrees and Irshad , 2014) reported that genetic markers of polymorphism due to a mutation in the genome loci or alteration of nucleotide and make it possible to identify genetic diversity between species. The authors concluded that the high polymorphism indicates that evaluating genetic diversity in the species.

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