

## Cytogenetic Studies in Some Species of *Medicago* L. in Iran

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### Abstract

A karyological study using the Image Analysis System was conducted of eight taxa of the genus *Medicago* L. namely *M. radiata* L., *M. intertexta* (L.) Mill. *M. orbicularis* (L.) Bart. , *M. laciniata* (L.) Mill., *M. coronata* (L.) bartal, *M. rigidula* (L.) All., *M. polymorpha* L. and *M. scutellata* (L.) Mill. used as forage plants from different geographic origins of Fars province from Iran. We found the two usual basic chromosome numbers in the genus,  $x=7$  and  $x=8$ . In the group with  $x=7$ , two diploid ( $2n=14$ ), one tetraploid ( $2n=28$ ) species and in the group with  $x=8$ , five diploid ( $2n=16$ ) species were found. Detailed karyotype analysis allows us to group the different species and to postulate relationships among them.

**Keywords:** Chromosome, *Fabaceae*, karyology, *Medicago*, taxonomy

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### Introduction

*Medicago* genus belongs to the tribe Trifolieae (*Fabaceae*, family). According to the IPNI reports the taxon includes about 396 annual and perennial species that have a widespread distribution from the Mediterranean to central Asia (Nixon 2006). This genus includes the widely cultivated major forage crop and weedy species *M. sativa* L. (alfalfa, lucerne) and the legume model species *M. truncatula* Gaertn. (Cannon et al. 2006). Taxonomically, *Medicago* along with *Melilotus* Mill. (sweet clovers) and *Trigonella* L. were included in the tribe Trigonellinae, first recognized by Schultz (1901), but as circumscribed this tribe was not accepted by most taxonomists. Instead, most authors recognized the tribe Trifolieae, which included these three genera and *Trifolium* L. (Rechinger 1984). Relationships within the genus are not yet sufficiently resolved, contributing to difficulty in understanding the evolution of a number of distinguishing characteristics such as aneuploidy and polyploidy, life history, structure of cotyledons, and number of seeds per

fruit. Both polyploids and aneuploids are found within *Medicago* genus (Lesins and Lesins 1979).

Polyploidy and chromosome rearrangement in the genus *Medicago* have resulted in the genetic isolation of genome groups. Diploid, tetraploid and hexaploid species all exist. Also, the union of two chromosomes in the genome and the loss of one centromere region has produced species with basic chromosome numbers of both  $x=8$  and  $x=7$ . Some annual species have the base number  $x = 7$  (Goldblatt 1981). The ploidy levels combined with differences in chromosome number have together provided a complete interbreeding barrier between members of this genus (Lesins et al. 1970).

The first report about chromosomal number of *Medicago* was  $2n=16$  for *M. disciformis* and *M. Arabica* (Goldblatt 1975). It also recorded  $2n=16$  for *M. marina* and *M. minima* (Goldblatt 1978). The chromosome numbers of the *M. rigidula* rouges are different from  $2n=14$  (Goldblatt 1978) to  $2n=16$  (Goldblatt 1975). Different species of *Medicago* have

been recorded from diploid in *M. orbicularis* ( $2n=16$ ) to tetraploid in *M. scutellata* ( $2n=32$ ) (Goldblatt 1975).

*Medicago radiata* or ray-podded medick is an annual plant species of the genus *Medicago* and belongs to the group of generic intermediates. It is found throughout the eastern Mediterranean and in Asia. *Medicago intertexta* or Calvary medick is an annual plant of the family *Fabaceae*. It is found primarily in the western Mediterranean basin area having spiny seed pods and leaves with dark spots. *Medicago orbicularis* is a winter annual plant with leafy stems, fimbriate stipules, oval non-hairy leaflets with toothed margins, orange-yellow flowers, and flat, coiled seed pods with no spines. It is found throughout the Mediterranean basin. It is also common along the European black sea coast. Common names include button clover and round-fruited medick. *Medicago laciniata* is a procumbent or ascending annual plant species of the genus *Medicago*. It is found primarily in the southern Mediterranean basin. Common names include cut-leaved medick and tattered medick. It grows in the Sinai, Egypt and is closely related to *M. aschersoniana* and may be hybridized with *M. sauvagei* (Singh and Lesins, unpublished). *Medicago scutellata* is an annual plant species of the genus *Medicago*. It is found throughout the Mediterranean basin. Common names include shield medick and snail medick. Deviating chromosome counts were given by Abdelguerfi and Guittonneau (1979) with  $2n = 28$  and Bauchan and Elgin (1984, cited in Goldblatt 1988: 109) with  $2n = 30$  chromosomes. *Medicago coronata* or crown medick is an annual plant species of the genus *Medicago*. It is found throughout the Mediterranean basin.

*Medicago rigidula* is an annual plant currently interpreted as a Mediterranean species, indigenous to North Africa, Europe and Asia, and spontaneous in the new world and elsewhere (Small et al. 1990). The most intensive analysis of intraspecific variation of *M. rigidula* is that of Heyn in 1963 and 1984, who concluded her account by recording the great variability

as well as the two recorded chromosome numbers ( $2n=14, 16$ ). *Medicago polymorpha* is a Decumbent or ascending annual broadleaf plant species of the genus *Medicago*. Deviating chromosome counts were given (Fernandes et al. 1977; Fernandes and Queiros 1978; Labadie 1979, 1980) with  $2n = 16$  and (Dahlgren et al. 1971; Luque et al. 1988) with  $2n=14$ . Common names include Bur clover and Bur medick. It is forage for livestock, but the fruit is prickly. It makes for a poor lawn in the late summer, when the leaves have yellowed and the fruit sets into the 7 mm seed heads that are covered with hooked prickles.

Generally taxa will cross more readily when brought to the same ploidy level. Exceptions, however, are encountered where crossability at uneven levels is more successful. The  $2n$  number alone, however, is of little use in determining the species relationship. For that purpose, karyotype studies may provide more information. Chromosome information is an important key for taxonomy, phylogeny, and evolution, genetic and breeding in *Medicago* plants. Here we present the report of the chromosome numbers, ploidy levels and comparison of karyotypic traits of some annual species of *Medicago* genus in Iran. Our results are useful for a better understanding of its taxonomy and breeding purposes such as inter and intraspecific hybridization and genetic variation induction.

## Materials and methods

### *Plant material*

Complete specimens were collected in several field trips from various locations of the Fars Province (Iran) during their period of fruiting (spring and summer) and dried with the specific methods relating to herbarium specimens. Voucher specimens were deposited in the Herbarium of natural research center of Fars, Iran. The mature Seeds of eight taxa were taken from the herbarium materials (Table 1).

**Table 1.** The origin of materials used in chromosome studies of *Medicago*

species	Origin and collector	Altitude	Herbarium code
<i>M. radiata</i> L.	Fars: Lar, Hatami	800 m	9642
<i>M. intertexta</i> (L.) Miller	Fars: Mamessani, Babameidan, Hatami	800 m	12821
<i>M. orbicularis</i> (L.) Bart.	Fars: Kazeroon, Baladeh, Hatami	800 m	10279
<i>M. laciniata</i> (L.) Mill.	Fars: Lar, Hatami	800 m	9659
<i>M. coronate</i> (L.) bartal	Fars: Mamessani, Golgoon, Hatami	1000 m	5123
<i>M. rigidula</i> (L.) All.	Fars: Mamessani, Golgoon, Hatami	1000 m	6423
<i>M. polymorpha</i> L.	Fars: Kazeroon, Baladeh, Hatami	800 m	9789
<i>M. scutellata</i> (L.) Mill.	Fars: Kazeroon, Dadin, Hatami	800 m	4370

### Chromosomal studies and data analysis

The mitotic metaphase chromosomes were investigated in the root tips of the seeds. The germination of seeds was done on damp filter paper in petri dishes at 22°C temperature. When the root tips reached 1-1.5 cm they were pretreated in saturated 8-hydroxyquinoline for 3.5 hours at +4°C. After this first procedure, the root tips were fixed in (3:1) alcohol – glacial acetic acid for 16-20 hours and then the root tips were hydrolyzed in 1N HCL at 60°C for 10-20 min and aceto-orcein (2%) was used for chromosome staining. Slides were then prepared by squashing in a droplet of 45% acetic acid, metaphases were captured using an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of about 2000x. The best metaphase plates were selected and used to prepare the karyotype by Adobe Photoshop 7.0 software, finally karyotypic characters were measured by Micro Measure 3.3 software (Reeves and Tear 2000). In each mitotic metaphase (at least 5 plates) the arm's length of each chromosome was measured and all parameters were estimated in each metaphase plate to characterise the karyotypes, according to the previous studies (Hesamzadeh Hejazi and Rasuli 2006; Hesamzadeh Hejazi and Ziaei Nasab, 2009, 2010; Hesamzadeh Hejazi 2011; Javadi et al. 2009; Irani et al. 2014; Salehi et al. 2014).

Both indices  $A_1$  and  $A_2$  in the Romero Zarco 1986 formula were independent to chromosome number and size. Karyotypic evolution has been determined using the symmetry classes

of Stebbins (SC) (Stebbins 1971). Karyotype formula was determined by chromosome morphology based on centromere position according to the classification of (Levan et al. 1964). For each species, karyograms and haploid idiograms were drawn based on the length of chromosome size (arranged large to small). In order to determine the variation between species, one – way balanced ANOVA was performed on normal data and parameter means were compared by Duncan's test. The principal components analysis (PCA) was performed to evaluate the contribution of each karyotypic parameter to the ordination of populations. Clustering was performed using the single linkage method after calculation of Cophenetic correlation coefficient ( $r$ ) to examine karyotype similarity among populations. Numerical analyses were performed using SAS ver. 6.12, 1996; JMP ver. 3.1.2, 1995; and StatistiXL ver 1.7, 2007 software's.

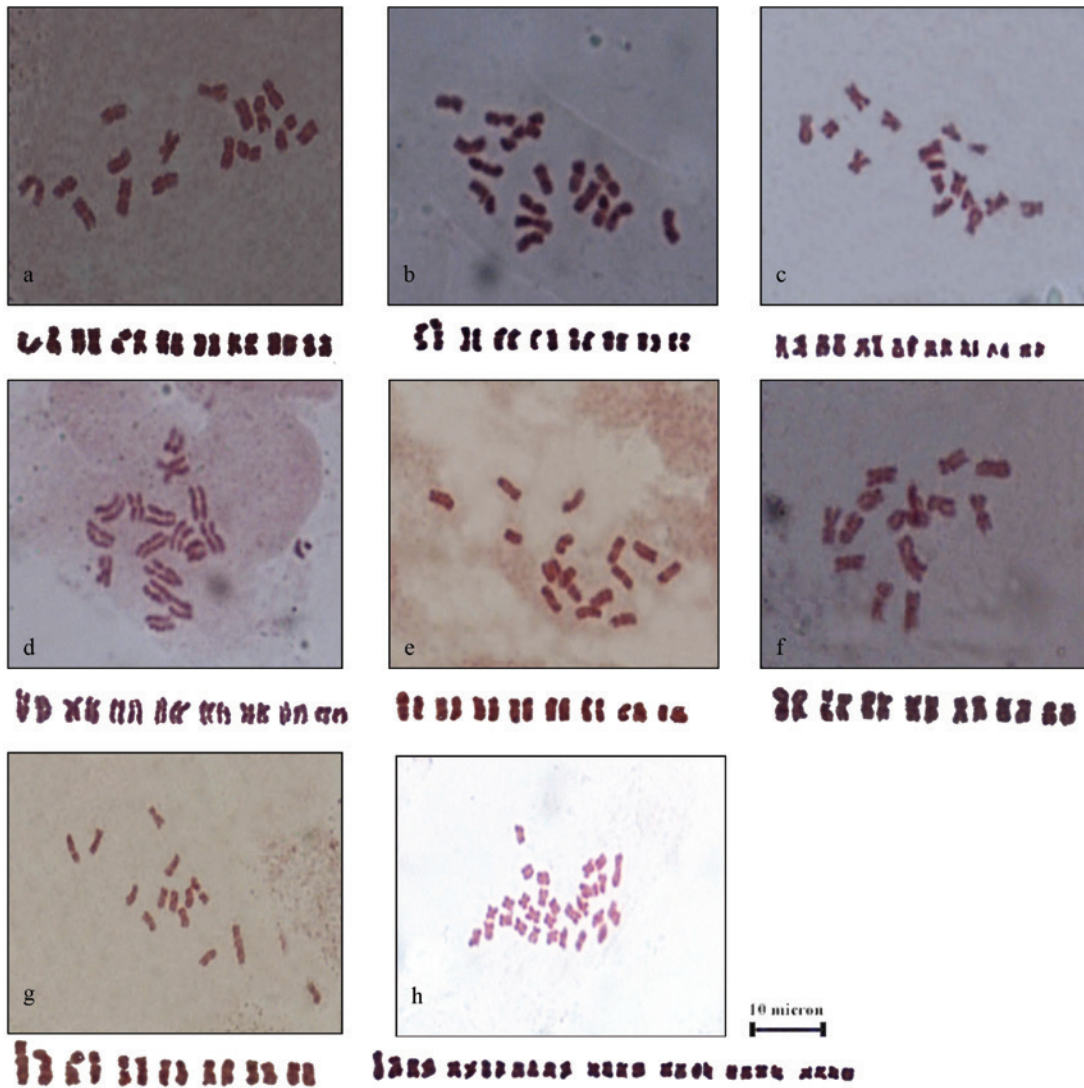
### Results

The results showed that the basic chromosome number varied between  $x=7$  and  $x=8$ . The somatic chromosome numbers ( $2n$ ), karyotype formulae and parameters for the studied species are summarized in Table 2. Most of the species belong to taxa with the basic number  $x=8$ , the most common one is in the genus. In the group with  $x=7$ , two diploid ( $2n=14$ ), one tetraploid ( $2n=28$ ) species and in the group with  $x=8$ , five diploid species exist (Table 2). The karyotypes of diploid and tetraploid species are illustrated in Figure 1.

**Table 2.** Karyotype characteristics of eight species of *Medicago* genus

Species	2n	x	A <sub>1</sub>	A <sub>2</sub>	SC	DRL	%TF	VRC	KF
<i>M. radiata</i> L.	16	8	0.18	0.09	1A	3.50	44.99	3.03	8m
<i>M. intertexta</i> (L.) Miller	16	8	0.16	0.11	1A	4.28	45.56	2.68	8m
<i>M. orbicularis</i> (L.) Bart.	16	8	0.20	0.17	1A	6.24	43.99	2.26	8m
<i>M. laciniata</i> (L.) Mill.	16	8	0.26	0.11	1A	3.99	41.92	3.56	8m
<i>M. coronata</i> (L.) Bartal	16	8	0.16	0.14	1A	5.18	45.41	2.32	8m
<i>M. rigidula</i> (L.) All.	14	7	0.29	0.22	1A	9.85	41.08	2.57	7m
<i>M. polymorpha</i> L.	14	7	0.23	0.16	1A	6.72	43.09	2.74	7m
<i>M. scutellata</i> (L.) Mill.	28	7	0.14	0.12	1A	3.00	45.90	1.94	7m

Somatic chromosome number (2n), basic chromosome(x), asymmetry indexes (A<sub>1</sub>, A<sub>2</sub>) of Romero Zarco, symmetry classes (SC) of Stebbins, difference of range relative length (DRL), total form percentage (TF %), value of relative chromatin (VRC), karyotype formula (K.F.) (m: metacentric)



**Figure 1.** Mitotic metaphase of *Medicago* species accompanied by karyograms- a: *M. radiata*, 2n=16, b: *M. intertexta*, 2n=16, c: *M. orbicularis*, 2n=16, d: *M. laciniata*, 2n=16, e: *M. coronata*, 2n=16, f: *M. polymorpha*, 2n=14, g: *M. rigidula*, 2n=14, h: *M. scutellata*, 2n=28, Scale bars=10 μm



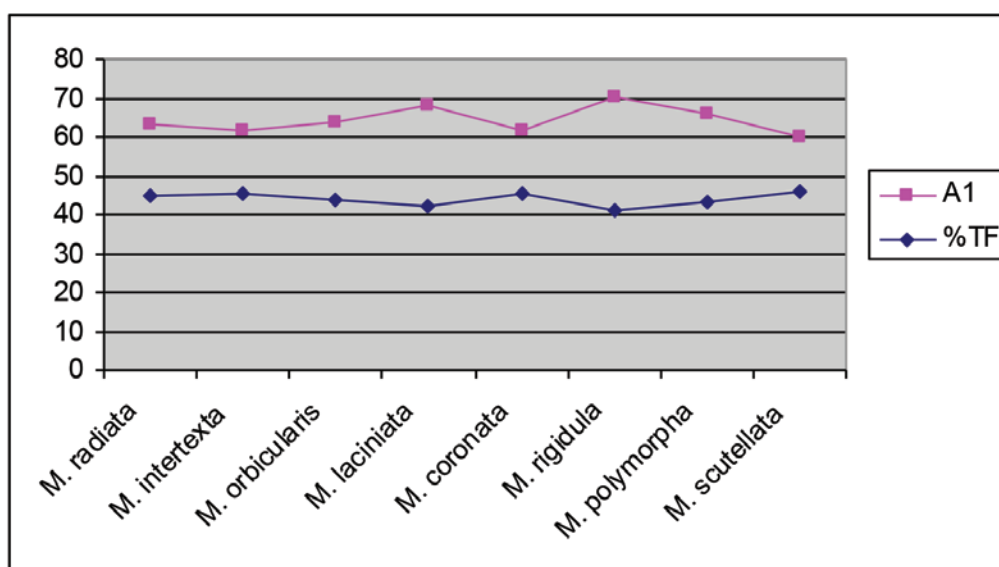
The mean value of chromosome's long arm varied from 2.07  $\mu\text{m}$  in *M. laciniata* to 1.05  $\mu\text{m}$  in *M. scutellata*. Averages of chromosome's short arm were different from 1.49  $\mu\text{m}$  in *M. laciniata* to 0.89  $\mu\text{m}$  in *M. scutellata*. The mean value of the chromosome's total length was varied from 3.56  $\mu\text{m}$  in *M. laciniata* to 1.94  $\mu\text{m}$  in *M. scutellata* and finally the mean value of the chromosome's arm ratio was changing from 1.43 in *M. rigidula* to 1.18 in *M. scutellata* (Table 4).

All of the chromosomes were metacentric (m) in all species and some species showed one (*M. intertextata*, *M. rigidula*, *M. polymorpha*) or two pairs (*M. scutellata*) of visible small or large satellites which were connected to the short arms of the chromosomes (Table 2).

The symmetry type of Stebbins (1971) and asymmetry indices of Romero-Zarco (1986) are given (Table 2). In terms of the Stebbins' system, all of the karyotypes of species seize 1A classes, which are considered in general to be primitive classes in this system (Table 2).

Romero's intrachromosomal asymmetry index ( $A_1$ ) expresses the arm ratio of each pair of homologous chromosomes. The interchromosomal asymmetry index ( $A_2$ ) corresponds to Pearson's coefficient of dispersion and gives an idea of the asymmetry caused by the different length of the chromosomes. By using the Romero-

Zarco asymmetry indices of  $A_1$  and  $A_2$  we can determine the more asymmetric karyotype among the populations which have the similar Stebbins classes of symmetry. For example in the species with 1A class, *M. rigidula* possesses the highest  $A_1$  value (0.29) and the lowest TF% value (41.08%), and therefore has a more asymmetric karyotype (Table 2) (Fig. 2). Also *M. rigidula* possesses the highest  $A_2$  value (0.22) and the highest DRL value (9.85), and therefore has a more asymmetric karyotype (Table 2) (Fig. 3). The total karyotype length, recorded from at least five cells, that roughly indicates the chromatin content amongst the studied diploid taxa with  $x=8$  was in range of 18.08  $\mu\text{m}$  in *M. orbicularis* ( $2n=16$ ; Table 2; Fig. 1) to 28.48  $\mu\text{m}$  in *M. laciniata* ( $2n=16$ ; Table 2; Fig. 1). Also, the total karyotype length among the diploid taxa tested with  $x=7$ , had a range from 17.99  $\mu\text{m}$  in *M. rigidula* ( $2n=14$ ; Table 2; Fig. 1) to 19.18  $\mu\text{m}$  in *M. polymorpha* ( $2n=14$ ; Table 2; Fig. 1). The highest VRC (value of relative chromatin) amongst all species was obtained for *M. laciniata* and the lowest was obtained for *M. scutellata* (Table 2). The asymmetry index %TF ranged from 41.08 to 45.90 and the intrachromosomal asymmetry index ( $A_1$ ) varied from 0.14 to 0.29, while the interchromosomal asymmetry index ( $A_2$ ) ranged from 0.09 to 0.22 (Table 2).



**Figure 2.** Intrachromosome asymmetry index and total form percentage trend in different *Medicago* species.

**Table 3.** The results of analysis of variance for karyotypic data based on CRD design

Source of variation	Degrees of freedom	Mean of squares								
		SA	LA	TL	AR	CI	A1	A2	DRL	TF%
species	7	0.20**	0.48**	1.25**	0.04**	0.002**	0.02**	0.01**	1.03**	16.24**
Error	32	0.010	0.015	0.044	0.004	0.0001	0.002	0.001	0.062	1.550
CV (%)		8.66	8.14	7.94	4.80	2.72	10.82	9.16	11.00	2.83

\*\* - significant at 1% level of probability

**Table 4.** Mean of chromosomes analysis of *Medicago* species

Species	SA	LA	TL	AR	CI	A <sub>1</sub>	A <sub>2</sub>	DRL	TF%
<i>M. radiata</i>	1.36 <sup>ab*</sup>	1.67 <sup>b</sup>	3.03 <sup>b</sup>	1.23 <sup>cd</sup>	0.45 <sup>a</sup>	0.18 <sup>cd</sup>	0.09 <sup>d</sup>	3.50 <sup>de</sup>	44.99 <sup>ab</sup>
<i>M. intertexta</i>	1.22 <sup>bc</sup>	1.46 <sup>bc</sup>	2.68 <sup>bcd</sup>	1.20 <sup>d</sup>	0.46 <sup>a</sup>	0.16 <sup>cd</sup>	0.11 <sup>cd</sup>	4.28 <sup>de</sup>	45.56 <sup>a</sup>
<i>M. orbicularis</i>	0.99 <sup>d</sup>	1.26 <sup>cd</sup>	2.26 <sup>ef</sup>	1.28 <sup>bcd</sup>	0.44 <sup>ab</sup>	0.20 <sup>bcd</sup>	0.17 <sup>ab</sup>	6.24 <sup>bc</sup>	43.99 <sup>abc</sup>
<i>M. laciniata</i>	1.49 <sup>a</sup>	2.07 <sup>a</sup>	3.56 <sup>a</sup>	1.39 <sup>ab</sup>	0.42 <sup>bc</sup>	0.26 <sup>ab</sup>	0.11 <sup>cd</sup>	3.99 <sup>de</sup>	41.92 <sup>cd</sup>
<i>M. coronata</i>	1.05 <sup>cd</sup>	1.27 <sup>cd</sup>	2.32 <sup>d<sup>ef</sup></sup>	1.20 <sup>d</sup>	0.45 <sup>a</sup>	0.16 <sup>cd</sup>	0.14 <sup>bcd</sup>	5.18 <sup>bcd</sup>	45.41 <sup>a</sup>
<i>M. rigidula</i>	1.05 <sup>cd</sup>	1.51 <sup>b</sup>	2.57 <sup>cde</sup>	1.43 <sup>a</sup>	0.41 <sup>c</sup>	0.29 <sup>a</sup>	0.22 <sup>a</sup>	9.85 <sup>a</sup>	41.08 <sup>d</sup>
<i>M. polymorpha</i>	1.18 <sup>c</sup>	1.56 <sup>b</sup>	2.74 <sup>bc</sup>	1.32 <sup>bc</sup>	0.43 <sup>bc</sup>	0.23 <sup>abc</sup>	0.16 <sup>bc</sup>	6.72 <sup>b</sup>	43.09 <sup>bcd</sup>
<i>M. scutellata</i>	0.89 <sup>d</sup>	1.05 <sup>d</sup>	1.94 <sup>f</sup>	1.18 <sup>d</sup>	0.46 <sup>a</sup>	0.14 <sup>d</sup>	0.12 <sup>cd</sup>	3.00 <sup>c</sup>	45.90 <sup>a</sup>

SA: short arm, LA: long arm, TL: total length, AR: arm ratio, CI: centromeric index, A<sub>1</sub>: intrachromosome asymmetry index, A<sub>2</sub>: interchromosome asymmetry index, DRL: Difference of Relative Length, TF%: Total Form percentage.

\* indicated Mean within each column followed by different lowercase letters are significantly different at the 1% level according to the Duncan's test.

**Table 5.** Eigenvectors from the first two Principal components for nine karyotype parameters to classify eight species of *Medicago*

Parameters	First component	Second component
SA	0.18559	0.49367
LA	0.29944	0.39414
TL	0.26073	0.43696
AR	0.41801	-0.05969
CI	-0.41421	0.09256
A1	0.41801	-0.0753
A2	0.19399	-0.49227
DRL	0.27292	-0.38069
%TF	-0.41803	0.07036
<b>Eigen Value</b>	5.5557	3.21
<b>Percentage of Variance</b>	61.7305	35.6668
<b>Cum Percentage of variance</b>	61.7305	97.3973

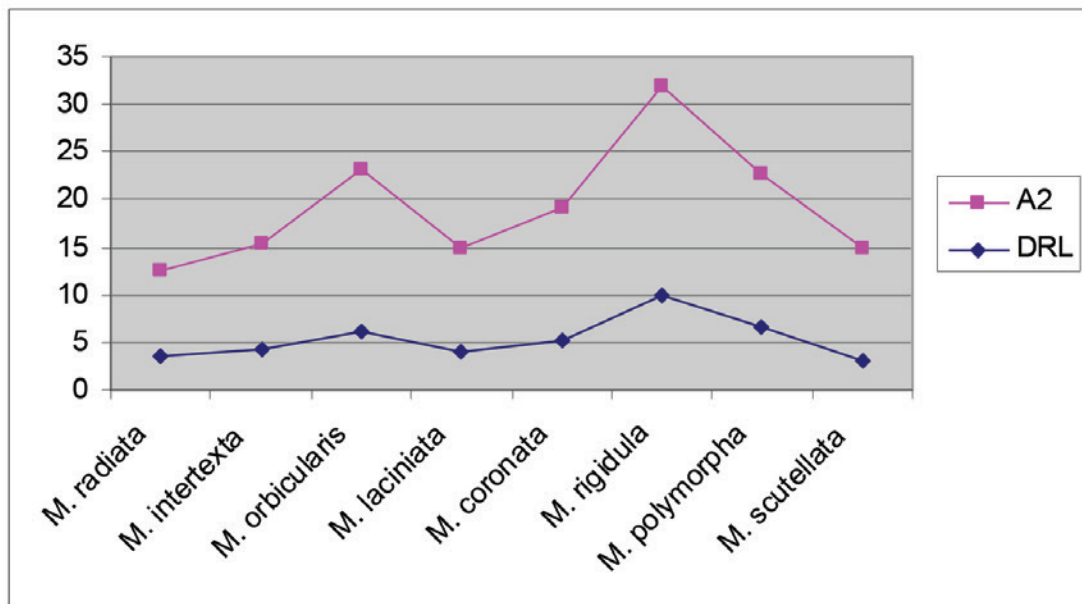
A statistical comparison based on completely randomized design demonstrates that there are significant differences among the species for all the measured traits ( $P < 0.01$ ) (Table 3). The

principal component analysis (PCA), of the karyotypic parameter shows that the first two principal components account for % 97.39 of total variance. Component one (%61.73) put

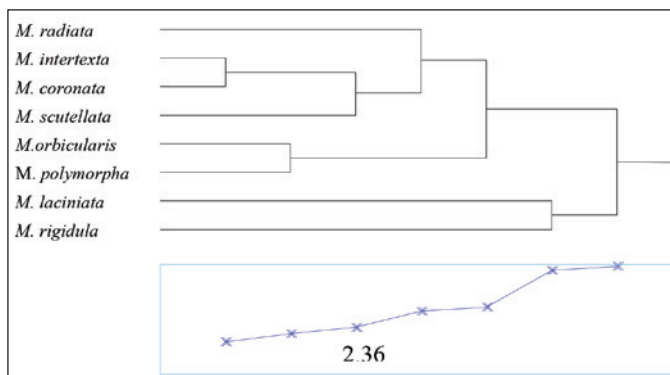
emphasis on the chromosome arm ratio, centromer index, A1 and TF% which had the highest coefficients of eigen vectors, while component two (%35.66) accentuates short arm length, long arm, total length, A<sub>2</sub> and DRL (Table 5). The grouping of the species are studied based on their relative karyotypic as well as mitotic characteristics (Table 4, Fig. 4). By cutting dendrogram resulted from cluster analysis Average methods with Cophenetic correlation coefficient ( $r=0.95$ ) in metric distance 2.36, the species classified under six

groups which certainly the first and the second components had the most significant role in separated classes. The highest metric distance was obtained between *M. radiata* and *M. laciniata* and the lowest metric distance was obtained between two species of *M. intertexta* and *M. coronata* (Fig. 4).

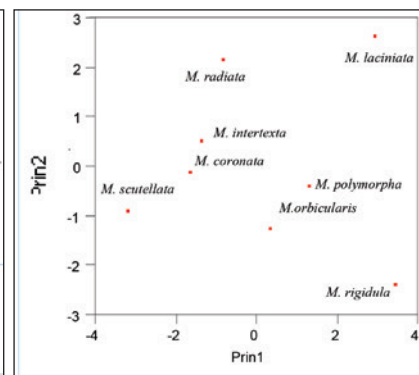
The diagram of the species dispersion, based on two first components showed the species separated in six groups, which completely fits with the results obtained through the average grouping analysis (Fig. 5).



**Figure 3.** Interchromosome asymmetry index and Difference of relative length trend in different *Medicago* species.



**Figure 4.** Dendrogram of eight species of *Medicago* by analyzing 9 karyotypic parameters using Average cluster analysis method. Cophenetic correlation  $\gamma=0.95$ .



**Figure 5.** Scatter plot of eight species for the first two principal components

## Discussion

Karyograms prepared from somatic chromosomes have provided some information on genetic relationships between groups of taxa.

In *M. Intertexta*, our count of  $2n = 16$  chromosomes is in agreement with numerous indications for this taxon (Fernandes et al. 1977; Abdelguerfi and Guittonneau 1979; Schlarbaum et al. 1984). A deviating count was reported from Portugal by Fernandes and Santos (1971) who found the tetraploid chromosome number of  $2n = 32$ .

It appears that self-incompatible plants occur in this taxon, which is not the case with other annual species. It is closely related to *M. ciliaris* and may be crossed with *M. muricoleptis*, which also belongs to the section Intertextae.

In *M. scutellata*, our count is in accordance with indications of tetraploid chromosome numbers made by several other authors with  $2n = 28$  (Abdelguerfi and Guittonneau 1979). Deviating counts were given by e.g. Nilsson and Lassen 1971 who found the tetraploid chromosome number of  $2n = 32$ , and Bauchan and Elgin (1984, cited in Goldblatt 1988: 109) who found the plant with  $2n = 30$  chromosomes. Fernandes et al. (1977), Fernandes and Queiros (1978), and Eraç and Tokluoglu (1983, cited in Goldblatt 1988: 109) found this species to occur also as a diploid cytotype.

Within the subsection Pachyspireae clade, we can find a polyploid species, *M. scutellata* of subsection Pachyspireae, that is  $2n = 30$  (Bauchan and Elgin 1984). *Medicago scutellata* is thought to be a polyploid derivative of a hybrid between a  $2n = 16$  species and a  $2n = 14$  species (Lesins and Lesins 1979; Bauchan and Elgin 1984), but the diploid species involved have not been identified. Strongly supported polymorpha clade as discussed above.

In *M. polymorpha*, our count of  $2n = 14$  chromosomes agrees with indications for this species made by several authors (Dahlgren et al. 1971; Luque et al. 1988). Deviating counts of  $2n = 16$  chromosomes reported by others (Fernandes et al. 1977; Fernandes and Queiros 1978; Labadie 1979, 1980) are considered to be either due to confusion with other *Medicago* species or presumably over-estimations due to the presence of a pair of chromosomes with large satellites.

Within section Spirocarpos (all annuals),

eight species are aneuploids and have a  $2n = 14$  chromosome number. Aneuploid reduction, whereby chromosome material from one small chromosome is added to another chromosome to form one larger chromosome instead of two smaller ones, has been hypothesized to result in the change from  $2n = 16$  to  $2n = 14$ .

In *M. rigidula* our count is in accordance with indications of diploid chromosome numbers made by several other authors with  $2n = 14$  (Heyn 1963; Lesins and Lesins 1979; Karadag 1997). Deviating counts were given by (Heyn 1963) who found the tetraploid chromosome number of  $2n = 16$ . The name *Medicago rigidula* has been applied to two groups that merit separate species status. Almost all of the plants of Europe and North Africa are preferable to one of these species, and almost all of the plants of Asia are preferable to the other. *Medicago rigidula* are recognized as European plants and *M. rigiduloides* are recognized as Asian plants (Small et al. 1990). The subsection *Pachyspireae* clade includes four species with  $2n = 14$ , *M. constricta*, *M. rigidula*, *M. rigiduloides*, and *M. sinkisiae* that form a monophyletic group when results from all workers are considered, and thus the chromosome number reduction from  $2n = 16$  to  $2n = 14$  may have taken place only once.

Four species *M. radiata*, *M. orbicularis*, *M. laciniata* and *M. coronata* possessed  $x=8$  ( $2n=2x=16$ ) chromosome numbers agrees with indications for this species made by several authors (Darlington 1961; Karadag 1997; Gazanchian 1993; Farsi 1989; Shariat 2001).

Results obtained from this research allow us to compare for the first time the karyotypes of several diploid and tetraploid species of *Medicago* genus. In view of the fact that, fewer DRL value illustrated more symmetry of karyotype, *M. scutellata* with DRL 3.00 values. Similarly, high DRL value leads to more changes in the construction of chromosomes. The Duncan's test applied to the chromosome morphometric traits (LA, SA, TL, AR and CI) showed a highly significant difference among all examined species belonging to different sections (Table 4). The ANOVA test showed the presence of significant differences ( $P < 0.01$ ) in the size of chromosomes as well as the ratio of long arms to short arms among diploid and tetraploid species. So these results indicate a significant quantitative



change in the amount of chromatin in *Medicago* species diversification (Table 3). Cluster analysis based on cytological data showed the species with the lowest metric distance may lead us to use species in crosses for inducing the highest genetic variations (Fig. 4). Grouping based on karyotypic data indicated all species based on ploidy level and base chromosome numbers stands on suitable branch except for *M. lacinita* that may be due to the different evolutionary history of cytological features and morphological characters in the species. The present study shows the change in the chromosomal traits as one of the mechanism of inter and intraspecies diversification in the *Medicago* genus as well as the earlier cytological reports. These genomic differences could be used for breeding purposes.

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