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Research Article
Comparative Studies Between Annual and Perennial Sesbania Using Karyological, Biochemical and Molecular Studies

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Abstract

Background and Objective: Genetic diversity of two *Sesbania* species (annual and perennial) was studied via using different attributes karyotype formula, biochemical study (SDS-PAGE) and molecular studies (RAPD and ISSR markers). This study was focused on *Sesbania* due to its medicinal importance from the previous literature. The objective of the paper is studying the genetic conservation of *Sesbania* and put light on the main difference between annual and perennial one. **Methodology:** Seeds and young leaves of two species of *Sesbania* were collected from ten locations in Egypt and then the seeds prepared into slides via different steps of fixation and squashing. SDS-PAGE was done using the seeds of investigated taxa, in addition to two molecular markers RAPD and ISSR using five primers in each were used based on fresh young leaves of the investigated taxa. **Results:** This present investigation confirmed the chromosome number of two species of *Sesbania* (annual *Sesbania sericea* and perennial *Sesbania sesban*) was diploid 2n = 2x = 12. The karyotype parameters showed slight difference between ten taxa, where *Sesbania sericea* (Ssa_5) had the lowest values of TF (%) and Syi index in addition to the highest value of A_1, whereas, *Sesbania sesban* (Ssp_1) had the highest values of TF (%) and Syi index and also the lowest value of A_1. The SDS-PAGE analysis showed that two unique bands with molecular weight of 69 and 71 KDa were characteristic for annual *Sesbania* (*Sesbania sericea*), these two bands differentiate the annual species from the other studied perennial taxa. The RAPD and ISSR molecular markers yielded a total number of 57 bands out of which 31 were polymorphic ones and only 26 bands were found to be monomorphic. **Conclusion:** Karyological parameters beside biochemical and molecular attributes had the ability to study the genetic diversity of *Sesbania* species and can be used in the conservation of these species in gene banks.

Key words: *Sesbania sesban* (L.) Merr., *S. sericea* (Willd.) Link., Karyotype, SDS-PAGE protein profile, RAPD, ISSR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The choice of appropriate genetic markers assumes a great significance for genetic diversity studies. Although morphological aspects have been used traditionally to characterize levels and patterns of diversity, these characters alone represent only a small part of plant genome and influenced by the environmental changes[1,2]. In recent times, use of molecular marker for study of genetic diversity is increasing[3,4]. The genus *Sesbania* Scop. (Leguminosae) contains about 50 species, which are widely distributed in warm and usually wet regions of the world[5]. The majority of *Sesbania* species are annuals and some are relatively short-lived woody perennials. In Egypt, only two species of genus *Sesbania* were reported: *S. sesban* (L.) Merr., shrub or small tree (perennial) and *S. sericea* (willd.) Link which annual, casual in cultivated ground and along canal banks[5].

Sesbania sesban (L.) Merr. is the most productive multipurpose tree widely distributed in tropics and subtropics. Different parts of *S. sesban* is reputed for various purposes such as weed control, phytoremediation, anti-inflammation and anti-oxidant effect, anti-microbial activity, fire wood source, livestock feed and pasture improvement[6]. Having these and other multiple uses, nutrition and health security. In Egypt, only two species of genus *Sesbania* were reported: shrub or small tree (perennial) *S. sesban* (L.) Merr. located on Nile, canal banks and edges of cultivation and annual *S. sericea* (willd) Link., casual in cultivated ground and along canal banks[5]. The species of *S. sericea* has been found in Egypt, annual or short-lived perennial, casual in cultivated ground and along canal banks[5] and *S. sericea* seeds can also be tested as poultry feed because this seed has high protein value to chicken.

Karyotype analysis provides insights into genome organization at the chromosome level and into chromosome evolution. A karyotype describes the phenotypic aspects of the chromosome complement of a species in terms of number, size, arm ratio (or centromere position) and other landmark features of its chromosomes[7]. Among biochemical techniques, studying protein profile using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is widely used due to its validity and simplicity for describing genetic structure of crop germplasm[8]. The changes in protein banding pattern might have played a vital role in inducing differences[9,10].

Molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level and without interferences from environmental factors and where they involve techniques that provide fast results to detailing genetic diversity[11,12]. The RAPD markers have been widely used by various workers for identification of various crops and testing genetic purity of hybrids. They have been used in *Chrysanthemum*[13], Barley[14] and Chili[15]. The ISSR markers allow simple and cost-effective method to assess genetic variability and similarity within and among cultivars and amplify different regions of genomes. The best molecular markers for genome mapping, marker assisted selection, phylogenetic studied and crop conservation has law cost and labor requirements and high reliability is an inter simple sequence repeat (ISSR)[16]. Egypt have a wide range of plant and species diversity due its location in Mediterranean basin. Study the genetic diversity of wild plants considered a key for giving information that can be used about genetic sustainability and plant management of the species. The aim of the present investigation is to examine the genetic variability of two species of genus *Sesbania* Scop. (*S. sesban* (L.) Merr. and *S. sericea* (Willd.) Link.) in Egypt collecting from different natural habitats using various tools (chromosome number and karyological analysis, biochemical and molecular analysis).

MATERIALS AND METHODS

Sample collections: Two species of wild *Sesbania* annual (*Sesbania sericea*) and perennial (*Sesbania sesban*) (five taxa from each) were collected from three Governorates and 10 locations in Egypt in 2015. The paper was carried out in the duration from summer 2016-2017. Names of investigated taxa, codes and its locations were illustrated in Table 1.

Cytological analysis: The cytological investigation of the selected taxa was followed[17]. The nomenclature of chromosome type was determined by Abraham and Prasad[18]. Karyotype analysis was carried out using micro-measure computer program[19]. The mean measurements of five cells for each taxon were used to construct the karyotype.

Protein analysis: The method of discontinuous SDS-PAGE technique was based on Laemmli[20] to identify the relationships between the annual taxa of *Sesbania sericea* and perennial taxa of *Sesbania sesban*.

DNA extraction: Genomic DNA was extracted from the fresh young leaves of studied plant samples according to Dellaporta *et al.*[21].

Random amplified polymorphic DNA (RAPD-DNA): The RAPD reactions were performed according to Williams *et al.*[22] with some modifications PCR amplifications were performed using five primers. The sequence of these primers was given in Table 2.

Table 1: List of investigated taxa, codes and its locations

Species	Code	Location	Governorate	GPS	
				Longitude (E)	Latitude(N)
Sesbania Sericea (Annual)	Ssa_1	Al-Enshasia	El-Dakahlia	14°31'24.07"	50°30'24.07"
	Ssa_2	Belqas	El-Dakahlia	21°31'37.94"	12°31'59.12"
	Ssa_3	Damietta	Damietta	48°31'51.99"	25°31'3.14"
	Ssa_4	Mit-abo El-hussein	El-Dakahlia	20°31'44.11"	51°30'24.48"
	Ssa_5	Orman-Talkha	El-Dakahlia	22°31'36.90"	31°4'37.36"
Sesbania sesban (Perennial)	Ssp_1	Aga	El-Dakahlia	19°31'52.64"	53°30'57.75"
	Ssp_2	Borg El-Nor	El-Dakahlia	20°31'13.23"	56°30'2.35"
	Ssp_3	Mit-Ghamr	El-Dakahlia	15°31'40.17"	42°30'55.87"
	Ssp_4	Talkha	El-Dakahlia	22°31'48.87"	31°3'17.69"
	Ssp_5	Zagazig	Al-sharqia	30°31'14.64"	34°30'35.54"

Table 2: List of RAPD and ISSR primers name and the sequences used in this study, number of total bands, polymorphic bands and percentage of polymorphism of each primer generated

Marker	Primer name	Sequence	Size range(bp)	Polymorphic bands	Total bands	Polymorphism (%)
RAPD	OP-A01	5'-CAG GCC CTT C-3'	300-970	4	6	66.67
	OP-A07	5'-GAA ACG GGT C-3'	400-830	1	3	33.33
	OP-A13	5'-CAG CAC CCA C-3'	440-860	2	4	50.00
	OP-B04	5'-GAT GAC CGC C-3'	250-1060	3	8	37.50
	OP-C04	5'-CCG CAT CTA C-3'	280-1100	6	9	66.67
ISSR	14A	(CTC)3(TCT)2 TTG	350-800	2	4	50.00
	44B	(CTC)3(TCT)2 TGC	280-620	2	4	50.00
	HB10	(GAG)2(AGA)2TGC CC	350-940	3	6	50.00
	HB12	(CAC)3 GC	250-1160	8	8	100.00
	HB15	(GTG)3 GC	460-1000	0	5	0.00

Inter-simple sequence repeats DNA (ISSR-DNA): A total five primers were tested to amplify the isolated DNA. These primers listed in Table 2.

Data analysis: All gels were photographed and analyzed using Bio-Rad video documentation system, Model Gel Doc 2000. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0, respectively).

Cluster analysis and Biplot mapping were conducted to generate the possible relationships among ten taxa based on karyological, biochemical and molecular attributes using the SYSTAT version 7.0 program[23].

RESULTS

Cytological analysis: All ten studied taxa (five annual and five perennial) was diploid 2n = 2x = 12, metaphase surface view illustrated in Fig. 1. Karyotype parameters were reported in Table 3. The highest value of diploid chromosome length (DCL) in annual *Sesbania sericea* was found in Ssa_5 (681.216) and the lowest value in Ssa_4 (263.218). For perennial *Sesbania sesban*, the lowest value of DCL was present in Ssp_4 (241.487) and the highest value was recorded in Ssp_5 (719.451). The highest value of total chromosome volume (TCV) was found in *S. sesban* (Ssp_3) 3165.121 μ^3 on the other hand the lowest value was illustrated in *S. sericea* (Ssa_4) (171.339 μ^3).

For intrachromosomal asymmetry (A_1), the highest value was reported in Ssa_5 (0.549), while the lowest value was present in Ssa_3 (0.122). On the other hand, interchromosomal asymmetry (A_2) was varied from 0.173 in Ssa_5 to 0.379 in Ssp_2.

Sesbania sericea (Ssa_5) had the lowest values of TF (%) and Syi index in addition to the highest value of A_1, whereas, *S. sesban* (Ssp_1) had the highest values of TF (%) and Syi index and also the lowest value of A_1. For chromosome measurements, karyogram demonstrated karyotype formula in all ten taxa of *Sesbania* (annual and perennial) (Fig. 2) as nearly metacentric (-) (nsm -) and nearly metacentric (nm) were found in all taxa with different in number. Ideograms of haploid complement of *Sesbania* was shown in Fig. 3.

Seed protein electrophoresis: Protein electrophoresis analysis for ten taxa of two species of *Sesbania* was accomplished using SDS-PAGE method. The banding patterns of SDS-PAGE gel was represented in Fig. 4, while Table 4 illustrated the band pattern for the investigated taxa. There are 10 total bands were generated from the protein gel with molecular weight ranged from 19-122 KDa. These bands divided into 5 monomorphic bands and 5 polymorphic bands. Two unique bands with molecular weight of 69 and 71 KDa were characteristic for annual *Sesbania* (*Sesbania sericea*), these two bands differentiate the annual species from the other studied perennial taxa. Band of molecular weight 70 Kda

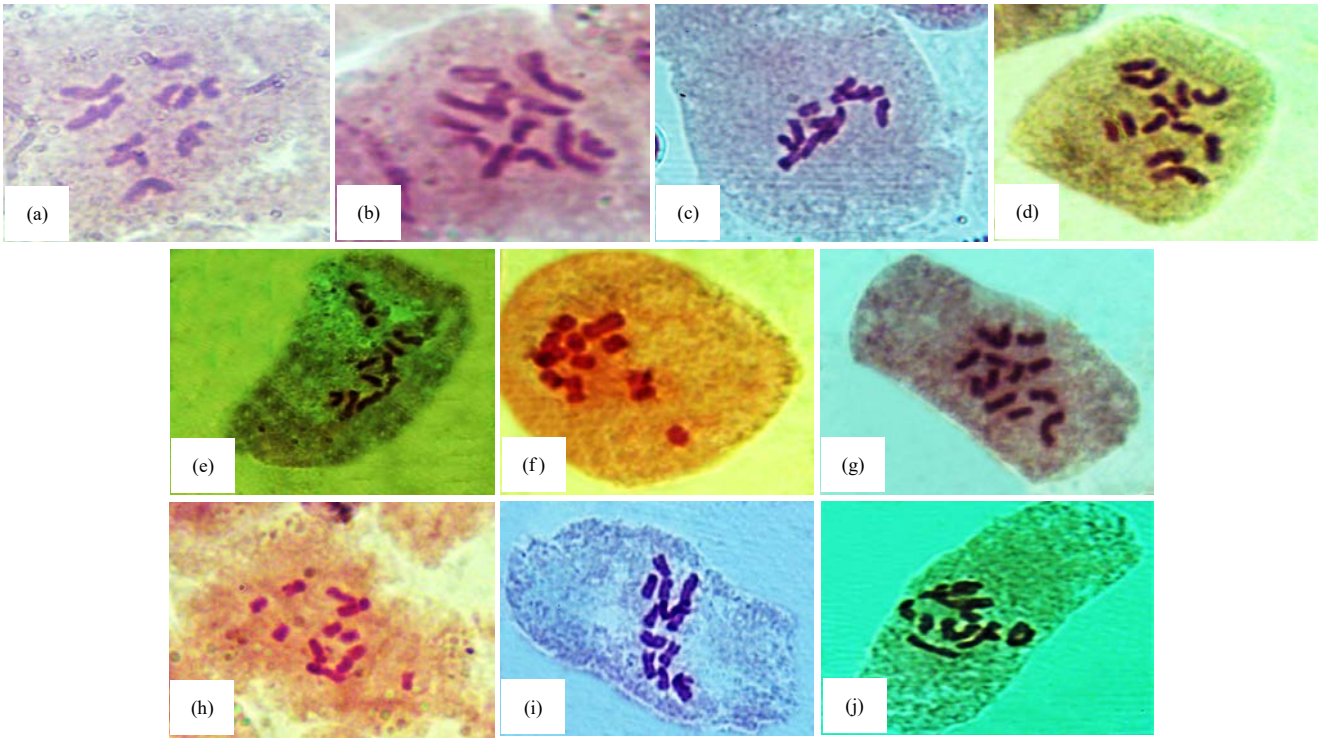

Fig. 1(a-j): Chromosomes of ten taxa of *Sesbania* species (*S. Sericea* and *S. sesban)* (X = 1000), (A) = Ssa_1: *Sesbania sericea* (annual) collected from Al- Enshasia (El-Dakahlia Governorate), (B) = Ssa2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (C) = Ssa3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (D) = Ssa4: *Sesbania sericea* (annual) collected from Mit-Abo El hussein (El-Dakahlia Governorate), (E) = Ssa5: *Sesbania sericea* (annual) collected from Orman -Talkha (El-Dakahlia Governorate), (F) = Ssp1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (G) = Ssp2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (H) = Ssp3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (I) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), (J) = Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

Table 3: Karyotype parameters of somatic chromosomes for the studied accessions of annual and perennial *Sesbania*

No.	Taxa	DCL	MCL	TCV	S%	TF%	A1	A2	Syi index	Rec index	Karyotype formula
1	Ssa_1	323.209	53.868	284.110	28.810	37.813	0.433	0.188	60.807	73.712	4nsm(-)+8nm
2	Ssa_2	654.241	109.040	1080.940	22.717	39.536	0.343	0.367	65.388	61.905	8nsm(-) +4nm
3	Ssa_3	614.072	102.345	2349.390	33.546	42.127	0.211	0.268	72.791	63.232	2 nsm(-) +10 nm
4	Ssa_4	263.218	34.869	171.339	27.636	42.485	0.221	0.262	69.699	64.624	2 nsm(-) +10 nm
5	Ssa_5	681.216	113.536	2117.240	25.878	31.981	0.549	0.173	47.018	71.659	10 nsm(-) +2 nm
6	Ssp_1	247.031	41.172	364.345	30.484	42.759	0.122	0.312	73.868	63.346	2 nsm (-) +10 nm
7	Ssp_2	326.313	54.385	287.414	24.344	41.072	0.231	0.379	69.694	56.071	2 nsm(-) +10 nm
8	Ssp_3	647.940	107.991	3165.121	27.245	39.007	0.256	0.297	63.953	62.377	4 nsm(-) +8 nm
9	Ssp_4	241.487	40.248	203.151	26.149	39.318	0.262	0.318	64.794	64.239	4 nsm(-)+8 nm
10	Ssp_5	719.451	119.923	1867.383	22.023	39.638	0.362	0.359	61.557	61.268	4 nsm(-)+8 nm

DCL: Diploid complement length, MCL: Mean chromosome length, TCV: Total chromosome volume, S (%): Symmetry (%), TF (%): Total form (%), A_1: Intrachromosomal asymmetry index, A_2: Interchromosomal asymmetry index, Syi: Symmetric indices, Rec: Resemblance between chromosomes. (1) = Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (2) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (3) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (4) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo El hussein (El-Dakahlia Governorate), (5) = Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), (1) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (2) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (3) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (4) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), (5) = Ssp_5: *Sesbania sesban* (perennial)collected from Zagazig (Al-Sharkia Governorate)

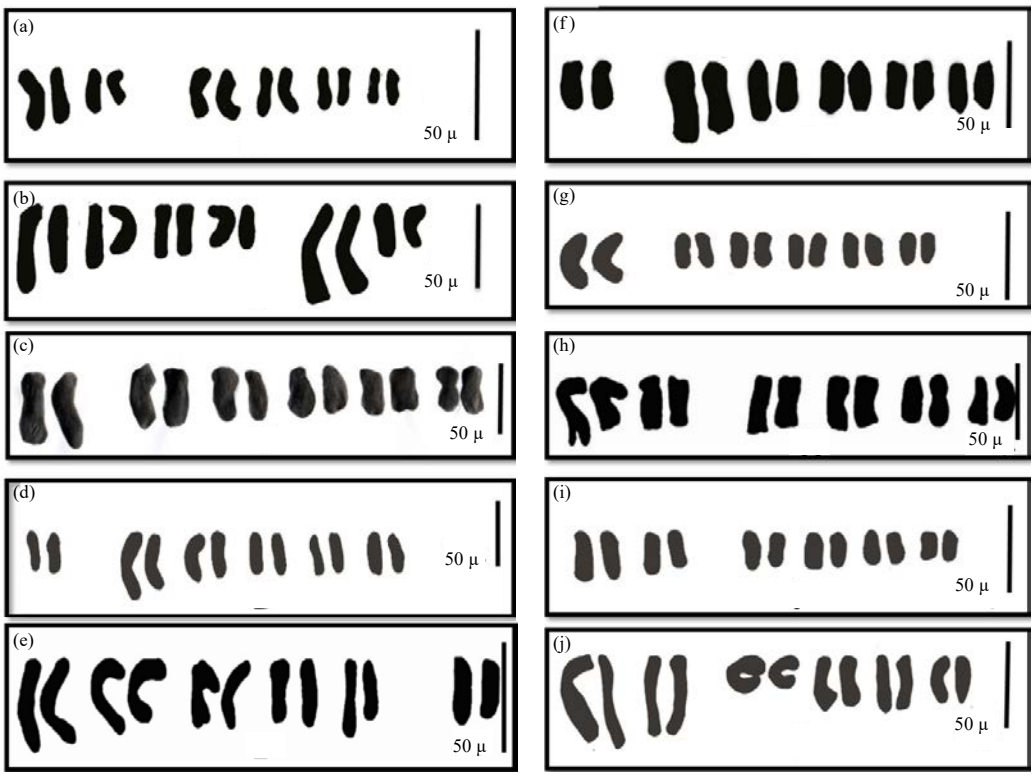

Fig. 2(a-j): Karyogram of ten taxa of *Sesbania*. (a) = Ssa_1 *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (b) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (c) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (d) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), (e) = Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), (f) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (g) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (h) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (i) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate) and (j) = Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

Table 4: SDS-PAGE of protein banding patterns for the studied accessions of annual and perennial *Sesbania*

		Sesbania sericea					*Sesbania sesban*				
Band No.	Codes (KDa)	1	2	3	4	5	6	7	8	9	10
1	122	1*	1*	1*	1*	1*	1*	1*	1*	1*	1*
2	73	1*	1*	1*	1*	1*	1*	1*	1*	1*	1*
3	71	1	1	1	1	1	0	0	0	0	0
4	70	0	0	0	0	0	1	1	1	1	1
5	69	1	1	1	1	1	0	0	0	0	0
6	65	1*	1*	1*	1*	1*	1*	1*	1*	1*	1*
7	62	1	1	1	0	1	0	1	1	1	1
8	25	1	1	1	0	1	1	1	1	1	1
9	22	1*	1*	1*	1*	1*	1*	1*	1*	1*	1*
10	19	1*	1*	1*	1*	1*	1*	1*	1*	1*	1*
Total bands	9	9	9	7	9	7	8	8	8	8	
Polymorphism for two species (%)		22.2%					12.5%				

(1) = Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (2) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (3) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (4) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (Dakahlia Governorate), (5) = Ssa_5: *Sesbania sericea* (annual) collected from Orman Talkha (El-Dakahlia Governorate), (6) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (7) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (8) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit Ghamr (El-Dakahlia Governorate), (9) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), (10) = Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate). *Monomorphic band

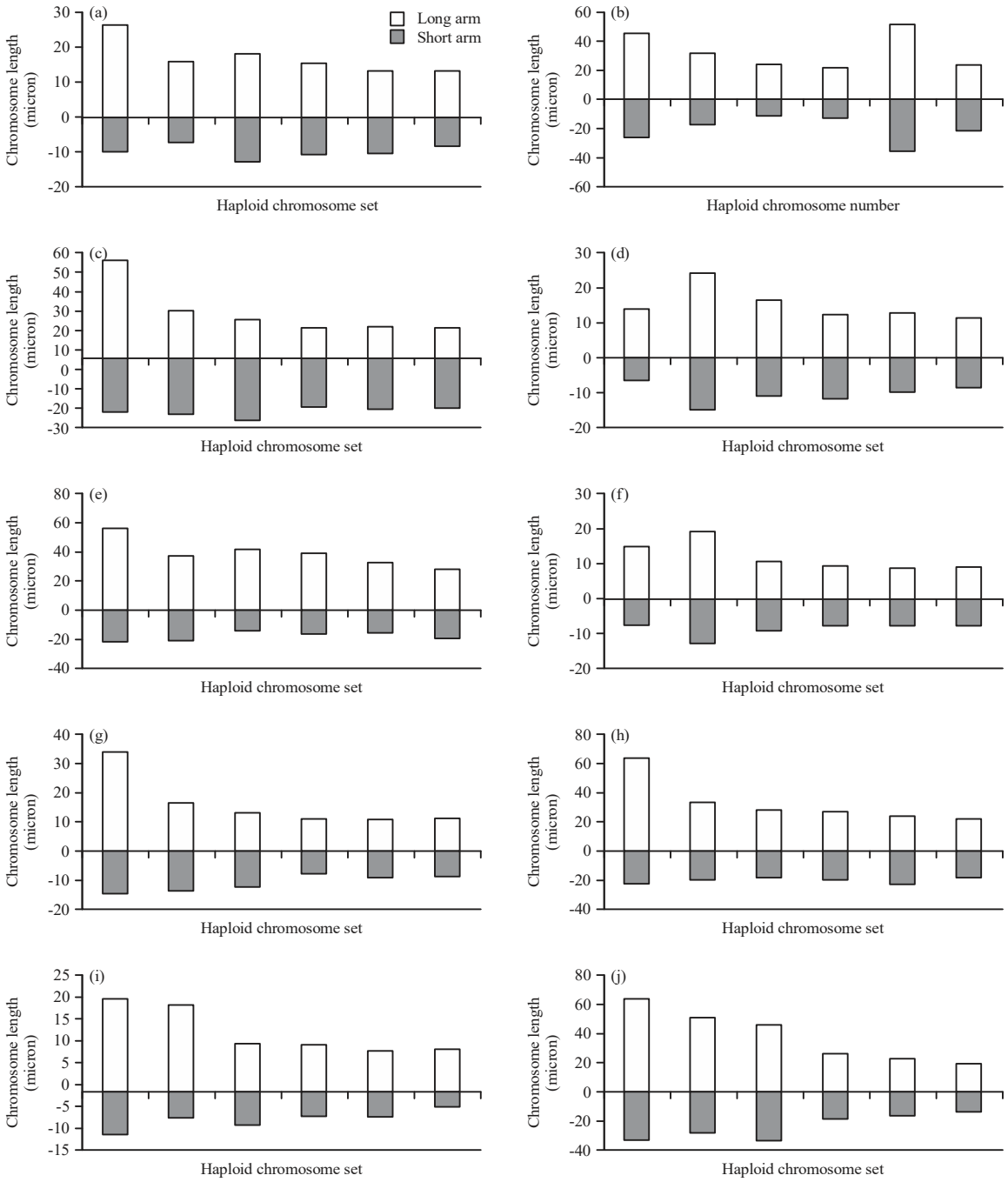

Fig. 3(a-j): Ideograms of haploid complement of *Sesbania*. (a) = Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (b) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (c) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (d) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), (e) = Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), (f) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (g) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (h) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (I) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate) and (j) = Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

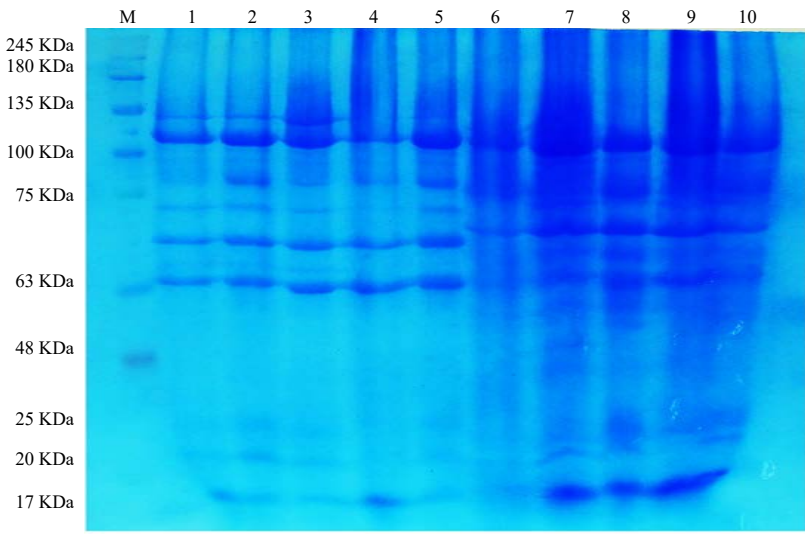

Fig. 4: SDS-PAGE of protein banding patterns gel for the studied accessions of annual and perennial *Sesbania*. M = Marker. (1) = Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (2) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (3) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (4) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), (5) = Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), (6) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (7) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (8) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (9) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), (10) = Ssp_5: *Sesbania sesban* (perennial)collected from Zagazig (Al-Sharkia Governorate)

was recorded for perennial *Sesbania* (*Sesbania sesban*) and absent in the annual taxa. The highest percentage of polymorphism 22.2% was reported in annual *Sesbania* (*Sesbania sericea*), while the lowest percentage of polymorphism 12.5% was found in perennial *Sesbania* as shown in Table 4.

Molecular analysis (RAPD and ISSR): For RAPD and ISSR molecular marker, five primers were used in each marker. The range of band products, number total bands and polymorphic bands produced by different primers were illustrated in Table 2, RAPD-PCR and ISSR-PCR product profiles gel in Fig. 5.

For RAPD molecular, a total 30 bands were amplified with the size range 2500-1100 bp, these bands were divided into 16 polymorphic and 14 monomorphic bands with percentage of polymorphism (55.33%) (Table 5). Regarding primer OP-C04 of RAPD, band of molecular weight 660 bp was recorded in five taxa of *S. sericea* (annual) and absent in the taxa of *S. sesban* (perennial). The highest number of total bands was generated using primer OP-C04 in addition to the highest percentage of polymorphism (66.67%) and also presented using primer OP-A01. The lowest value of polymorphism (33.33%) generated from using primer OP-A07 as shown in Table 2.

For ISSR marker, out of a 27 total bands with 12 monomorphic bands and 15 polymorphic bands were produced using five primers, the polymorphism of ISSR marker was 55.56% as shown in Table 5. Regarding to primer 44B, the band of molecular weight 500 bp was recorded for *S. sesban* only as unique band and absent from the taxa of *S. sericea*. Also band of molecular weight 500 bp produced using HB-10 primer was characteristic for five taxa of *S. sesban.* Regarding to primer HB-12, characteristic band of molecular weight 860 bp was found in all taxa of *S. sericea* distinguished this species from perennial *S. sesban*. The highest number of total bands (8) in addition to the highest value of polymorphism (100%) were recorded in primer HB-12, while the lowest polymorphism (0%) was produced using primer HB-15 Table 2.

Data analysis: To illustrate the genetic relationships among the studied taxa, genetic distances were measured based on karyotype parameters in addition to biochemical and molecular attributes. Biplot mapping by using perceptual mapping (PERMAP) showed the importance and the ability of some parameters as: A1, A2, TF%, Syi index and protein polymorphism beside OP-A01 and 44B primers to separate the

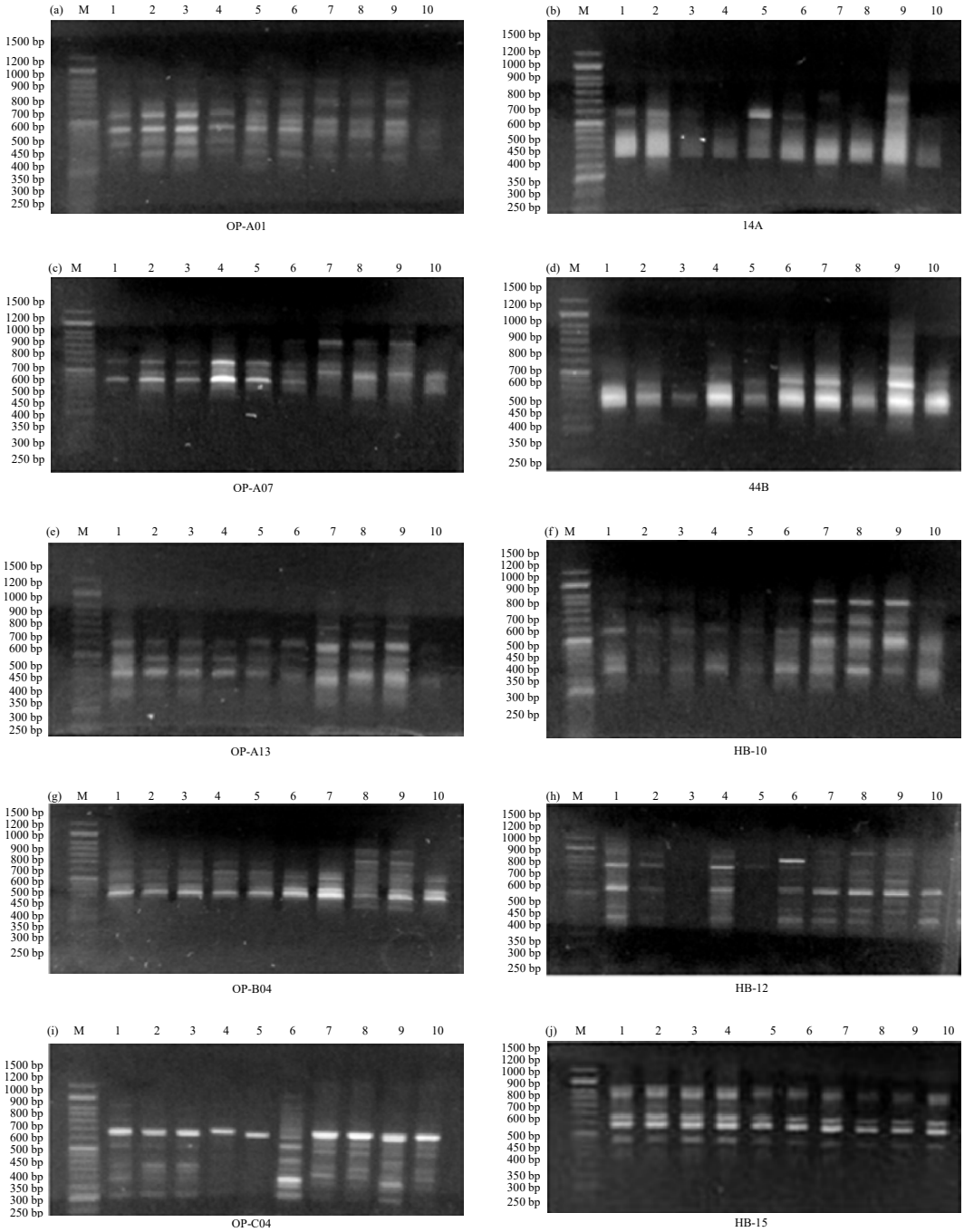

Fig. 5(a-j): RAPD-PCR and ISSR-PCR product profiles of annual and perennial species of *Sesbania*. M = Maker. (a) = Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), (b) = Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), (c) = Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), (d) = Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), (e) = Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), (f) = Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), (g) = Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), (h) = Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), (i) = Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate) and (j) = Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

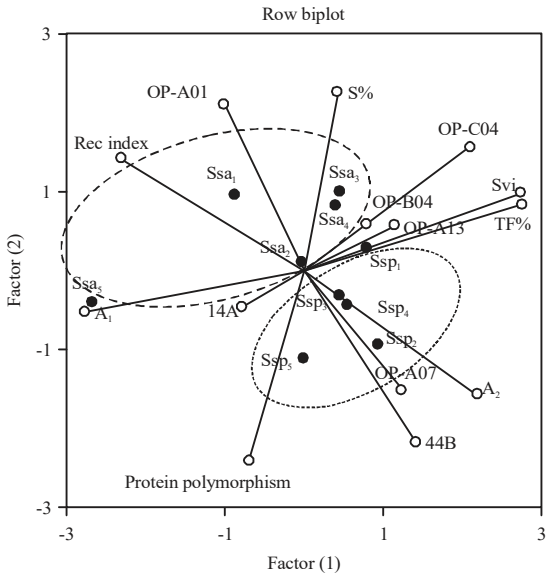

Fig. 6: Biplot (perceptual mapping) of the studied taxa for *Sesbania* species based on karyological, biochemical and molecular attributes. Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate). Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

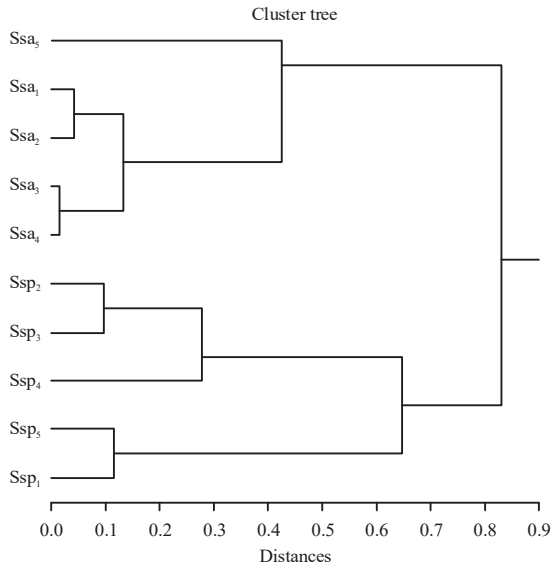

Fig. 7: Custer analysis showing the relationships between the studied taxa of *Sesbania* species using 1-Pearson correlation coefficient Ward minimum variance method. Ssa_1: *Sesbania sericea* (annual) collected from Al-Enshasia (El-Dakahlia Governorate), Ssa_2: *Sesbania sericea* (annual) collected from Blqas (El-Dakahlia Governorate), Ssa_3: *Sesbania sericea* (annual) collected from Damietta (Damietta Governorate), Ssa_4: *Sesbania sericea* (annual) collected from Mit-Abo-El hussein (El-Dakahlia Governorate), Ssa_5: *Sesbania sericea* (annual) collected from Orman-Talkha (El-Dakahlia Governorate), Ssp_1: *Sesbania sesban* (perennial) collected from Aga (EL-Dakahlia Governorate), Ssp_2: *Sesbania sesban* (perennial) collected from Borg El-Nor (EL-Dakahlia Governorate), Ssp_3: *Sesbania sesban* (perennial) collected from Mit-Ghamr (El-Dakahlia Governorate), Ssp_4: *Sesbania sesban* (perennial) collected from Talkha (El-Dakahlia Governorate), Ssp_5: *Sesbania sesban* (perennial) collected from Zagazig (Al-Sharqia Governorate)

Table 5: Bands characteristics produced by molecular markers (RAPD and ISSR) in ten taxa of *Sesbania*

Parameters	RAPD	ISSR		
Studied taxa	10	10		
No. of primers	5	5		
Marker range (bp)	250-1100	250-1160		
Total bands	30	27		
Monomorphic bands	14	12		
Polymorphic bands	16	15		
Unique bands				
No. of unique bands	1	3		
Size range	660	500	500	860
Primers	OP-C04	44B	HB-10	HB-12
Polymorphism (%)	53.33	55.56		

studied taxa of *Sesbania* into two groups the first group included all taxa *S. sesban*, the second group included *S. sericea* (Fig. 6).

Cluster analysis was conducted to generate a dendrogram (Fig. 7) illustrating the relationships among the ten studied taxa of genus *Sesbania*. The investigated data were divided into two groups at 0.648, the first group also divided in to subgroups at 0.426, first sub group included *S. sericea* (Ssa_5) collected from Orman-Talkha in separate group and the other taxa of *S. sericea* in second sub group. The second group included *S. sesban*. The pearson correlation showed the

Table 6: Pearson correlation between ten taxa of *Sesbania* Scop. Species

Code	Ssa_1	Ssa_2	Ssa_3	Ssa_4	Ssa_5	Ssp_1	Ssp_2	Ssp_3	Ssp_4	Ssp_5
Ssa_1	1.00									
Ssa_2	0.958	1.00								
Ssa3	0.90	0.932	1.00							
Ssa_4	0.917	0.93	0.985	1.00						
Ssa_5	0.67	0.793	0.722	0.656	1.00					
Ssp_1	0.747	0.789	0.698	0.721	0.664	1.00				
Ssp_2	0.69	0.693	0.782	0.763	0.57	0.608	1.00			
Ssp_3	0.69	0.673	0.787	0.768	0.502	0.504	0.903	1.00		
Ssp_4	0.733	0.705	0.61	0.628	0.492	0.74	0.745	0.789	1.00	
Ssp_5	0.546	0.57	0.528	0.528	0.583	0.884	0.659	0.57	0.748	1.00

highest similarity (0.985) between *S. sericea* (Ssa_3) collected from Damietta and *S. sericea* (Ssa_4) collected from Mit-Abo-El Hussein. The lowest similarity (0.492) was recorded between *S. sericea* (SS_5) collected from Orman-Talkha and *S. sesban* (Ssp_4) collected from Talkha (Table 6).

DISCUSSION

Genetic diversity is a great interest for investigating plant systematic biology and conservation[24,25]. Cytological, biochemical and molecular markers have been widely used for genetic diversity studies[26]. In plant taxonomy, breeding and genetic studies, information about chromosome karyotype can be useful in species identification and analysis of populations[27,28] (Karyotype characteristics especially chromosome number, chromosome length, total chromosome/complement length, relative length of particular chromosomes, arm ratio, presence, number and position of satellites are often compared to find the relationship between different taxa or differences within a taxon or for other biosystematics reasons[29].

The karyotype studies also are of a particular importance in the interpretation of evolutionary pathways[30]. All ten studied taxa (five annual *Sesbania sericea* and five perennial *Sesbania sesban*) was diploid 2n = 2x = 12. This result agreed with previous reports[31-34]. The basic chromosome number[35] of *Sesbania* is x = 6.

Stebbins[36] and Moore[37] had reported that the karyotype is varied in the degree of symmetry among the plant genera and species. Symmetrical karyotypes are considered more primitive than asymmetrical ones. The evolution of the latter from the former has been recorded in several genera of Fabaceae, for example *Trifolium*[38], *Medicago*[39].

The TF (%) index was bigger in *S. sesban*, this indicated a great proportion of metacentric chromosomes in this species. Asymmetry in karyotypes may be due to variation either in chromosome lengths or centromere position in different chromosomes of complement. The S% and relative length estimates give an idea about the variation in length of different chromosomes. The species of high A_1 values more advanced than others[40]. The TF% and Syi index values decrease with increasing asymmetry, while A1 increase with increasing asymmetry[41,42].

In the present study, according to TF% and Syi index, perennial *S. sesban* (Ssp1) may be had the most symmetrical karyotype. According to and A_1, *S. sesban* (Ssp1) may be considered the most symmetrical (more primitive) and annual *S. sericea* (Ssa5) may be had the most asymmetrical karyotype (more advanced) than other studied species. This is further supported by the high values of TF (%), which gives an estimate of mean position of centromere in different chromosomes.

The use of seed proteins as a taxonomic marker is well established[43]. Polyacrylamide gel electrophoresis (PAGE) is employed to detect differences in polymorphic proteins in different species[44], to evaluate the genetic variation among the accessions of the wild species[45] and to determine of genetic diversity between and within different plant species[46]. The electrophoretic analysis showed 10 total bands with a two unique band with molecular weight 69 and 71 KDa characteristic for annual *S. sericea*, while one unique band of molecular weight 70 KDa characteristic for perennial *S. sesban* as positive marker. The highest polymorphisms (71.4%) of this study using protein profile bands were greater than the percentage of *Trifolium* species (63.63 %) in the same family[38].

Molecular markers are widely used to evaluate both cultivated and wild species with different ploidy levels[47,48]. Different molecular markers used for genetic fingerprinting and deriving phylogenetic relationship include random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) in genetic variability assessment and breeding programmes[49].

In the present investigation, ten taxa from two species of *Sesbania* were fingerprinted using RAPD and ISSR markers. Five RAPD primers used, only one primer can differentiate between annual and perennial species. Similar observation was earlier made in the legume sub-tribe Millettieae[50], in contrast there are 19 RAPD primers had distinguished and give at is factory amplification between six species of *Sesbania*[51]. RAPD and ISSR markers yielded a total number of 57 bands

out of which 31 were polymorphic ones and only 26 bands were found to be monomorphic. This is indicative that high degree of polymorphism exists in genus *Sesbania*. Jamnadass *et al.*[52] observed high level of polymorphism in populations of *S. sesban* from Africa. The ISSR marker, three primers had the ability to differentiate between the annual and perennial *Sesbania*, 44B and HB-10 characteristic for *S. sesban*, while HB-12 distinguished for *S. sericea*. These results clarified the ability of ISSR analysis to differentiate among the investigated species of *Sesbania*.

Data analysis showed that investigated taxa separated into two groups one group contain annual *S. seiceae* and the other group included perennial *S. sesban*.

CONCLUSION

The content of this genetic information can be used for genetic analysis to obtain information about genetic sustainability and plant management of the species. The genetic diversity two *Sesbania* species reported important information on the conservation strategies for this species that conservation program, such as the construction of gene banks should be designed to capture the widest genetic variability among samples within populations.

SIGNIFICANCE STATEMENT

This study discovered the perennial *Sesbania sesban* may be considered the most symmetrical (more primitive) than annual *S. sericea* (more advanced can be beneficial for plant taxonomy; in addition to the ISSR molecular markers more efficient than RAPD markers with regards to polymorphism detection using in genetic diversity between *S. sericea* and *S. sesban*. This study will help the researchers to uncover the critical areas of plant conservation study that many researchers need to explore.

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