International Journal of Botany

Karyotype Analysis and Systematic Relationships in the Egyptian *Astragalus* L. (Fabaceae)

[1]Abdelfattah Badr and [2]Sherif M. Sharawy
[1]Department of Botany, Faculty of Science, Tanta University, 31529 Tanta, Egypt
[2]Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt

Abstract: In this study the karyotype criteria of 35 taxa representing 24 species of Egyptian *Astragalus* have been analyzed and their impact on the systematic delimitation of the studied species is discussed. Chromosome numbers, based on x = 8 have been found in the majority of *Astragalus* species in Egypt. A diploid number (2n = 16) was recorded in 22 taxa representing 17 species and polyploid numbers are recorded in six taxa representing three species. Numbers based on x = 7 were recorded in four taxa of which three counts are tetraploid with 2n = 28 representing *A. annularis*, *A. mareoticus* and *A. vogelii*. In addition, numbers based on x = 6 were encountered in *A. trimestris* (2n = 12 and 2n = 24) and *A. boeticus* (2n = 30). The chromosomes in the studied species of *Astragalus* are generally small with a mean size ranging between 0.82 and 1.59 μm. Short chromosomes were particularly found in *A. vogelii* (MCL = 0.82 μm) and *A. boeticus* (MCL = 0.87 μm), whereas longer chromosomes were scored in *A. sinaicus* (MCL = 1.59 μm). The karyotype in the studied taxa is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio that ranges between 1.35 in *A. vogelii* and 2.03 in *A. asterias*. The degree of karyotype asymmetry is indicated by high values of TF% that ranges between 36.11% in *A. asterias* and 47.48% in *A. tribuloides*. The A_1 value ranges between 0.40 in *A. vogelii* and 0.90-0.92 in samples of *A. asterias*. Distance trees illustrating the relationships of the studied taxa, based on the analyses of karyotype features, have been constructed using Dice and Jaccard similarity coefficients. The grouping of the examined species, in these trees, is discussed in the light of their previous systematic treatments.

Key words: *Astragalus*, Egyptian flora, chromosomes, karyotype, systematics

INTRODUCTION

The genus *Astragalus* L. of the family Fabaceae is the largest genus of flowering plants (Polhill, 1981) comprising over 2000 annual or perennial herbaceous or subshruby species grouped in 150 sections (Podlech, 1986). In Egypt, the genus is represented by 32-35 species, delimited in several sections and distributed in different phytogeographical regions of the country (El-Hadidi and Fayed, 1995; Boulos, 1999). Many species of *Astragalus* exhibit valuable economic values. *Astragalus hamosus* L., *A. multiceps* Benth and *A. tribuloides* Del., are medically useful. *Astragalus cicer* L. is a promising legume species for forage production (Towensend, 1981). Some species yield gum tragacantha, which is used by printers and dyers (Ali, 1961). However, several species of *Astragalus* have poisonous effects on grazing animals (James, 1983; Daniel *et al.*, 1984; Panter and Hartley, 1989).

The first deliberate study on the chromosomes of *Astragalus* was made by Ledingham (1960) who reported chromosome numbers for 84 species giving counts for 53 species that were not previously made. He found that the species from the old world have a basic chromosome number of x = 8, while those from the new world have x = 11, 12 and 13. That report was substantiated by another report by Ledingham and Rever (1963) in which further counts for 83 species were given. The studies on the cytology of *Astragalus* have ever since been made on species in different geographic regions of the World (www.mobot.mobot.org).

Chromosome counts, based on x = 8 have been reported in the vast majority of Old World species. In addition, counts based on base numbers of x = 7 or x = 6 have been encountered in few species (Maassoumi, 1987; Badr *et al.*, 1996; Malallah *et al.*, 2001). Meanwhile, studies on the cytology of *Astragalus* in America (Ledingham and Pepper, 1973; Martinez, 1974; Liston, 1990; Dopchiz *et al.*, 1995) confirmed the existence of basic numbers ranging between 11 and 15. The preponderance of species with a basic number of x = 8 led Badr *et al.* (1996) to conclude that it is the primary basic number in *Astragalus*. They further assumed that the x = 7 and x = 6 numbers have been derived from x = 8 by

aneuploid loss of chromosomes. However, comprehensive studies on the karyotype criteria of *Astragalus* species in relation to their systematic treatment are generally lacking.

The importance of chromosomal information in plant systematics and evolution has attracted the attention of several workers. At the generic level and below chromosome features have provided a range of possibilities for understanding the affinities of taxa. Examples illustrating the role of chromosomal data in solving systematic problems in plant genera are found in *Allium* (Badr and Elkington, 1978), *Plantago* (Badr and El-Kholy, 1986), *Ulex* (Fernandez *et al.*, 1993), *Sesbania* (Abou El-Enain *et al.*, 1998; El-Shazly and Abou El-Enain, 1999) and several others. As reported by several authors Ledingham (1960), Ledingham and Rever (1963) Ledingham and Pepper (1973), Martinez (1974), Dopchiz *et al.* (1995) and Badr *et al.* (1996), variation in chromosome number in *Astragalus*, differentiate Old World species from those of America.

The cytology of the Egyptian *Astragalus* was first studied by Badr *et al.* (1996) who described the karyotype for 14 taxa representing eight species. Chromosome counts for species of Egyptian *Astragalus* were substantiated by Sharawy (2001) who reported counts for 22 species. However, detailed karyotype analysis has not been made. In this study, we describe the karyotype criteria for 35 taxa representing 24 species of the Egyptian *Astragalus* and discuss the impact of the analyses of variation in these criteria on the systematic treatment of species.

MATERIALS AND METHODS

Material of 35 taxa representing 24 species, two subspecies and five varieties of the Egyptian *Astragalus* L. were collected, through 2000 to 2005, as mature flowering plants, from different localities in Egypt (Table 1). Herbarial sheets for all taxa are deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. To obtain seeds for cytological preparations, pods of all taxa were collected from healthy plants; left to dry at room temperature, seeds were then obtained from dry pods and kept at 4°C until use. For cytological investigations, young root tips were obtained from seeds that had been germinated in Pteri-dishes, pretreated with 0.05% colchicine solution for 3 h and fixed in 3:1 ethanol: glacial acetic acid for 24 h.

For cytological preparations, root tips were hydrolyzed for 6 min in 1 M HCl at 60°C, washed briefly in dd H_2O and stained in Feulgen = s solution for 1-2 h. Squashed preparation were made in 2 drops of 45% acetic acid and made permanent by rinsing in absolute alcohol and mounting in Euparal. Examination of chromosomes was made under the high power of light microscope

using oil emersion lens. Photographs of well-spread chromosomes were made using Carl-Zeiss photomicroscope III, at a magnification of 2500 and chromosomal measurements were made from photographic prints. Somatic number of chromosomes (2n) as counted in cytological preparations.

Total chromosome length of the haploid genome and the mean chromosome length±standard error (MCL±SE). Mean arm ratio±standard error (arm ratio±SE) was calculated for each karyotype by dividing the sum of long arms length on the sum of short arms length. Based on the measurements of chromosome length and arm ration, a karyotype has been constructed and a karyotype formula has been calculated, for each taxon, by arranging the chromosomes in homologous pairs or groups in order of decreasing length and arm ratio as proposed by Levan *et al.* (1965). The degree of karyotype asymmetry was also determined by calculating total form percent (TF %) using the equation of Huziwara (1962) as follows:-

$$TF\ (\%) = \frac{\text{Sum of short arm length}}{\text{Sum of total chromosome length}} \times 100$$

In addition, asymmetry based on the ratio between the chromosome arms ratio and length has been estimated for each taxon using the equation of Zarco (1986) as follows:-

$$A_1 = 1 - \Sigma \frac{b_i}{B_i} \div n_i$$

where:

A_1 = Intrachromosomal symmetry index that ranges from zero to one,

n_i = Number of homologous chromosome pairs or groups,

b_i = Average length for short arms in every homologous chromosome pair or group and

B_i = Average length for their long arms. The equation is formulated in order to obtain lower values when chromosomes tend to be metacentric.

In order to find out relationships based on the karyotype features of the studied taxa, total chromosome length per genome of all taxa has been plotted against the measures of karyotype asymmetry values (mean arm ratio, TF% and A_1 value). In addition, the recorded karyotype features have been coded and analyzed with the software NTSYS-pc 2.1 (Rohlf, 1993) using UPGMA (Sokal and Michener 1958) and Neighbor-joining (Saitou and Nei, 1987) methods in order to produce distance trees that illustrate the relationships among the studied taxa.

Table 1: A list of the taxa used in the present study, assigned to species and sections and summary of their karyotype features

Taxa and their sections	Localities	2n	x	TCL (μm)	MCL (μm) ±SE	Mean r-value ±SE	Karyotype asymmetry		Karyotype formula		
							TF%	A₁	m	sm	A
Section Ankylobus (Stev.) Bge.											
A. hispidulus DC.	Burg El Arab, 26 km south west of Alexandria										
A. hispidulus spp. Kralikianus	Wadi El Arish, Sinai	16	8	11.84	1.46±0.04	1.49±0.32	46.24	0.43	7	1	-
(Coss.) Täckh. ex Boulos.		16	8	11.68	1.48±0.06	1.50±0.20	45.80	0.48	5	3	-
Section Buceras Bge.											
A. hamosus L.	Alexandria-Matruh road, 115 km east of Matruh	32	8	10.00	1.25±0.04	1.40±0.22	41.66	0.52	6	2	-
A. hamosus v. *brachyceras* Ledeb.	Alexandria-Matruh road, 80 km east of Matruh Wadi	32	8	10.88	1.36±0.12	1.44±0.31	41.40	0.58	5	3	-
A. hamosus v. *brachyceras* Ledeb.	El Arish, Sinai	64	8	10.64	1.33±0.09	1.42±0.11	44.33	0.60	4	3	-
A. hamosus v. *buceras* Rouy.	Burg El Arab, 26 km south west of Alexandria	32	8	10.40	1.30±0.04	1.37±0.18	42.04	0.51	4	4	-
Section Carpini DC.											
A. carpinus L.	Matruh-Sallum road, 10 km west of Matruh	16	8	11.04	1.38±0.09	1.41±0.11	41.89	0.52	7	1	-
Section Chronopus Bge.											
A. dactylocarpous Boiss. spp. *acinaciferus* (Boiss)E	Saint Catherine, Wadi Sahab	16	8	11.76	1.47±0.06	1.53±0.09	40.92	0.56	5	3	-
A. fruticosus Forssk.	El Arish-Rafah road (El Kharouba)	16	8	11.92	1.49±0.14	1.51±0.18	39.96	0.49	5	3	-
A. sieberi DC.	Saint Catherine, Wadi Tabuk	16	8	11.68	1.46±0.11	1.52±0.22	40.64	0.45	5	3	-
A. trigonus DC.	Alexandria-Matruh road, 20 km east of Matruh	16	8	11.36	1.42±0.05	1.51±0.16	41.03	0.58	7	1	-
Section Cyamodes Bge.											
A. boeticus L.	Matruh-Sallum road, 10 km west of Matruh	30	6	5.22	0.87±0.05	1.66±0.26	37.56	0.84	3	2	1
Section Eremophysa Bge.											
A. kahiricus DC.	Gabal Labna, 60 km south of El Arish	16	8	11.76	1.47±0.13	1.55±0.07	39.45	0.57	6	2	-
Section Falcinellus Bge.											
A. eremophilus Boiss.	Cairo-Hurghada road, 100 km north of Hurghada	16	8	12.08	1.51±0.05	1.56±0.13	39.50	0.58	4	4	-
Section Haematodes Bge.											
A. annularis Forssk.	Alexandria–Matruh road, 115 km east of Matruh	14	7	10.71	1.53±0.17	1.51±0.18	39.93	0.56	6	2	-
A. annularis Forssk.	Wadi El Arish, Sinai	28	7	10.50	1.50±0.11	1.56±0.20	37.57	0.60	5	3	-
Section Harpilobus Bge.											
A. corrugatus Bert.	Saint Catherine, Wadi Sahab	32	8	11.44	1.43±0.15	1.48±0.35	41.27	0.52	5	3	-
A. hauarensis Boiss.	Saint Catherine, Wadi Sahab	16	8	10.56	1.32±0.06	1.48±0.28	41.67	0.50	6	1	1
A. mareoticus Del.	Cairo-Suez road, 85 km east of Cairo	28	7	9.66	1.38±0.09	1.61±0.47	38.86	0.48	4	1	1
A. trimestris L.	Rashid fixed sand dunes	24	6	8.58	1.43±0.07	1.47±0.24	41.68	0.49	6	-	-
A. trimestris L.	Port Said-El Arish road, 120 km west El Arish	12	6	8.94	1.49±0.08	1.41±0.32	41.41	0.43	6	-	-
Section Herpocaulos Bge.											
A. vogelii (Webb.) Bornm.	Hurghada-Safaga road, 40 km south Hurghada	28	7	5.74	0.82±0.04	1.35±0.16	42.68	0.40	7	-	-
Section Platyglottis Bge.											
A. bombycinus Boiss.	Cairo-Suez road, 85 km east of Cairo	16	8	11.04	1.38±0.13	1.60±0.21	38.92	0.51	5	3	-
A. bombycinus v. *sinaicus* Sirj.	Saint Catherine, Wadi El Arbeen	16	8	10.88	1.36±0.14	1.61±0.33	37.27	0.50	5	3	-
A. peregrinus Vahl.	El Zaranik Protectrate, 20 km west El Arish	16	8	10.32	1.29±0.05	1.62±0.12	38.53	0.48	8	-	-
Section Poterium Bge.											
A. spinosus (Forssk) Muschl.	Taba, Sinai	16	8	10.72	1.34±0.07	1.48±0.35	41.02	0.50	6	2	-
Section Sesamei DC.											
A. asterias Stev. ex Ledeb.	Burg El Arab, 26 km south west of Alexandria	24	8	11.36	1.42±0.12	2.03±0.30	36.71	0.90	2	5	1
A. asterias Stev. ex Ledeb.	Wadi El Arish, inside the airport	16	8	11.52	1.44±0.06	1.93±0.41	36.11	0.92	2	5	1
A. schimperi Boiss.	Saint Catherine, Wadi Sahab.	16	8	10.24	1.28±0.06	1.44±0.19	41.35	0..55	7	1	-
A. sinaicus Boiss.	Wadi El Arish, inside the airport	16	8	12.72	1.59±0.08	1.66±0.41	38.87	0.74	6	2	-
A. stella L.	Port Said-El Arish road, 120 km west El Arish	16	8	10.08	1.26±0.04	1.72±0.12	43.56	0.61	5	2	1
A. tribuloides Del.	Saint Catherine, Wadi Tabuk	16	8	11.04	1.38±0.13	1.79±0.19	47.48	0.65	6	1	1
A. tribuloides v. *mareoticus* Sirj.	Burg El Arab, 26 km south west of Alexandria	16	8	11.12	1.39±0.06	1.77±0.14	44.82	0.66	6	1	1
A. tribuloides v. *minutus* Boiss.	Burg El Arab, 26 km south west of Alexandria	16	8	10.72	1.34±0.04	1.89±0.49	44.99	0.63	5	2	1
A. tribuloides v. *minutus* Boiss.	Wadi El Arish, inside El Arish airport	16	8	10.56	1.32±0.03	1.81±0.47	43.27	0.61	5	1	1

TCL = Total Chromosome Length, MCL = Mean Chromosome Length, Mean r-ratio = Mean arm ratio, SE = Standard Error, TF% = Total Form percent, m = Metacentric chromosome, SM = Submetacentric chromosome and A = Acrocentric chromosome

RESULTS AND DISCUSSION

The cytological data for the examined taxa is summarized in Table 1 and their karyotypes are illustrated in Fig. 1-3. Chromosome numbers based on the basic number of x = 8 are found in the majority of the studied taxa, a diploid number of 2n = 16 is recorded in 22 taxa, whereas a triploid number (2n = 24) is recorded in one sample of *A. asterias* (section Sesamei). In addition, a tetraploid number (2n = 32) is counted in *A. corrugatus,* of section Harpilobus and in three samples of *A. hamosus* (section Buceras) and a hexaploid (2n = 64) is recorded in *A. hamosus* var. *brachyceras*. Numbers based on x = 7 are found in *A. annularis* (two samples) and *A. mareoticus* of section Harpilobus and in *A. vogelii* (section Herpocaulos); all these samples are tetraploid (2n = 28).
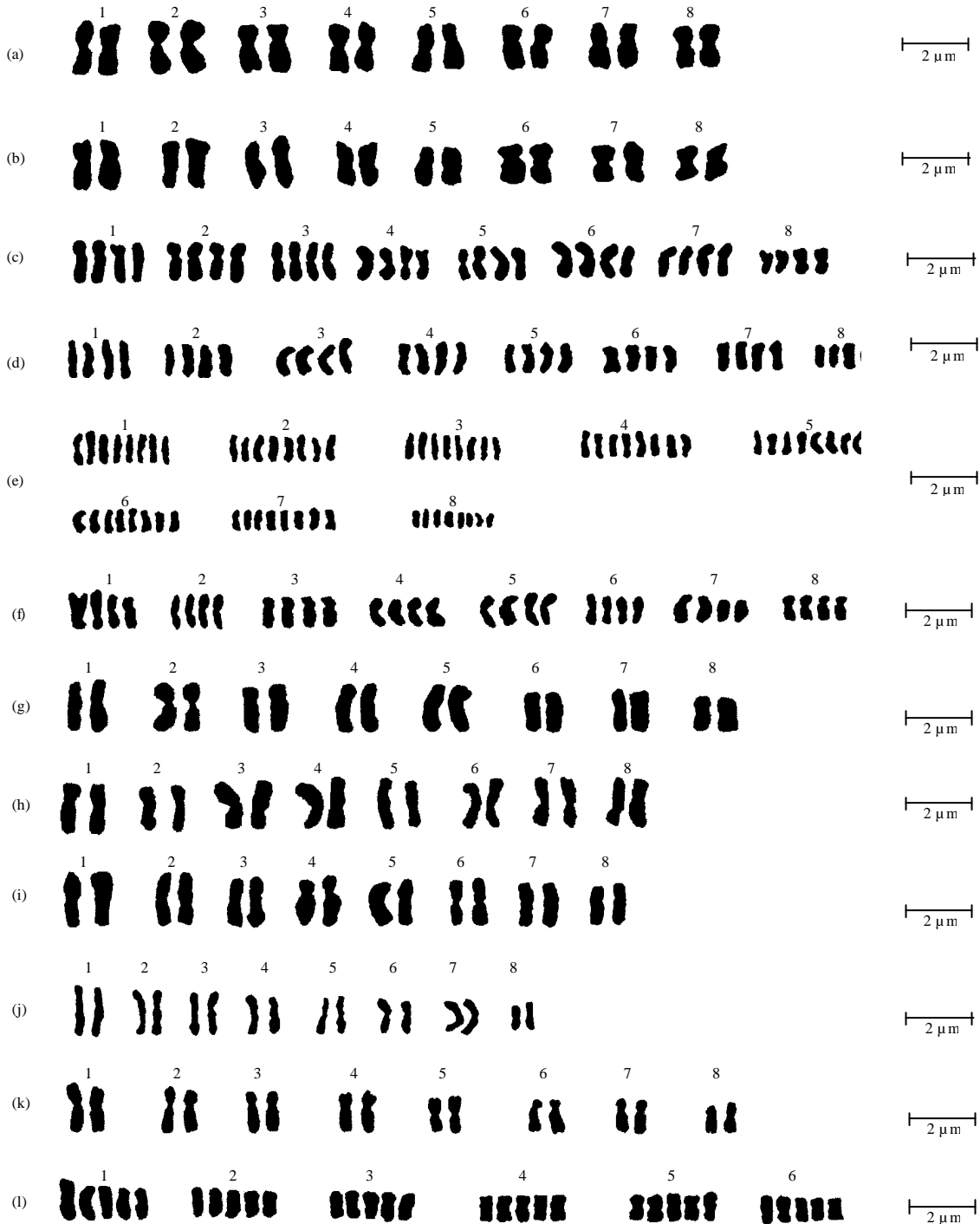
Fig. 1: Karyotypes of 12 taxa of Egyptian *Astragalus* L. a: *A. hispidulus*, b: *A. hispiauus* spp. *Kralikianus*, c: *A. hamosus*, d: *A. hamosus* var. *brachyceras*, e: *A. hamosus* var. *brachyceras*, f: *A. hamosus* var. *buceras*, g: *A . carpinus*, h: *A. fruticosus*, i: *A. sieberi*, j: *A. trigonus*, k: A. *dactylocarpous* spp. *acinaciferus* and l: *A. boeticus*
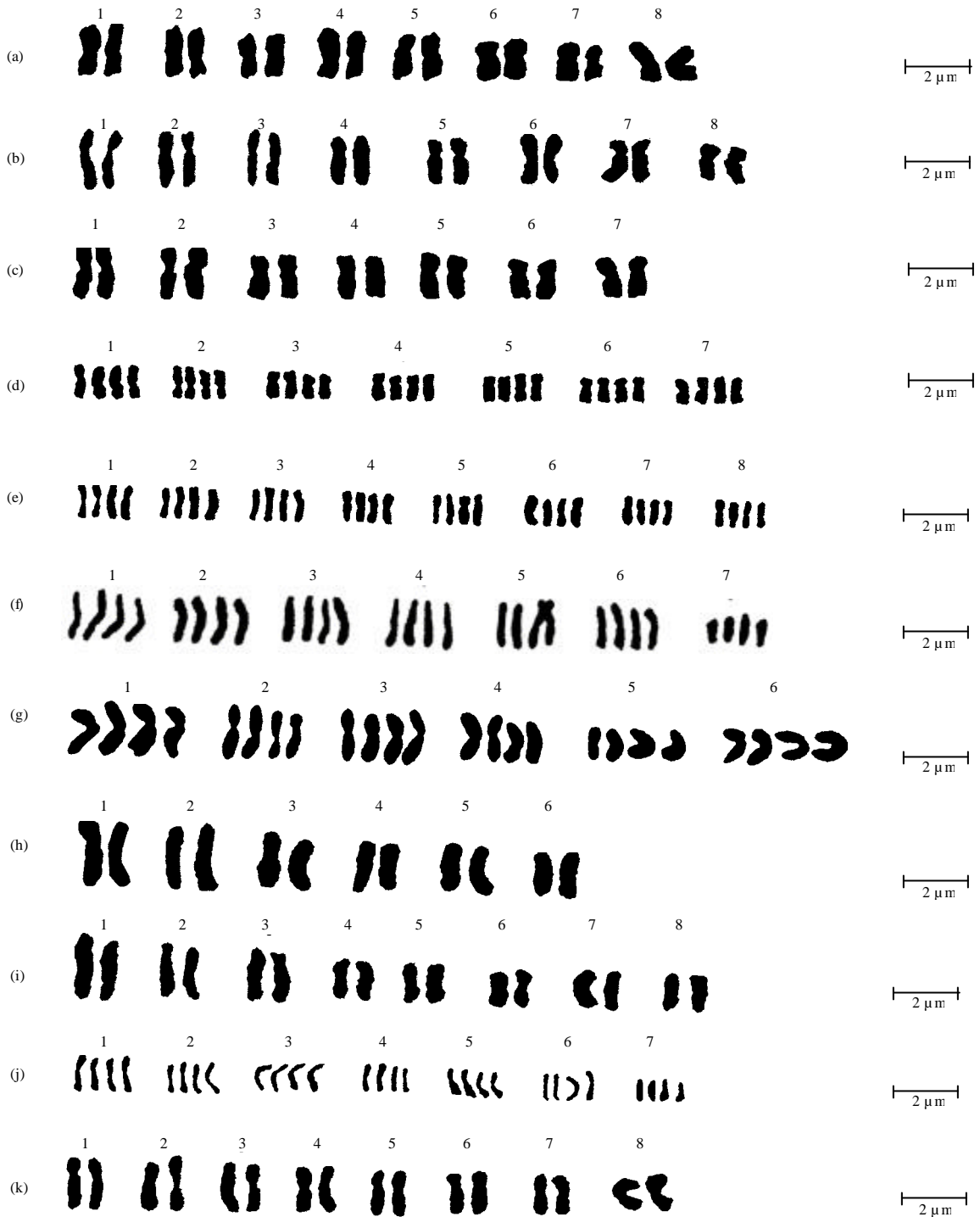
Fig. 2: Karyotypes of 11 taxa of Egyptian *Astragalus* L. a: *A. kahiricus*, b: *A. eremophilus*, c: *A. annularis*, d: *A. annularis*, e: *A. corrugatus*, f: *A. mareoticus*, g: *A. trimestris*, h: *A. trimestris*, i: *A. hauarensis*, j: *A. vogelii* and k: *A. bombycinus*

Fig. 3: Karyotypes of 12 taxa of Egyptian *Astragalus* L. a: *A. bombycinus* var. *sinaicus*, b: *A. peregrinus*, c: *A. spinosus*, d: *A. asterias*, e: *A. asterias*, f: *A. schimperi*, g: *A. sinaicus*, h: *A. stella*, i: *A. tribuloides*, j: *A. tribuloides* var. *mareoticus*, k: *A. tribuloides* var. *minutes* and l: *A. tribuloides* var. *minutes*. (Arrow indicates the position of the satellite)

However, one additional sample of *A. annularis* is found diploid with 2n = 14. Meanwhile, numbers based on x = 6 are encountered in two samples of *A. trimestris* of section Harpilobus; one diploid with 2n = 12 and the other tetraploid with 2n = 24 and in *A. boeticus* (section Cyamodes) where a pentaploid number (2n = 30) is recorded.

The chromosomes of the examined taxa of *Astragalus* are generally small. Total chromosome length (TCL) varies about two folds between species (Table 1). *Astragalus boeticus* of section Cyamodes (TCL = 5.22) and *A. vogelii* of section Herpocaulos (TCL = 5.74 μm) exhibit much shorter chromosomes compared to other species. Meanwhile, longest TCL (12.72 μm) have been found in *A. sinaicus* (x = 8) of section Sesamei. In the remaining taxa, TCL ranges between 8.58-894 μm in the two samples of *A. trimestris* and 12.08 μm in *A. eremophilus*. Similarly, shortest MCL was scored in *A. boeticus* (0.82±0.05 μm) and longest MCL in *A. sinaicus* (1.59±0.08 μm).

The karyotype in the studied taxa is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio. This ratio ranges between 1.35±0.16 in *A. vogelii* and 1.93±0.30 to 2.03±0.30 in *A. asterias* (Table 1). The low value of the Standard Error (SE) for mean arm ratio values indicates low degree of karyotype asymmetry in the studied taxa. The degree of karyotype asymmetry is also indicated by high values of TF% that ranges between 36.11% in *A. asterias* and 47.48% in *A. tribuloides*; the examined samples of the latter species and the two samples of *A. hispidulus* show higher TF% compared to other species. Similarly, the A_1 ranges between 0.40 in *A. vogelii* and 0.90-0.92 in the two samples of *A. asterias*. These values confirm the low karyotype asymmetry as indicated by the values of arm ratio and TF%.

The plotting of TCL against arm ratio (Fig. 4a) distinguish most of the species that have base numbers of x = 6 and x = 7. In particular, this figure clearly distinguish *A. boeticus* (2n = 30, x = 6) of Section Cyamodes and *A. vogelii* (2n = 28, x = 7) of Section Herpocaulos, the two samples of *A. trimestris* (2n = 12; 24, x = 6) of section Harpilobus and the two samples of *A. asterias* (2n = 16; 24, x = 8) and *A. sinaicus* (2n = 16) of section Sesamei. The plotting diagram of total chromosome length against the A_1 values (Fig. 4b) also clearly distinguished *A. boeticus*, *A. vogelii*, *A. trimestris*, *A. asterias* and *A. sinaicus*. The plotting diagram of total chromosome length against the values of total form percent (Fig. 4c) also clearly distinguished *A. boeticus*, *A. vogelii* and the two samples of *A. trimestris* and also the two samples of *A. hispidulus* (2n = 16, x = 8) of Section Ankylobus, the octaploid sample of *A. hamosus* (2n = 64,
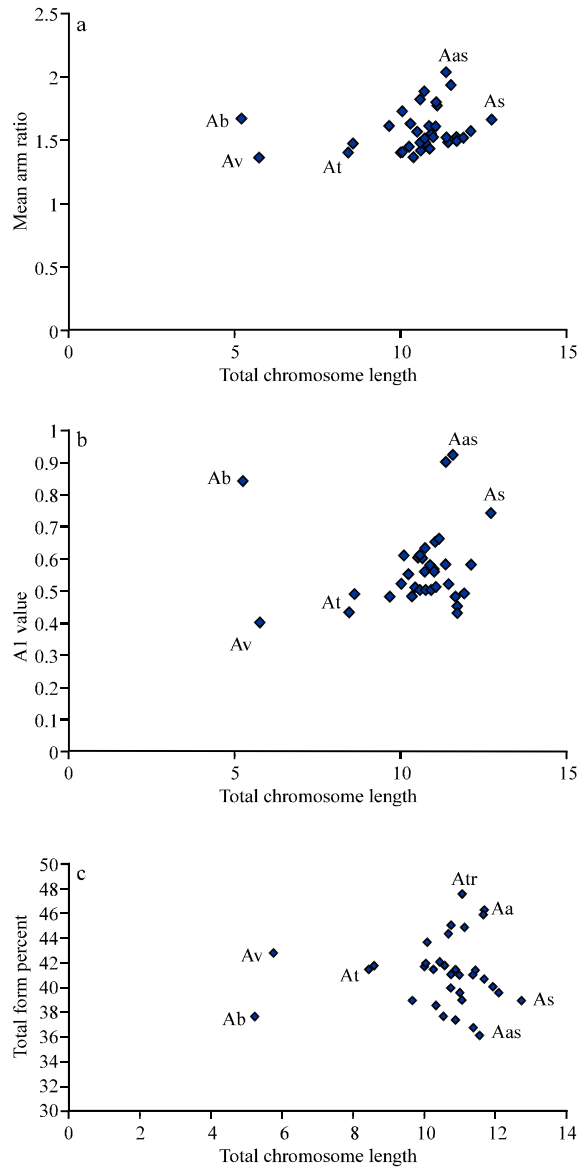

Fig. 4: The relationship between chromosome length and each of mean chromosome arm ratio (a), A_1 values (b) and total form percent (c). Aa = *A. annularis*, Aas = *A. asterias*, Ab = *A. boeticus*, As = *A. sinaicus*, At = *A. trimestris*, Atr = *A. tribuloides* and Av = *A. vogelii*

x = 8) of section Buceras and the four samples of *A. tribuloides* (2n = 16; 24, x = 8) of section Sesamei. The remaining taxa, that mostly have a numbers based x = 8 are not sufficiently differentiated by the plotting of chromosome length against the arm ratio or A_1 values.

The neighbor joining tree illustrating the relationships between the studied samples of *Astragalus*

is illustrated in Fig. 5. In this tree, *A. mareoticus* (x = 7), *A. boeticus* (x = 6) and *A. vogelii* (x = 7) and the samples of *A. hamosus*, with polyploid numbers based on x = 8 are separated, as different clusters, from a large group that comprises other taxa. In the latter group, the two samples of *A. trimestris* (x = 6), *A. corrugatus* (x = 8) and the two samples of *A. annularis* (x = 7) are also distinguished separate clusters at high distance. The remaining taxa (all with x = 8) are divided in two major groups; the first comprises two subgroups; a large one that includes *A. sinaicus*, *A. stella*, *A. tribuloides*, *A. schimperi* and *A. asterias* and a smaller one comprised of *A. peregrinus* and *A. bombycinus*. In the other group, *A. eremophilus* and *A. kahiricus* as well as *A. spinosis* and *A. haurensis* are differentiated from a group comprising the remaining seven samples. This group is differentiated into two clusters; one including *A. trigonus*, *A. dactylocarpous* and *A. carpinus* and other *A. sieberi*, *A. fruticosus* and the two samples of *A. hispidulus*.

The UPGMA tree illustrating the relationships between the studied samples of *Astragalus* (Fig. 6) also separated *A. mareoticus*, *A. boeticus* and *A. vogelii* and the samples of *A. hamosus*, as different clusters from other taxa. The two samples of *A. trimestris* are also clearly distinguished from other taxa. However, in this tree *A. corrugatus* and the two samples of *A. annularis* are not separated, as in the NJ tree whereas, the two samples of *A. hispidulus* are clearly delimited as a distinguished cluster. The remaining taxa are divided in two major groups; the first comprises two subgroups; a larger one that includes *A. stella*, *A. sinaicus*, *A. tribuloides*, *A. schimperi* and *A. asterias* The other group is divided into three subgroups; the first comprises *A. corrugatus* and the two samples of *A. annularis*; the second of *A. peregrinus*, *A. bombycinus*, *A. eremophilus* and *A. kahiricus* and the third of *A. spinosis* and *A. haurensis* and a cluster comprised of *A. sieberi*, *A. fruticosus*, *A. trigonus*, *A. dactylocarpous* and *A. carpinus*.
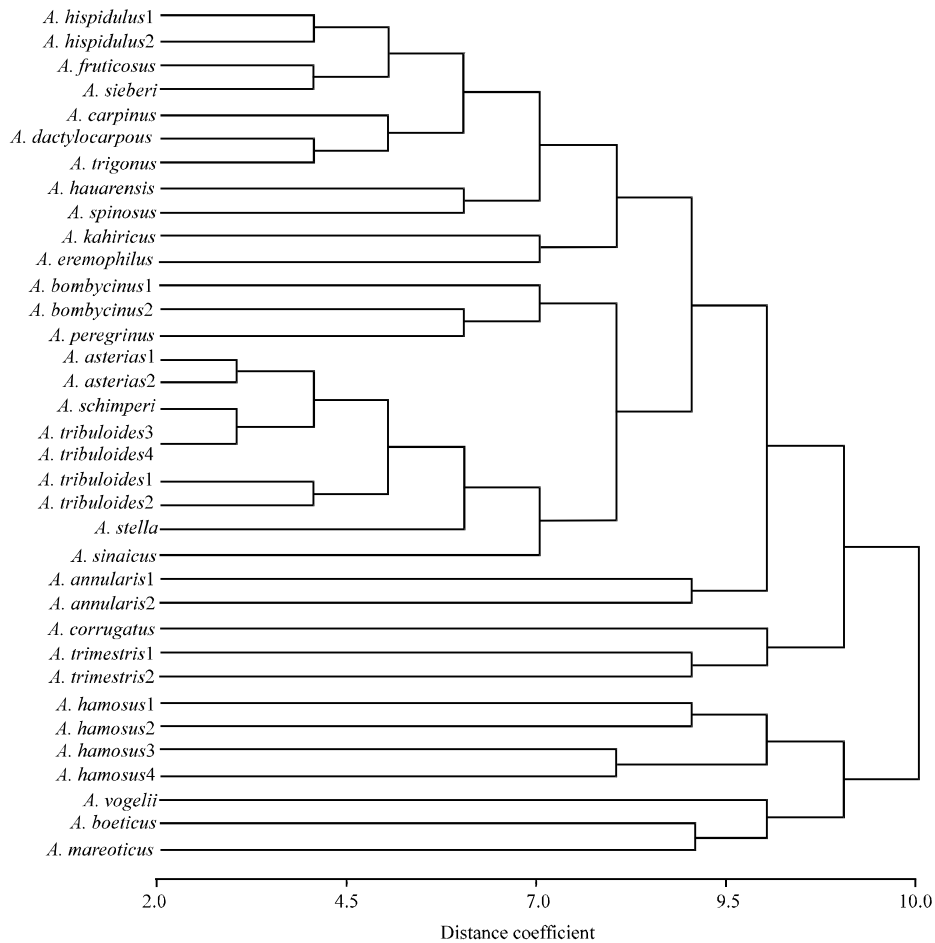

Fig. 5: A neighbor joining tree illustrating the relationships among the studied 35 taxa of *Astragalus*, based on the analysis of karyotype criteria
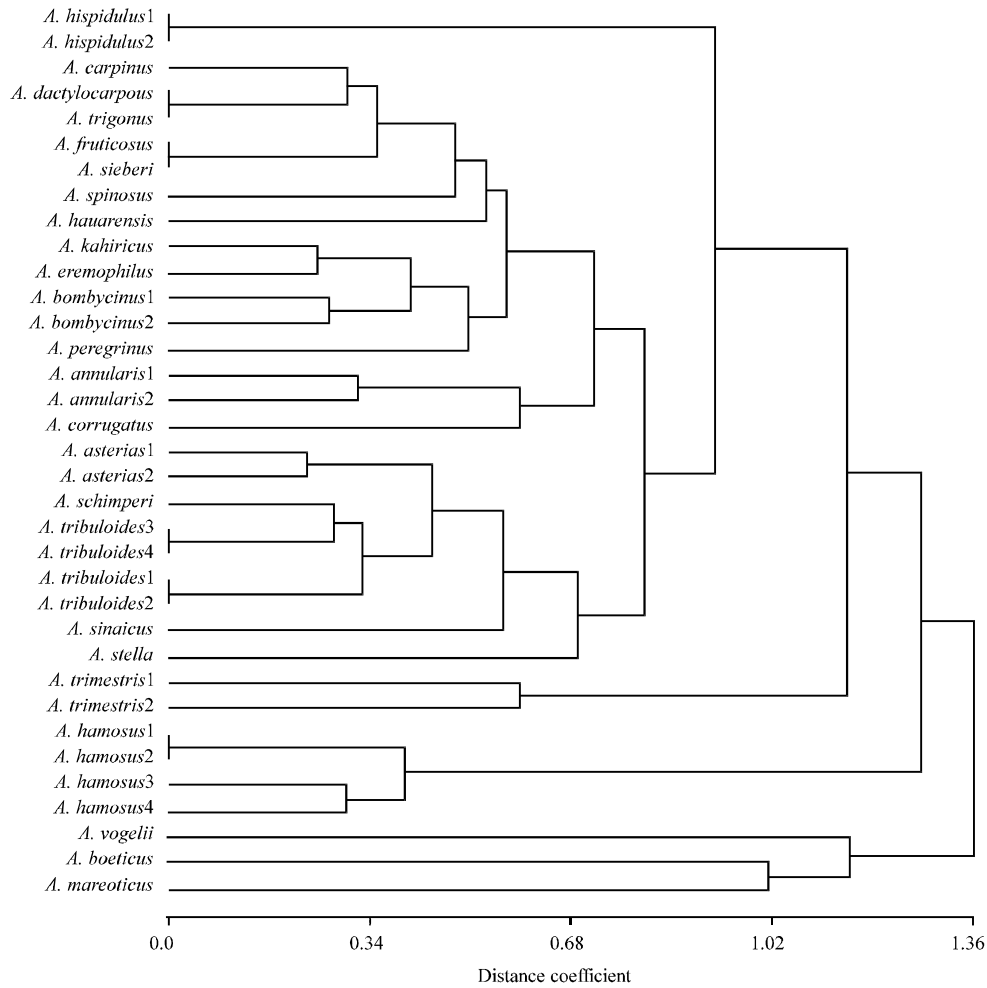
Fig. 6: UPGMA tree illustrating the relationships among the studied 35 taxa, of *Astragalus* based on the analysis of karyotype criteria

The studied species of Egyptian *Astragalus* are delimited in 13 sections (Podlech, 1986; 1991, Table 1). Section Ankylopus is represented by two samples of *A. hispidulus*, one representing the type *A. hispidulus* and the other *A. hispidulus* ssp. *kralikianus*. Both samples have 2n = 16 with metacentric and submetacentric chromosomes (Fig. 1a and b), the two samples also have closely similar chromosome length and mean arm ratio but slightly different A_1 value (Table 1). The subspecies *kralikianus* differs from the type by shorter and broader pods and smaller number of seeds (Boulos, 1999). Podlech and Aytac (1998) favored the consideration of *A. hispidulus* ssp. *kralikianus* as a separate species i.e., *A. kraliki* Batt.; a view supported by the electrophoretic profile of storage seed protein (Al-Nowaihi *et al.*, 2002). However, the karyotype features of the examined samples do not justify the proposed delimitation. The UPGMA tree appear to justify the delimitation of *A. hispidulus* in a

separate section but in the NJ tree this species is associated with the species in section Carpini (*A. carpinus*) and section Chronopus (*A. dactylocarpous*, *A. fruticosus*, *A. sieberi* and *A. trigonus*). All these species have 2n = 16 and similar TCL and mean arm ratio but slightly different A_1 value (Table 1).

Karyological similarities are evident among the examined four species of section Chronopus i.e., *A. dactylocarpous*, *A. fruticosus*, *A. sieberi* and *A. trigonus*. All four species have 2n = 16 with closely similar chromosome length, arm ratio TF% and A_1 (Table 1). The resemblance in chromosome criteria among these species is correlated with similarities in their seed protein electrophoretic profiles (Al-Nowaihi *et al.*, 2002). However, morphological and anatomical features distinguished *A. fruticosus* from the other three species (Sharawy, 2001). Podlech (Personal Communication, 2001) favored the delimitation of this species in subsection

Astragalus and the other three species in section Chronopus. However, this view is not substantiated by the karyotype features reported here. On the contrary, karyotype analysis and the analysis of chromosome data support close relationships between these species. Furthermore, the NJ tree, based on these data, indicates close relationships among the species of section Chronopus and *A. carpinus* of section Carpini and *A. hispidulus* (section Ankylopus).

Section Buceras is represented by four samples of *A. hamosus*; all have polyploid chromosome numbers based on x = 8, three samples are tetraploid with 2n = 32 and one sample of *A. hamosus* spp. *brachyceras* has 2n = 64 (Table 1). The tetraploid number has been frequently reported for *A. hamosus* but pentaploid (2n = 40), hexaploid (2n = 48) and other aneuploid numbers were also recorded in this species (Loeve and Kjellquisst, 1974; Horjales, 1976; Diaz Lifante *et al.*, 1992). In Egyptian material, Badr *et al.* (1996) recorded 2n = 32 in *A. hamosus* and 2n = 40 in *A. hamosus* ssp. *buceras*, however, 2n = 64 in *A. hamosus* ssp. *brachyceras* is recorded here for the first time. The chromosomes of all samples of *A. hamosus* are metacentric and sumbetacentric (Fig. 1c-f) with similar TCL, MCL, arm ratio, TF% and A_1 values (Table 1).

Podlech and Aytac (1998) regarded *A. hamosus* a polymorphic species in morphological characters; an observation supported by differences in chromosome number among different samples. However, the variation, in chromosome number among different samples of *A. hamosus*, is not associated with morphological and anatomical resemblances (Sharawy, 2001) and similarities in the electrophoretic profiles of seed protein (Al-Nowaihi *et al.*, 2002). The samples of *A. hamosus* are clearly delimited as a distinguished group in the NJ and UPGMA distance trees. The analysis of chromosome features thus support the position of *A. hamosus* in section Buceras as proposed by Podlech (1986 and 1991).

The chromosome number of 2n = 30 reported in *A. boeticus* of section Cyamodes is most likely pentaploid, based on x = 6 and is similar to previous counts reported for this species by several authors (Ledingham, 1960; Ledingham and Rever, 1963; Martinez, 1974; Fernandes and Quiros, 1978; Badr *et al.*, 1996). The karyotype is comprised of metacentric and submetacentric short chromosomes. The chromosomes of this species, like that of *A. vogelii* (2n = 28) of section Herpocaulos, are extremely short compared to other species. However, the karyotype of *A. boeticus* is more asymmetric as judged by higher mean arm ratio, TF% and A_1 value compared to that of *A. vogelii* (Table 1).

The analyses of karyotype data separate *A. vogelii* and *A. boeticus* as well as *A. mareoticus* (2n = 28), of section Harpilobus, from other species, at high distance coefficients between them. In the UPGMA tree, these three species are clearly distinguished from all other taxa. The high distance coefficients among these species, in both the NJ and UPGMA trees, may be congruent with the delimitation of *A. boeticus* in section Cyamodes and *A. vogelii* in section Herpocaulos as proposed by Podlech (1986 and 1991) and agree with differences between them in morphological, anatomical and seed protein criteria as described by Sharawy (2001) and Al-Nowaihi *et al.* (2002).

Both the NJ and UPGMA trees clearly indicated the separation of *A. mareoticus* from other species of section Harpilobus. This section is represented in this study by four species that have different chromosome numbers and exhibit variable arm ratio, TF% and A_1 values. Numbers based on x = 8 are found in *A. corrugatus* (2n = 32) and *A. hauarensis* (2n = 16) and numbers based on x = 6 in *A. trimestris* (2n = 12; 24). Meanwhile, a tetraploid number, based on x = 7 (2n = 28) is found in *A. mareoticus*. The chromosome counts and karyotype features of these four species do not support their grouping in section Harpilopus (Podlech, 1991) and is contrary to similarities in their seed protein electrophoretic profile (Al-Nowaihi *et al.*, 2002). In the NJ tree, *A. trimestris* and *A. corrugatus* are associated together at high distance coefficients between them. In the UPGMA tree, *A. corrugatus* appeared associated, at relatively high distance coefficient, with *A. annularis* (section Haematodes). In both trees *A. hauarensis* is grouped with *A. spinosus* of section Poterium; both species have 2n = 16 and similar karyotype features (Table 1). The karyotype features and the analyses of chromosomal data thus do not support the grouping of the above four species in section Harpilopus as proposed by Podlech (1986 and 1991).

Two samples of *A. annularis* (section Haematodes) have been examined in this study; one has a diploid number of 2n = 14 and the second a tetraploid number of 2n = 28 (Fig. 2c and d). The diploid number was recorded in Egyptian material by Badr *et al.* (1996) and the tetraploid number is recorded here for the first time. The chromosomes of the two samples have similar length and arm ratio but the diploid sample shows slightly higher TF% and lower A_1 value (Table 1). The karyotype criteria for *A. annularis* support the morphological and anatomical characters (Sharawy, 2001) and seed protein electrophoretic profile (Al-Nowaihi *et al.*, 2002) that justify the delimitation of this species in section Haematodes as proposed by Podlech (1986). This is confirmed by the position of the two samples of *A. annularis* in the NJ tree.

The association of this species with *A. corrugatus* in the UPGMA tree may be merely reflecting similarities in chromosome measurements.

A diploid number of 2n = 16 is reported for *A. kahiricus* of section Eremophysa (Fig. 2a) and *A. eremophilus* of section Falcinellus (Fig. 2b). The same number was reported for material of *A. eremophilus* from Saudi Arabia (Badr and Gassim, 1992) and Egypt (Badr *et al.*, 1996). The karyotype of both species is comprised of metacentric and submetacentric. The two species also have similar chromosome length, arm ratio, TF% and A_1 value (Table 1). These resemblances are reflected in the NJ tree; in which the two species are delimited together, at a relatively high distance coefficient. The grouping of these two species in the NJ tree is contrary to their separation in two sections by Podlech (1986 and 1991). In the UPGMA tree, *A. kahiricus* and *A. eremophilus* are grouped at low distance coefficient but delimited with *A. peregrinus* and *A. bombycinus* of section Platyglottis; all four species have 2n = 16 but the former two species have longer chromosomes, lower arm ratio and higher FF% and A_1 value.

Section Platyglottis is represented in this study by two samples of *A. bombycinus* and one sample of *A. peregrinus*; all three samples are diploid with 2n = 16. The same number was reported in both species by Ledingham and Rever (1963) and in *A. peregrinus* by Brullo *et al.* (1991) and Badr *et al.* (1996). The karyotype of both samples of *A. bombycinus* is composed of metacentric and submetacentric chromosomes (Fig. 2k and 3a) and that of *A. peregrinus* have eight pairs of metacentric chromosomes (Fig. 3b). However, both species have similar arm ratio, TF% and A_1 value, but the latter species has shorter chromosomes (Table 1). The delimitation of *A. bombycinus* and *A. peregrinus*, as one group, in the NJ tree, agree with similarities among them in morphological and anatomical characteristics and pollen type (Saad and Taia, 1988). These data combined with similarities among these two species in spermoderm characters (Sharawy *et al.*, 2003) and seed protein electrophoretic profiles (Al-Nowaihi *et al.*, 2002) support their delimitation together in section Platyglottis (Podlech, 1991).

Chromosome numbers based on x = 8 have been recorded in nine taxa representing five species of section Sesamei (Table 1). One sample of *A. asterias* has a triploid number of 2n = 24 and the other eight samples have a diploid number of 2n = 16. The same number was only reported, in Egyptian material, for *A. sinaicus* by Badr *et al.* (1996). The karyotype of the nine taxa is

illustrated in Fig. 3d-l. They differ in chromosome length that ranges from 1.59±0.08 μm in *A. sinaicus* to 1.26±0.04 μm in *A. stella*. In the karyotype of the latter species (Fig. 3h) a satellite is observed on the short arms of the chromosome pair numbered 3. The two samples of *A. asterias* are distinguished by higher mean arm ratio and A_1 value that indicates asymmetric karyotype (Table 1).

In the NJ and UPGMA trees, the species of section Sesamei are clearly distinguished as one group; only *A. sinaicus* and *A. stella* are slightly distinct from the samples representing the other three species. This distinction is in agreement with the view of Gazer (1993), who proposed four groups for section Sasamei typified by *A. asterias*, *A. sinaicus*, *A. stella* and *A. schimperi* respectively. *Astragalus asterias* possesses sessile leaves and fruits with double indumentum (Sharawy, 2001). Saad and Taia (1988) also found that this species has *A. palaestinus* pollen type that differs from the pollen types in section Sasamei. Moreover, evidence from seed protein electrophoretic analysis indicated the grouping of *A. asterias* with *A. tribuloides* (Al-Nowaihi *et al.*, 2002) that is correlated with similarities between these two species in spermoderm characteristics (Sharawy *et al.*, 2003). However, in this study samples representing both species are grouped with *A. schimperi* indicating close relationship between these three species. Chromosomal criteria and analysis of karyotype data, as presented here, support the grouping of the five species that has been delimited in section Sesamei by Podlech (1986 and 1991).

REFERENCES

Abou El-Enain, M.M., H.H. El-Shazly and M.A. El-Kholy, 1998. Karyological studies in some African species of the genus *Sesbania* (Fabaceae). Cytologia, 63: 1-8.

Ali, S.I., 1961. Revision of the Genus *Astragalus* L. from W. Pakistan and N.W. Himalayas. Biolosia, 7: 7-92.

Al-Nowaihi, A.S., S.F. Khalifa, A. Badr and S.M. Sharawy, 2002. Species relationships of *Astragalus* L. in Egypt based on storage seed protein electrophoretic criteria. Proc. 2nd Int. Conf. Biol. Sci. Faculty of Science, Tanta University. 27-28 April 2002, 2: 174-188.

Badr, A. and T.T. Elkington, 1978. Variation in Giemsa C-banded and fluorochromes banded karyotypes and relationships in *Allium* subgenus *Molium*. Plant Syst. Evol., 128: 23-35.

Badr, A. and M.A. El-Kholy, 1986. Chromosomal studies in the Egyptian flora, II. Karyotype studies in *Plantago* L. Cytologia, 52: 725-731.

Badr, A. and A. Gassim, 1992. Chromosomal studies on some plants in the flora of Madinah region Saudi Arabia. KAU J. Sci. Res., 4: 23-35.

Badr, A., M. Hamoud and H. El-Rabey, 1996. Chromosomal studies in the Egyptian flora V. Chromosomal relationships in the genus *Astragalus* L. (Fabaceae) and their taxonomic inferences. Cytologia, 61: 105-111.

Boulos, L., 1999. Flora of Egypt. Al-Hadara Publishing, Cairo, Egypt, 1: 320-336.

Brullo, S., A. Guglielmo, P. Pavone and M.C. Terrasi, 1991. Numeri cromosomici per la flora Italiana: 1251-1266. Bot. Ital., 23: 39-46.

Daniel, P.F., C.D. Warren and L.F. Games, 1984. Swainsonine-introduced oligosaccharide exerted in sheep. Biochem. J., 221: 601-607.

Diaz, Lifante, Z., T. Luque and C. Santa Barbara, 1992. Chromosome numbers of plants collected during Iter Mediterraneun II, Israel. Bocconea, 3: 229-250.

Dopchiz, L., S. Comez and L. Poggio, 1995. Karyotype and nuclear DNA content of six species of *Astragalus* (Leguminosae). Cytologia, 60: 329-335.

El-Hadidi, M.N. and A. Fayed, 1995. Materials for excursion flora of Egypt. Taeckholmia, Cairo University Herbarium.

El-Shazly, H.H. and M.M. Abou El-Enain, 1999. Chromosomal criteria of some *Sesbania* species and their taxonomic inferences. Bull. Fac. Sci. Assiut Univ., 28 (2-D): 123-132.

Fernanades, A. and M. Queirors, 1978. Contribution a la connaissance cytotaxinomique des spermatophyte du Portugal. IV. Leguminosae (Suppl. 3) Bol. Soc. Brot., 52: 79-164.

Fernandez, J., H. Nava, M. Vera, M. Alvareg, T. Diaz, M.I. Fernandez, M.C. Fernandez and M. Gutierrez, 1993. Chromosome numbers and geographic distribution of Ulex gallii and U. minor (Leguminosae). Botanical J. Linnean Soc., 112: 43-49.

Gazer, M., 1993. Revision of *Astragalus* L. Sect. Sesamei DC. (Leguminosae). Mitteilungen-der-Botanischen-Staatssammlung-und-des-Instituts-für-Systematische-Botanik-der-Universität-München, 1:69-155.

Horjales, M., 1976. Contribucion al Estudio Citotaxonomico de la flora Espanola. Trab. Dep. Bot. Y.F. Veg. (Madrid), 9: 13-18.

Huziwara, Y., 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of *Aster*. Am. J. Bot., 49: 116-119.

James, L.F., 1983: Poisonous plants aliphatic nitro containing *Astragalus*. Range Lands, 5: 256-257.

Ledingham, G.F., 1960. Chromosome number in *Astragalus* and *Oxytropis*. Can. J. Genet. Cytol., 2: 119-128.

Ledingham, G.F. and B.M. Rever, 1963. Chromosome number of some South West species of *Astragalus* and *Oxytropis*. Can. J. Genet. Cytol., 5: 18-32.

Ledingham, G.F. and B.M. Pepper, 1973. Chromosome number of some North American species of *Astragalus*. Kurtziana, 7: 27-37.

Levan, A., K. Fredga and A.A. Sanders, 1965. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

Liston, A., 1990. Chromosome counts in *Astragalus* (Fabaceae). Madrono, 37: 59-60.

Loeve, A. and E. Kjellqvist, 1974. Cytotaxonomy of Spanish plants. Lagascalia, 4: 3-32.

Maassoumi, A.A., 1987. Notes on the genus *Astragalus* in Iran. I. Cytotaxonomic studies on some species. Iran J. Bot., 3: 117-128.

Malallah, G.A., M. Muhammed and M. Al-Dosari, 2001. Chromosome numbers of Kuwaiti flora, III. Willdenowia, 31: 411-418.

Martinez, A.P., 1974. In IOPB chromosome number reports. Taxon, 23: 801-812.

Panter, K.E. and W.J. Hartley, 1989. Transient testicular degeneration in rams fed locoweed (*Astragalus lentiginosus*). Vet. Hum. Toxicol., 31: 42-46.

Podlech, D., 1986. Taxonomic and phytogeographical problems in *Astragalus* of the old World and south west Asia. Proc. Roy. Soc. Edinburgh, 89: 37-43.

Podlech, D., 1991. The systematics of annual species of the genus *Astragalus* L. (Leguminosae). Flora et. Vegetatio Mundi, 9: 1-18.

Podlech, D. and Z. Aytac, 1998. Two new species of *Astragalus* L. sect. Disstiflori DC. (Fabaceae) from Turkey. Mitteilungen-der-Botanischen-Staatssammlung-und-des-Instituts-für-Systematische-Botanik-der-Universität-München, 5:265-268.

Polhill, R.M., 1981. Gelegeae in Advances in Legume Systematics. Polhill, R.M. and P.H. Raven (Eds.), Royal Botanic Gardens, Kew, England, pp: 357-363.

Rohlf, F.J., 1993. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics Inc., New York.

Saad, S.I. and W.K. Taia, 1988. Palynological studies of some species in the genus *Astragalus* L. Leguminosae in Egypt. Arab. Gulf. J. Sci. Res., B6: 227-243.

Sharawy, S.M., 2001. Taxonomic studies on interspecific and infraspecific relationships in the genus *Astragalus* in Egypt. Ph.D Thesis, Ain Shams University, Cairo, Egypt.

Sharawy, S.M., M.M. Mourad and A.S. Al-Nowaihi, 2003. The assessment of the morpho-anatomical characters of the spermoderm in the delimitation of some *Astragalus* taxa growing in Egypt. Bull. Fac. Sci. Assiut. Univ., 32: 325-346.

Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 28: 1409-1438.

Towensend, C.E., 1981. Breeding *Cicer* Milkvetch for improved forage yield. Crop Sci., 21: 362-366.

www.mobot.mobot.org., 2007. Index to Plant Chromosome Numbers (IPCN), Missouri Botanic gardens, USA.

Zarco, C.R., 1986. A new method for estimating karyotype asymmetry. Taxon, 35: 526-530.