Short Communication

CHROMOSOME STUDIES OF IRANIAN MEMBERS OF TRIBE *SOPHOREAE* (FAMILY LEGUMINOSAE)

M. Noori[1.2.3], M. S. J. Simmonds[2] and M. Ingrouille[1*]

[1] *Department of Biology, Birkbeck College, University of London, Mallet Street, WC1E 7HX, UK*
[2] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Sorrey TW9 3AB,UK*
[3] *Department of Biology, Faculty of Science, University of Arak, P. O. Box 38156-879, Arak, Islamic Republic of Iran*

Abstract

The tribe *Sophoreae* sensu Polhill [9,10] is a large and diverse assemblage comprising the ancient and primitive ancestral stocks of Papilionoideae. The most frequent chromosome basic numbers in this tribe are x = 11 and x = 9 but chromosome numbers range from x = 8-14 are also known. In this study chromosome numbers and karyotype variation of Iranian members of tribe *Sophoreae* are reported. Iranian taxa in the *Sophoreae* are *Sophora alopecuroides* ssp. *alopecuroides* L., *S. alopecuroides* ssp. *tomentosa* (Boiss.) Yakovlev, *S. pachycarpa* Schrenk ex C.A. Meyer, *S. mollis* ssp. *griffithii* (Stocks) Ali, *S. mollis* ssp. *mollis* Graham, *Ammothamnus lehmanni* Bunge and *Ammodendron conollyi* Fische. *S. alopecuroides* and *S. pachycarpa* are 2n=36 and exhibit wide variation in chromosome size within karyotypes. The other taxa are 2n=18. The two subspecies of *S. mollis* show relatively little variation in chromosome type within the karyotype. *Ammodendron conollyi* had the smallest mean size of chromosome and *Ammothamnus lehmanni* had the biggest mean chromosome size. The significance of these results in relation to the evolution of the group and in comparison to some previously reported results is discussed. These results agree with Goldblatt's count for *A. lehmanni* and *A. conollyi* and also agree with Jahan's count for *S. m.* ssp. *griffithii* and another taxon was reported for the first time.

Keywords: *Sophora; Ammodendron*; *Ammothamnus*; Papilionoideae; Leguminosae; Sophoreae; Karyotype; Iran

Introduction

The *Sophoreae* sensu Polhill [9,10] are a large and diverse assemblage comprises the ancient and primitive

* *E-mail: m-noori@araku.ac.ir*

ancestral stocks of Papilionoideae. The genus *Sophora* L. is by far the largest and most diverse genus in the tribe and is probably a paraphyletic group of species representing a range of basal or ancestral conditions.

In the *Sophora* group n = 14 is probably the most common haploid number but x = 9 is common as well. The genus is clearly ancient and diverse. Cytological data support fragmentation as proposed by Yakovlev [13]. Darlington and Wylie [2] reported haploid chromosome numbers of *S. davidi* Tschechov, *S. flavescens* Ait., *S. microphylla* Soland. ex Ait., *S. secundiflora* (Ort.)DC, *S. tetraptera* J. F. Mill and *S. tomentosa* Linn. as 9 (2n = 18), *S. moorcroftiana* (Grah.) Benth. ex Baker as 8 (2n = 16) and *S. chinensis* D. Don and *S. japonica* Linn. as 14 (2n = 28).

Goldblatt [3,4] confirmed a chromosome number of 2n = 28 for *S. japonica*. Also he got 2n = 28 for *S. affinis* Torr. and *S.*(*Echinosophora*) *korensis* Nakai, and 2n = 18 for *S. arizonica* Watson, *Ammothamnus lehmanni* (Bge.) and *Ammodendron conollyi* (Bge.).

Palmino *et al* [8] reported a basic chromosome number x = 9 (2n = 18) for three species: *S. secundiflora* (Ort.) DC, *S. velutina* var. *zimbabweensis* Klotz and *S. tomentosa* L.. Karyotypes of five species of the genus *Styphnolobium* (Schott.) Tsoong showed a basic chromosome number x = 14 (2n = 28). These results agree with Sousa and Rudd's [12] proposal to include species with 2n = 28 in the genus *Styphnolobium.*

Jahan *et al.* [5] reported a basic chromosome number x = 9 (2n = 18) for *Sophora mollis* ssp. *griffithii*.

The aims of this work were to understand variation between species and populations in Iranian members of tribe *Sophoreae*. Also looking chromosome number, size, shape and comparison of them.

Materials and Methods

Seeds were collected from various parts of Iran during 1998 and 1999 (Table 1). Experiments carried out in Jodrell Cytogenetic Laboratory of Royal Botanic Gardens, Kew and University of Arak in Iran. Seeds were germinated on petri dishes in 30 ± 2C° after scarification. Also a few seeds were transferred to soil in pots after scarification. Fresh root tips were collected from petri dishes and pot plants for karyotypic studies.

Stains and pretreatments were prepared according to methods of Darlington and La Cour [1]. A number of different pretreatments were tried. The best results were obtained using α-bromonaphtalene (ABN) as a pretreatment (24 h at 4°C). Fixation was then carried out in freshly prepared solution of 3:1 EtOH: HoAC. After fixation for a minimum of 24 h at 4°C, the roots were hydrolyzed in 1 M HCl for 11.5-12 min at 60°C, and stained in Feulgen (Schiff's regent) in the dark at the room temperature for 1 h. Acetocarmine proved to be a poor stain for this material.

The root meristem was dissected onto a clean slide and squashed in 45% acetic acid or 2% acetic Orcein to enhance staining after examination. Well-separated metaphase plates were selected. Photographs of suitable cells were taken under phase contrast conditions using a Zeiss Photo-microscope III on Ilford Pan F film. Chromosome counts and measurements were repeated three times on each nucleus and from ten different metaphase plates for each plant.

Chromosome preparations were made during 1998 and 1999 in Iran and at the Jodrell laboratory in 1999. On each occasion 5-10 seeds were sampled from each population. 10-20 slides were prepared from each seedling providing a minimum of ten counts for each seedling. The best 3 metaphase spreads were used for measuring chromosome size.

Voucher samples are kept in Royal Botanic Gardens, Kew, Herbarium of Research Institute of Forests and Rangelands [Tehran (TARI) P. O. Box: 13185-116] and personal samples.

Results and Discussion

For all species somatic chromosomes were small and stained poorly, with a tendency not to spread very well. The chromosome numbers and chromosome sizes obtained for the Iranian *Sophoreae* are shown in Table 1. A sample metaphase spreads are shown in Figure 1.

Two subspecies of *S. alopecuroides* (*S. alopecuroides ssp. alopecuroides* and *S. alopecuroides ssp. tomentosa*) and *S. pachycarpa* were tetraploid (polyploid) and had a chromosome number of 2n = 36. All remaining species (two subspecies of *S. mollis*, *A. lehmanni* and *A. conollyi*) were diploid and had a chromosome number of 2n=18.

The base number of all these taxa is x = 9, which places them among some of the more derived members of *Sophora*. Although the chromosomes are small, it is possible to see the presence of some large metacentric chromosomes in some karyotypes which may represent the fusion of two smaller telo- or acrocentric chromosomes from the ancestral x = 14 condition.

Diploid samples are shrubby and suffrutescent: the two subspecies of *S. mollis* are shrubby, and *Ammothamnus lehmanni* and *Ammodendron conollyi* are suffrutescent. Chromosome size was relatively uniform.

Polyploid samples were herbaceous and had the most variability in chromosome size. Polyploid taxa had also a greater tendency to produce chlorotic and otherwise abnormal plants but there was no evidence of any hybridization in chromosome studies. Polyploidy was a common phenomenon in angiosperms and high rates of polyploidy have been reported for other leguminous taxa of the semi-arid areas, like these members of the *Sophoreae* (Ingrouille, communication). For example, in

Table 1. Samples used in chromosome studies, 2n and means size of chromosomes

No.	Taxon	Locality	Latitude and Longitude		2n	Mean size range (μm)
	Sophora alopecuroides					
Noori 01	ssp.*tomentosa*	Gavar Road-Iran	34° 02′ N	49° 36′ E	36	0.89-1.75
Noori 02	ssp.*tomentosa*	Gerdou Mountains-Iran	34° 05′ N	49° 42′ E	36	0.88-1.70
Noori 03	ssp.*tomentosa*	Entezam Garden-Iran	34° 05′ N	49° 42′ E	36	0.89-1.70
Noori 04	ssp.*tomentosa*	Karahroud-Iran-Iran	34° 03′ N	49° 38′ E	36	0.89-1.75
Noori 010	ssp.*tomentosa*	Khomyn Road-Iran	33° 39′ N	50° 04′ E	36	0.88-1.75
Noori 016	ssp.*tomentosa*	Mashad-e′ Ardehar-Iran	34° 03′ N	51° 00′ E	36	0.90-1.80
Noori 027	ssp.*tomentosa*	Darband-e′ Astaneh-Iran	33° 53′ N	49° 22′ E	36	0.88-1.70
Noori 029	ssp.*tomentosa*	SE of Arak -Iran	34° 05′ N	49° 42′ E	36	0.89-1.80
Noori 032	ssp.*tomentosa*	West of Karahroud-Iran	34° 02′ N	49° 37′ E	36	0.90-1.80
Noori 033	ssp.*tomentosa*	East of Azna-Iran	33° 25′ N	49° 31′ E	36	0.90-1.75
Noori 039	ssp.*tomentosa*	Komijan-Iran	34° 40′ N	50° 22′ E	36	0.90-1.70
Noori 026	ssp.*alopecuroides*	Hosainabad-e′ Joukar-Iran	34° 25′ N	48° 40′ E	36	1.16-2.04
	Sophora mollis					
Noori 034	ssp.*griffithii*	Kermestan Village-Iran	26° 25′ N	58° 18′ E	18	1.20-2.00
Noori 038	ssp.*griffithii*	Firuzabad-e′ Fars-Iran	28° 10′ N	55° 49′ E	18	1.20-2.00
Noori 05	ssp. *mollis*	Esphahan-Iran	32° 37′ N	51° 41′ E	18	1.40-2.60
Noori 013	*Sophora pachycarpa*	S of Kerman-Iran	30° 17′ N	57° 05′ E	36	1.09-1.9
Noori 014	*Sophora pachycarpa*	Kerman-Iran	30° 17′ N	57° 05′ E	36	1.08-1.92
Noori 021	*Ammothamnus lehmanni*	Sarakhs Road-Iran	36° 30′ N	61° 16′ E	18	1.70-2.38
Noori 035	*Ammodendron conollyi*	Rigabad-e′ Khash-Iran	28° 13′ N	61° 13′ E	18	0.95-1.43
Noori 036	*Ammodendron conollyi*	Torshabi-e Khash-Iran	28° 13′ N	61° 13′ E	18	0.95-1.43
Tavakoli 7608	*Ammodendron persicum*	Ghaen-Iran	33° 40′ N	60° 00′ E	18	0.95-1.43

Acacia closely related diploid and polyploid taxa have been reported and here polyploidy has been reported to be associated with the evolution of distinct geographical variants [6].

No variation in count was observed within species (Table 1). Goldblatt's [3,4] counts of 2n = 18 for *Ammothamnus lehmanni* and *Ammodendron conollyi*, and the count of 2n = 18 for *S. m.* ssp. *griffithii* [5] were confirmed. The number 2n=18 was not exceptional for *Sophoreae*.

Ammothamnus lehmanii had the largest chromosomes with a mean size range of 1.70-2.38 μm and *Ammodendron conollyi* had the smallest with a mean size range of 0.95-1.43 μm. Chromosome size distributions from different taxa are shown in Figure 2. The distribution of chromosome size was uniform, within and among populations of the same taxon, but clearly distinct among taxa. Even between the very closely related taxa like *S. alopecuroides* ssp. *alopecuroides* and *S. alopecuroides* ssp. *tomentosa* there were some marked differences in size distribution. There was a very marked difference in chromosome size distribution between. *S. mollis* ssp. *mollis* and *S. mollis* ssp. *griffithii* as well. The evolutionary importance of such differences is difficult to explain but they may be related to the creation by unequal translocations of highly adaptive gene combinations linked together on the same chromosome.

Rechinger [11] recorded *A. persicum* as a species of uncertain status. Chromosome studies of *A. conollyi* and *A. persicum* here have confirmed that *A. persicum* is identical to *A. conollyi* because chromosome number, shape and size were the same for both. Also phytochemistry, leaf anatomy, climatological, ecological, macro- and micro-morphological studies confirmed this result [7].

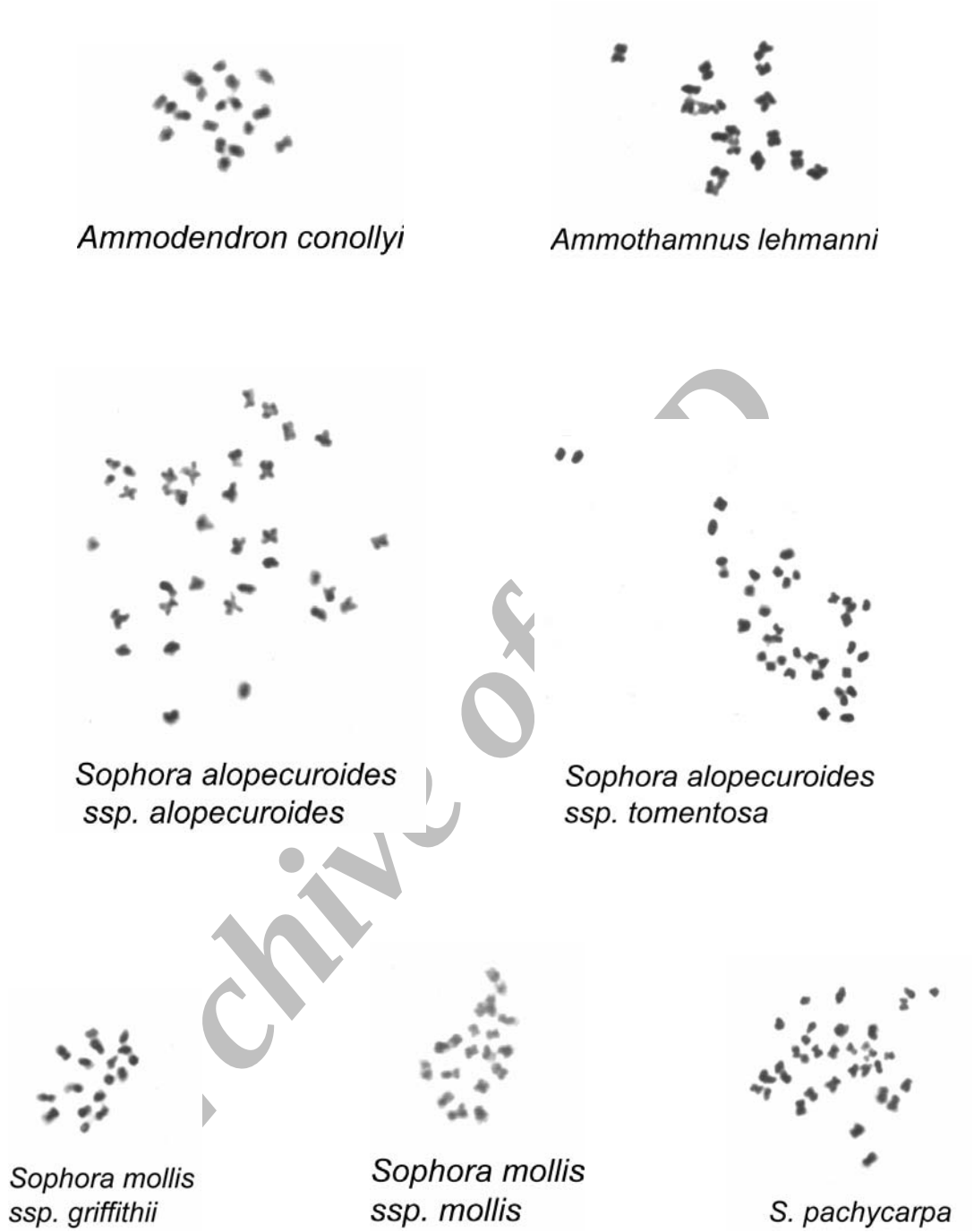

Figure 1. Metaphase in Iranian members of tribe *Sophoreae*. 10µm (Magnification of proof print=1,500, 10µm = 1.5 cm long).

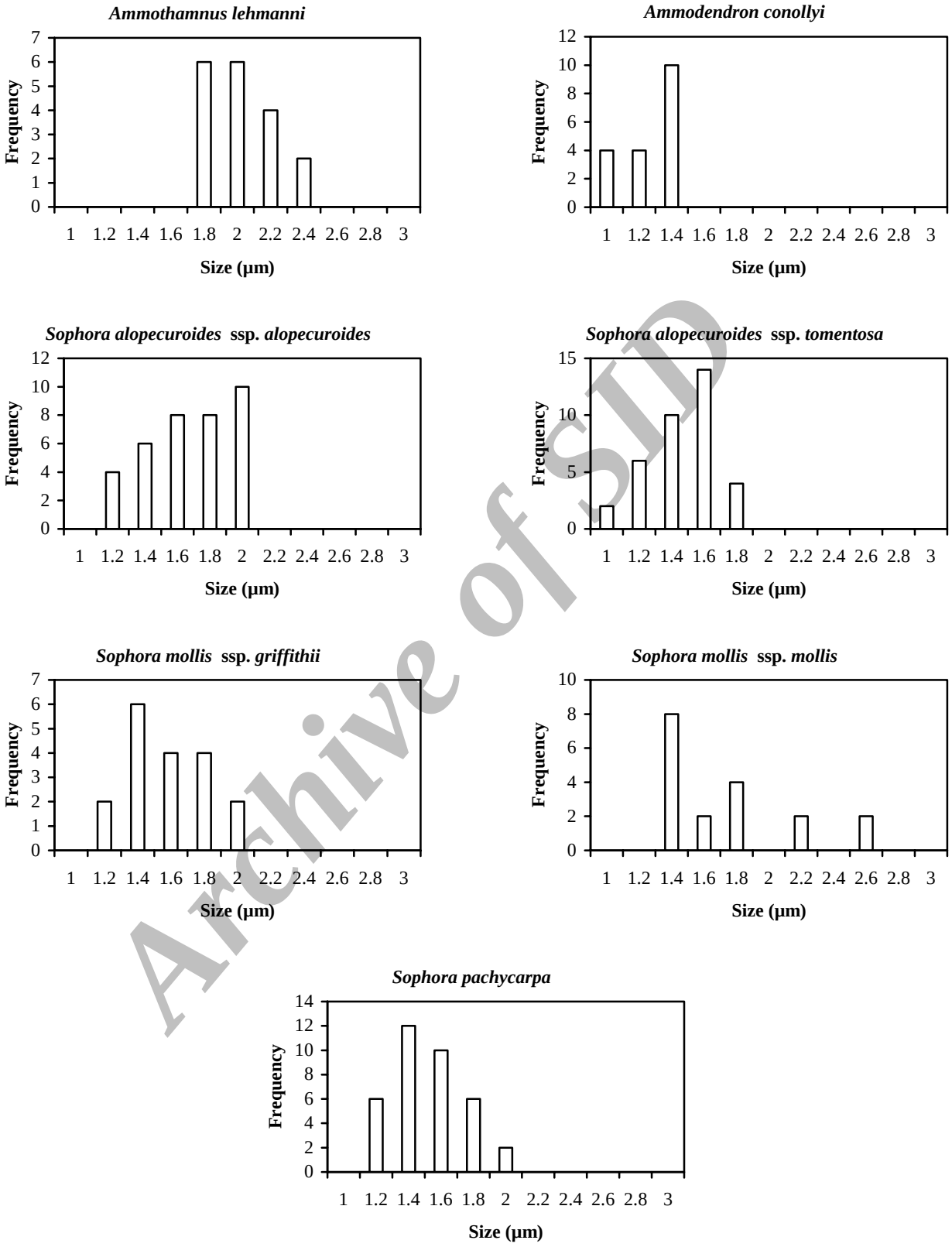

Figure 2. Chromosome size frequency in Iranian members of tribe *Sophoreae*.

Acknowledgements

We wish to thank I. Leitch, cytogenetics representatives and L. Hanson, assistant scientific officer.and P. Brandham for their help and direction in cytogenetic labs. We would like to acknowledge C. Foster, unit manager, living collections at Kew, and T. Bryant, higher botanical horticulturist in Jodrell glass house for preservation of our plants. We would also like to thank photographers in Media Resources of Royal Botanic Gardens, Kew for developing our chromosome pictures.

We would also like to thank personnel of the Research Institutes of Natural Sources and Domesticated Affairs of Tehran, Hormozgan, Sistan-Balochestan, Fars and Khorasan provinces in Iran.

References

1. Darlington, C. D. and La Cour L. F. The handling of chromosomes, London George Allen and Unwin LTD: 1-201, (1976).
2. Darlington, C. D. and Wylie, A. P. Chromosome Atlas of Flowering Plants. London George Allen and Unwin LTD, London, p.107, (1955).
3. Goldblatt, P. Chromosome numbers in Legume II. Annals of Missouri Botanical Garden, St. Louis, **68**: 551-57, (1981).
4. Goldblatt, P. Cytology and the phylogeny of Lguminosae, In: Polhill, R. M. and Raven, P. M. (Eds.), Advances in Legume Systematics, Royal Botanic Gardens, Kew, 1981, part **2**: 427-63, (1981).
5. Jahan, B., Vahidy, A. and Ali, S. I. Chromosome numbers in some taxa of Fabaceae mostly native to Pakistan. Annals of the Missouri Botanical Garden, **81**(4): 792-99, (1994).
6. Kordofani, M. and Ingrouille, M. J. Patterns of morphological variation in the Acacia species (Mimosaceae) of northern Sudan. Botanical Journal of the Linnaean Society, **105**: 239-56, (1991).
7. Noori, M., Ingrouille, M., Simmonds, M. and Azizian, D. Taxonomic studies of genus Ammodendron in Iran. Ninth Iranian Biology Conference, University of Tehran, (2000).
8. Palmino, G., Martinez, P., Bernel, C. and Sousa S. M. Adifferencias cromosomicas entre algunas especies delos generos Sophora L. Y Styphnolobium Schott. Annals of Missouri Botanical Garden St. Louis, **80**: 284-90, (1993).
9. Polhill, R. M. Sophoreae, In: Advances in Legume systematics. Polhill, R. M. and Raven, P. H. (Eds.), Royal Botanic Gardens, Kew, part **1**: 213-30, (1981).
10. Polhill, R. M. and Raven, P. H. Advances in Legume systematics. Royal Botanic Gardens, Kew, part **1**: 213-30, (1981).
11. Rechinger, K. H. Flora Iranica, Papilionaceae II. Wien and Aliis, No. 157(Dec): 15-23, (1984).
12. Sousa, S. M. and Rudd, V. E. Revision del genero Styphnolobium (Leguminosae: Papilionoideae: Sophoreae). Annals of the Missouri Botanical Garden, **80**(1): 270-83, (1993).
13. Yakovlev, G. P. Contributions to the system of the order Fabalses. Bot. Zhurn., **57**: 585-95, (1972).