Acta Bot. Croat. 78 (1), 82–90, 2019
DOI 10.2478/botcro-2019-0010

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

A cytogenetic and pollen study of annual *Medicago* species from Soummam Valley (Northeastern of Algeria)

Linda Djafri-Bouallag*, Malika Ourari, Mohamed Sahnoune

Laboratory of Ecology and Environment, Department of Environment Biological Sciences, Faculty of Nature and Life Sciences, Université de Bejaia, Targa Ouzemmour, 06000 Bejaia, Algeria

Abstract – This paper reports a cytogenetic study of eight *Medicago* L. species sampled from the Soummam Valley (northeastern Algeria). Chromosome numbers and meiosis irregularities during microsporogenesis were explored. Pollen viability rate and pollen size were also examined. The studied taxa are diploid and display bivalent pairing and regular chromosome segregation during meiosis. Although meiosis appears regular, some anomalies were detected in relatively high cumulated rates (14.66%–26.14%). The most common meiotic abnormality examined here is related to cytomixis (from 14.66% in *M. littoralis* to 25.83% in *M. laciniata*). Other anomalies were also detected, including chromatic bridges, asynchronous divisions, micronuclei and multipolar cells. Consequently, the species exhibited varying percentages of pollen viability (from 70.11% in *M. laciniata* to 99.14% in *M. littoralis*). Pollen viability was negatively correlated with meiotic abnormalities (Pearson correlation coefficient $R = -0.72$, $p = 0.043$). The pollen grains were also heterogeneous in size. *Medicago truncatula* Gaertn. and *M. laciniata* (L.) Miller presented the most variable pollen size (relative standard deviation exceeding 19%). *Medicago littoralis* is distinguished from other species by possessing homogeneous and large sized pollen (relative standard deviation RSD = 6.73 %). The cytogenetic and pollen data provided by this study are discussed in the context of species systematics and in the perspective of genetic improvement.

Keywords: chromosome numbers, *Medicago*, meiotic abnormalities, microsporogenesis, pollen grain area, pollen viability, Soummam Valley

Introduction

Medicago L. is one of the largest genera in the Fabaceae family with 87 species including shrubs and herbaceous plants (Small 2011). *Medicago* species are characterized by flowers exhibiting a unique explosive pollination mechanism (Small 2011). Taxonomically, this genus belongs to the Trifolieae tribe and to the Trigonellinae sub-tribe. Basing their work on two nuclear genes (*CNGC5*, *β-cop*) and one mitochondrial DNA sequence (*rpS* 14-cob), Maureira-Butler et al. (2008) estimated the divergence time between *Medicago* and its sister clade *Trigonella* as having been ca 15.9 million years ago. The ancestral *Medicago* forms originated from the western Asia and Mediterranean regions and are supposed to be perennial and preferentially allogamous (Lesins and Lesins 1979). The derived annual species would have differentiated six to seven million years ago (Prosperi et al. 1995) and now have highly variable distributions depending of human interference. Annual species such as *M. truncatula*, *M. polymorpha*, *M. orbicularis* or *M. minima* are considered colonizers (Olivieri et al. 1991, Prosperi et al. 1995) and they are widely distributed in Mediterranean-type climates. Other species including *M. coronata* are rare or endemic and restricted to particular regions. With their ability to fix atmospheric nitrogen, *Medicago* species are of real interest in improving soil fertility, in mainly arid and semiarid areas. These species are also used in crop rotation and pest management plans to maintain a productive soil. In addition to their agricultural and ecological interests, *Medicago* species are used for animal nutrition (forage) and human consumption due to their high protein level. *Medicago* is recognized to be one of the most important genera of pasture plants in the world (Heyn 1963). Moreover, according to Small (2011), *Medicago* has potential for the production of biopharmaceuticals, bioplastics, cellulose and biofuel. *Medicago truncatula* is used as model plant for legume biology as a result of vari-

* Corresponding author, e-mail: djafribouallaglinda@gmail.com

ous biological characteristics (Cannon et al. 2006) including diploidy, small genome size, self-pollination, and rapid reproductive cycle.

The systematics of the genus is based on morphological and floral (Heyn 1963, Lesins and Lesins 1979, Small 2011) and biochemical (Classen et al. 1982, Jurzysta et al. 1992) traits. Pollen morphology (Small 2011) and interspecific hybridization (Simon 1965, Simon and Millington 1967, Lesins and Lesins 1979) were also studied. Recent phylogenetic studies involved chloroplast (*matK*, *trnK*/*matK*), mitochondrial (*rpS14-cob*) and nuclear (*GA3ox1*, *ITS*, *ETS*, *CNGC5*, *β-cop*) DNA sequences (Steele et al. 2010, Hu et al. 2014, de Sousa et al. 2016). However, homonyms and similarities still make species delineation and subspecies identification confusing. In Algeria, studies on this genus are scarce, being mainly conducted with regard to autoecology (Abdelguerfi et al. 1988b), physiology (Refoufi 1988, Amouri and Fyad-Lamèche 2012) morphology and biochemistry (Medoukali et al. 2015, Moulai and Fyad-Lamèche 2017), and *Rhizobia-Medicago* symbiotic interactions (Cheriet et al. 2014). The genus still remains under-investigated with regard to cytogenetics, largely due to both small chromosome size and technical difficulties encountered during staining procedures. Most of the studies conducted to date remain restricted to mitotic chromosome counts (Heyn 1963, Simon and Simon 1965, Lesins and Lesins 1979, Schlarbaum et al. 1984, Mariani et al. 1996). Very few karyological studies have involved Algerian *Medicago* populations (Abdelgeuerfi and Guittonneau 1979, Abdelguerfi et al. 1988a, Fyad-Lamèche et al. 2016) and even fewer have considered the haploid phase of these plants, as is generally the case in the whole *Medicago* genus worldwide. *Medicago* species exhibit significant diversity in vegetative morphology as a result of interspecific hybridization (Heyn 1963). Meiotic analyses may provide valuable information regarding the genetic or genomic causes of this diversity. Such studies could also provide significant insight into the cytological evolution of species that can be used in genetic improvement and in the conservation of genetic resources (Kumar and Singhal 2011).

In the present study, we analyzed natural populations of eight annual *Medicago* species sampled from different localities of the Soummam Valley in northeastern Algeria. The main aim of this paper, in addition to chromosome counts, is to examine the male meiotic course and the subsequent effects of meiotic abnormalities encountered during different stages of meiosis I and II on pollen grain size and pollen viability.

Materials and methods

Eight annual species, namely *M. truncatula* Gaertn., *M. littoralis* Rohde ex Lois., *M. intertexta* (L.) Miller, *M. ciliaris* (L.) Krocker, *M. arabica* (L.) Huds., *M. polymorpha* L., *M. laciniata* (L.) Miller and *M. minima* (L.) Bart. were sampled from wild populations of the Soummam Valley and neighborhoods (Northeastern Algeria). The geographical characteristics of the sampling sites are given in Tab. 1. Species identification was performed according to Lesins and Lesins (1979) and voucher specimens were deposited at ENSA Herbarium of the botany department of the National High School of Agriculture in Algiers (dpt.botanique@ensa.dz; www.ensa.dz).

Tab. 1. Geographical characteristics of the sampling sites of the Algerian *Medicago* L. species.

Taxa	Sampling site	Latitude, longitude	Altitude (m)
M. truncatula Gaertn.	Vahloul	36°32'03,65"N, 4°35'46,23"E	238
M. littoralis Rohde ex Lois.	Allaghane	36°23'42,76"N, 4°27'43,13"E	206
M. intertexta (L.) Miller	Ideraken (Timzrit)	36°37'48,78"N, 4°46'29,97"E	75
M. ciliaris (L.) Krocker	El Ghaba (Smaoune)	36°36'53,79"N, 4°50'30,86"E	305
M. arabica (L.) Huds.	Taddart Tamokrante (Amizour)	36°37'6.30"N 4°59'57.80"E	511
M. polymorpha L.	Allaghane	36°23'42,76"N 4°27'43,13"E	206
M. laciniata (L.) Miller	Allaghane	36°23'42,76"N 4°27'43,13"E	206
M. minima (L.) Bart.	Aftis	36°23'7.85"N 4°27'23.45"E	217

Flower buds at different stages of development before anthesis were collected, fixed in situ in Carnoy's fixative (glacial acetic acid – chloroform – absolute ethanol, 1:3:6) and preserved at 4 °C in a refrigerator until used.

Cytological analysis was performed on pollen mother cells (PMCs). Young flower buds were hydrolyzed in 1 N HCl at 60 °C in a water bath for three minutes. Under a binocular stereomicroscope, the removed immature anthers were immediately excised on a slide in a drop of 1% lactopropionic orcein and squashed under a coverslip. All stages of meiosis were analyzed in order to determine chromosome numbers, to study meiotic behavior and to evaluate meiotic abnormality frequencies. About fifty individuals per population were harvested and around 1000 PMCs per species were scrutinized.

Pollen viability was determined by staining mature flowers before anthesis using cotton blue in lactophenol (1% blue aniline in lactophenol) according to Mertens and Hammersmith (1998). Flowers were dissected to free up pollen grains from the anther and mounted in a drop of cotton blue. The staining takes from 20 to 30 minutes. Viable pollen grains are stained uniformly dark blue, whereas defective grains are weakly stained and appear vacuolated, plasmolysed, and atypical in size and shape. Photomicrographs were taken using a digital camera connected to an OPTIKA B-350 microscope. About 15 flowers were prepared for each population and up to 1000 pollen grains per flower were analyzed for evaluating pollen viability. An average pollen viability rate was estimated for each species. The pollen grain cell area was measured for 100 viable pollen grains per sample using Im-

ageJ 1.48v Image Processing and Analysis in Java (Rasband 2016). Pearson correlation coefficient (R) between meiotic abnormalities frequency and pollen viability rate as well as the relative standard deviation (RSD) of pollen grain area were estimated for each species sample. The relative standard deviation formula is: RSD = 100 s / $\bar{x}$; where s is the sample standard deviation, $\bar{x}$ is the sample mean. Fisher's exact test was applied to compare frequencies of meiotic abnormalities and pollen viability rates; and the least significant difference (LSD) test was used to check the homogeneity of pollen viability rates and pollen grain area mean values across samples. Before running LSD test, raw data were divided by SD in order to homogenize their variances. Statistical treatments were performed using Statistica 8.0 (StatSoft 2008).

Results

Meiotic chromosome counts revealed that the *M. truncatula* Gaertn., *M. littoralis* Rohde ex Lois., *M. intertexta* (L.) Miller, *M. ciliaris* (L.) Krocker, *M. arabica* (L.) Huds, *M. laciniata* (L.) Miller and *M. minima* (L.) Bart. populations were all diploid with a basic number x = 8 chromosomes. *M. polymorpha* L. proved also diploid but the basic chromosome number was x = 7 (Figs. 1, a-h). Our samples showed no tetraploid or aneuploid cytotypes.

The studied taxa displayed regular bivalent pairing and chromosome segregation at meiosis. The analysed PMCs show exclusively bivalent associations (Figs. 1, a-h). No multivalent or univalent associations were observed. Numerous anomalies in relatively high rates (≈ 25%) were detected, including cytomixis, the most common abnormality, chromatic bridges, asynchronous divisions, micronuclei and multipolar cells (Tab. 2). *M. littoralis* is distinguished by its lowest abnormality rate, restricted to cytomixis (14.66%). The samples show cytomixis rates varying from 14.66% in *M. littoralis* to 25.83% in *M. laciniata* (Tab. 2). *M. arabica*, *M. polymorpha*, *M. laciniata* and *M. minima* (Sect. Leptospirae) exhibited cytomixis rate values exceeding 22.13%, significantly higher than those of the remaining species (Tab. 2). The occurrence of cytomixis in our samples was recorded from early prophase I to microspore stage (Figs. 2, a-e). The chromatin transfer is performed either by one or by several strands in all the analysed species. It also occurs by direct contact between two or more cells (Figs. 2, a-b). Another abnormality recorded in *M. truncatula*, *M. intertexta*, *M. ciliaris*, *M. polymorpha* and in *M. minima* was chromatic bridges at rates ranging from 0.63% in *M. minima* to 1.45% in *M. ciliaris* (Tab. 2, Figs. 2, f). Asynchronous divisions were observed at anaphase II in *M. intertexta*, *M. polymorpha*, *M. laciniata* and in *M. minima* (Fig. 2, g). The rate recorded for this irregularity in the analysed samples varies from 0.31% in *M. laciniata* to 1.32% in *M. intertexta* (Tab. 2). Micronuclei were found in *M. truncatula* (1.88%), *M. intertexta* (0.79%), and in *M. minima* (0.42%) (Figs. 2, h-i). The last anomaly observed in the analysed taxa is the form of multipolar cells. In this case, the ultimate meiotic product corresponds to polyads instead of tetrads (Fig. 2, j-l). This abnormality was observed only in *M. truncatula* (2.56%) and *M. intertexta* (0.79%) (Tab. 2).

Pollen viability rates were variable among and within populations (Fig. 3). While *M. littoralis* appears to be invariable with a high percentage of viability (99.14%), *M. laciniata* shows significant variability ranging from 0 to 98.75% with a relatively low mean value (70.11%). Pollen viability rates seem to be inversely proportional to the cumulative rate of meiotic anomalies, since the Pearson coefficient correlation value is – 0.72 with p = 0.043. Pollen average area varies from 501.21 μm² ± 53.86 in *M. polymorpha* to 1074.93 μm² ± 72.32 in *M. littoralis* (Figs. 4, 5). *M. truncatula* and *M. laciniata* reveal heterogeneous pollen grain area; with a calculated relative standard deviation of pollen grain area (RSD) of around 19%. It is worth noting that *M. littoralis* is distinguished from

Tab. 2. Chromosome numbers and meiotic abnormality rates in the *Medicago* L. species from Northeastern Algeria. Abbreviations: PMC – pollen mother cells; CM – cytomixis; CB – chromatic bridges; AD – asynchronous divisions; MN – micronuclei; MC – multipolar cells; CA – cumulative abnormality frequency; PV – pollen viability; SD – standard deviation; PGA – pollen grain area; RSD – relative standard deviation; N – number of individuals (for each species). For each column separately, values with different letters are significantly different as revealed by Fisher's exact test (small letters) and LSD test (capital letters) at P = 0.05.

Species	Chromosome numbers and meiotic abnormalities								Pollen data		
	PMC	2n	CM (%)	CB (%)	AD (%)	MN (%)	MC (%)	CA (%)	PV±SD (%) (N=15)	PGA±SD (10^2 μm²) (N=100)	PGA RSD (%) (N=100)
M. truncatula	1170	16	18.29b	1.2c	0	1.88c	2.56c	23.93	0.79±0.16B	6.37±1.24C	19.56
M. littoralis	921	16	14.66a	0	0	0	0	14.66	0.99±0.01G	10.75±0.72F	6.73
M. intertexta	1137	16	15.04a	1.06c	1.32c	0.79b	0.79b	19.00	0.88±0.21B	7.01±0.99E	14.15
M. ciliaris	1035	16	14.78a	1.45c	0	0	0	16.23	0.96±0.05E	6.39±0.99D	15.58
M. arabica	1124	16	24.11cd	0	0	0	0	24.11	0.97±0.02F	6.81±0.95E	13.91
M. polymorpha	1323	14	22.45c	0.68b	1.13c	0	0	24.26	0.81±0.12C	5.01±0.53A	10.75
M. laciniata	1270	16	25.83d	0	0.31b	0	0	26.14	0.70±0.30A	7.22±1.37C	19.04
M. minima	1437	16	22.13c	0.63b	0.84c	0.42b	0	24.01	0.87±0.05D	6.34±0.73B	11.63

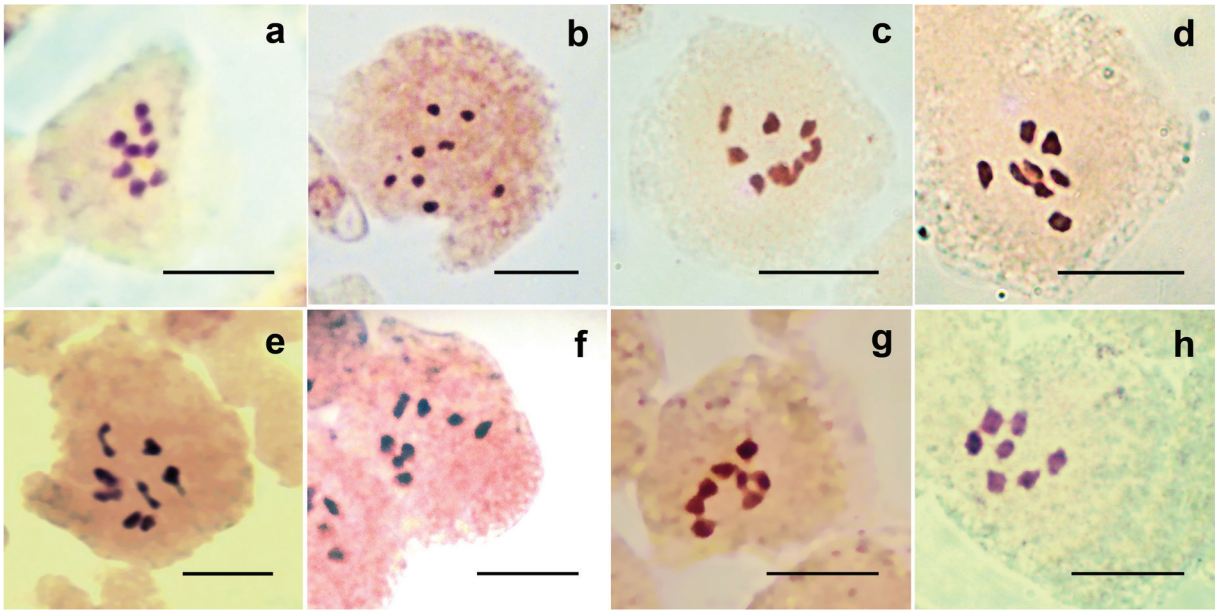

Fig. 1. Micromeiocyte metaphase plates in the *Medicago* L. species from Northeastern Algeria. a) *M. truncatula* (8 bivalents, 2n=2x=16); b) *M. littoralis* (8 bivalents, 2n = 2x = 16); c) *M. intertexta* (8 bivalents, 2n = 2x = 16); d) *M. ciliaris* (8 bivalents, 2n = 2x = 16); e) *M. arabica* (8 bivalents, 2n = 2x = 16); f) *M. laciniata* (8 bivalents, 2n = 2x = 16); g) *M. minima* (8 bivalents, 2n = 2x = 16); h) *M. polymorpha* (7 bivalents, 2n = 2x = 14). Scale bars = 10 μm.

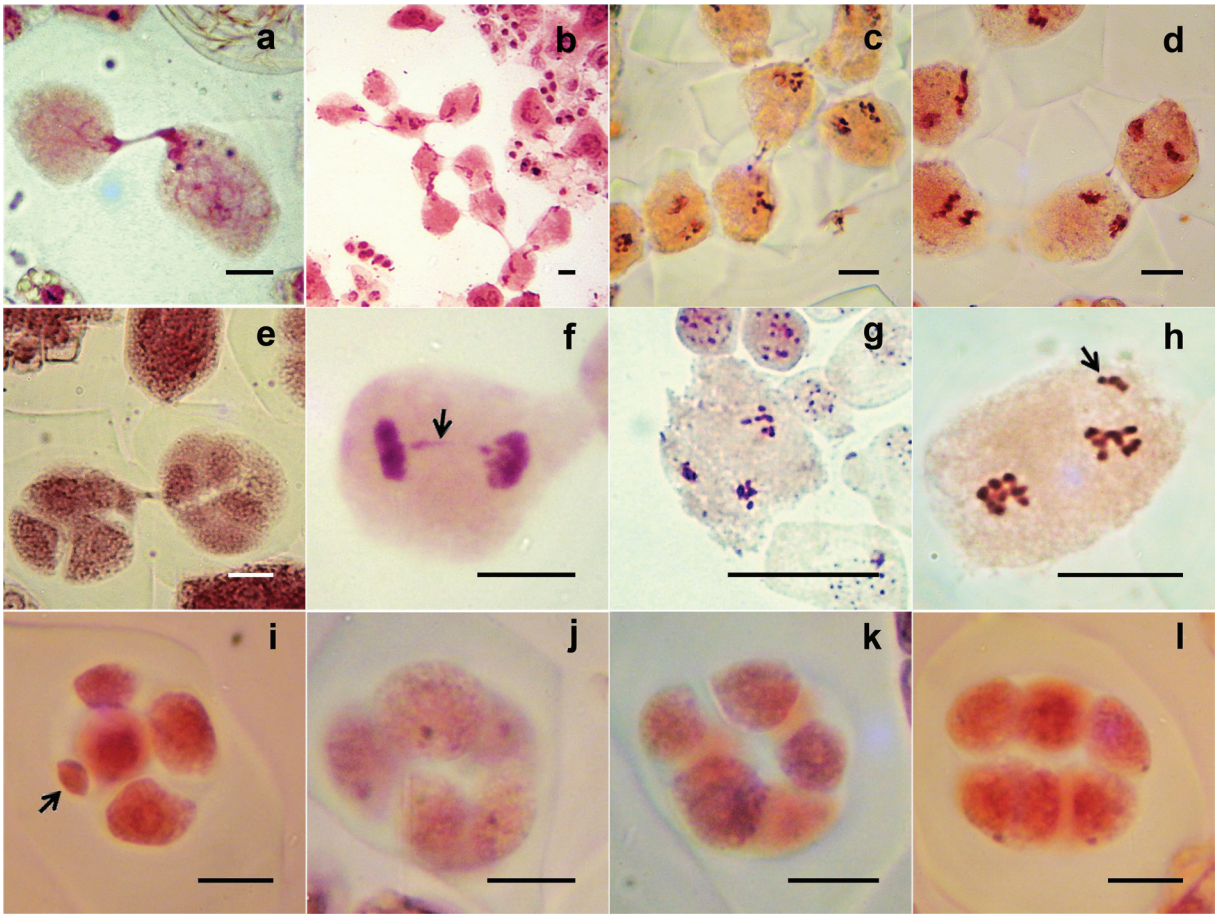

Fig. 2. Abnormal meiotic behaviour in the studied *Medicago* L. species from Northeastern Algeria. a) Cytomixis between two micromeiocytes at prophase I; b-d) cytomixis between several micromeiocytes at different stages; e) cytomixis between two microspores; f) chromatic bridge (arrow); g) asynchronous division; h-i) micronucleus (arrow); j-l) multipolar cells. Scale bars = 10 μm.

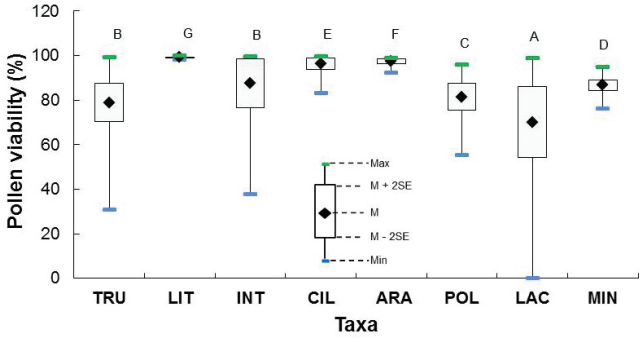

Fig. 3. Pollen viability in the *Medicago* L. species from Northeastern Algeria. Max – maximum, M – mean; SE – standard error, Min – minimum. TRU – *M. truncatula*, LIT – *M. littoralis*, INT – *M. intertexta*, CIL – *M. ciliaris*, ARA – *M. arabica*, POL – *M. polymorpha*, LAC – *M. laciniata*, MIN – *M. minima*. Different capital letters above bars denote significantly different results as revealed by LSD test (P = 0.05).

other species by the large size and the homogeneity of its pollen (RSD = 6.73 %) (Fig. 5).

Discussion

In this article, chromosome numbers, meiotic behaviour and meiotic abnormalities were performed by analysis of dividing pollen mother cells on natural populations in Northeastern Algeria. Pollen viability analyses were also carried out. The present results represent the first haploid phase cytogenetic observations reported for Algerian populations of *Medicago* species.

The encountered basic chromosome numbers (x = 8, x = 7) were previously reported in the genus *Medicago* by Heyn (1963), Simon (1965) and by Lesins and Lesins (1979). According to Steele et al. (2010), the basic chromosome number of x = 8 would be plesiomorphic in the Trifolieae tribe. The basic chromosome number of x = 7 was interpreted as an innovation deriving from x = 8 following chromosome rearrangements (Lesins and Lesins 1979). Based on the phylogeny of plastid and nuclear sequences (*trnK* / *matK*, *GA-3ox1*), the apomorphic base number x = 7 arose four times in the genus. Furthermore, this phylogeny showed that *M. polymorpha* and *M. murex* form a monophyletic group called 'Polymorpha clade' (Steele et al. 2010). As in *M. murex*, *M. polymorpha* genome would have arisen from a translocation of chromosome 8 on chromosome 3 by losing the centromeric region and thus forming de novo a long satellite (Lesins and Lesins 1979). The chromosome counts 2n = 2x = 14 and 2n = 2x = 16, obtained for the analysed species are in agreement with those made by several authors (Heyn 1963, Simon and Simon 1965, Lesins and Lesins 1979, Schlarbaum et al. 1984, Mariani et al. 1996). However, some authors reported deviating counts with different chromosome basic numbers. In fact, Humphries et al. (1978) and Vogt and Aparicio (1999) found 2n = 14 in *M. truncatula* Gaertn. In *M. polymorpha*, several authors (see Rani et al. 2012) found 2n = 16 with x = 8. In this taxon, the deviating count 2n = 16 is considered to be due to either species misidentification or presumably to the number over-estimation attributed to the presence of a pair of chromosomes with large satellites (Kamari et al. 1996). The absence of tetraploid or aneuploid cytotypes is consistent with the results obtained by the majority of authors for these species (Simon and Simon 1965,

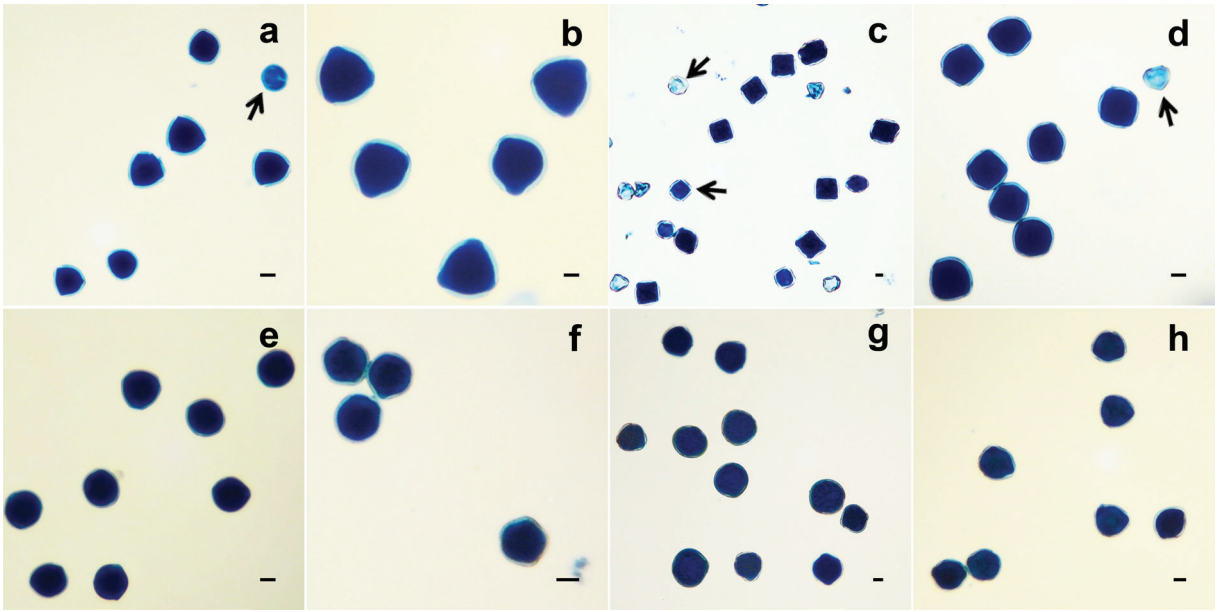

Fig. 4. Pollen grains of the *Medicago* L. species from Northeastern Algeria stained by cotton blue in lactophenol. a) *M. truncatula* (heterogeneous pollen); b) *M. littoralis* (homogeneous pollen); c) *M. intertexta* (homogeneous pollen); d) *M. ciliaris* (homogeneous pollen); e) *M. arabica* (homogeneous pollen); f) *M. polymorpha* (homogeneous pollen); g) *M. laciniata* (heterogeneous pollen); h) *M. minima* (homogeneous pollen). Arrows indicate defective pollen grains. Scale bars = 10 μm.

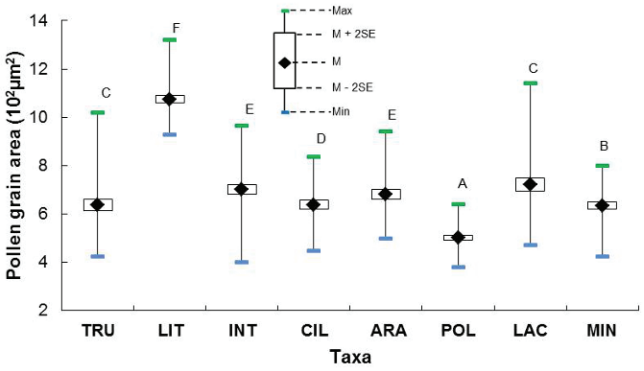

Fig. 5. Pollen grain area in the *Medicago* L. species from Northeastern Algeria. Max – maximum, M – mean; SE – standard error, Min – minimum. TRU – *M. truncatula*, LIT – *M. littoralis*, INT – *M. intertexta*, CIL – *M. ciliaris*, ARA – *M. arabica*, POL – *M. polymorpha*, LAC – *M. laciniata*, MIN – *M. minima*. Different capital letters above bars denote significantly different results as revealed by LSD test (P = 0.05).

Lesins and Lesins 1979, Schlarbaum et al. 1984, Mariani et al. 1996, Small 2011, Sadeghian and Hesamzadeh Hejazi 2014). However, polyploid levels were reported for two species (*M. intertexta*, *M. polymorpha*) by some authors. As reported by Fernandes and Santos De Fatima (Kamari et al. 1996) but not confirmed by Lesins and Lesins (1979), the individual of *M. intertexta* (L.) Miller from Portugal was tetraploid (2n = 32). Recently, in *M. polymorpha* L., a tetraploid level with 2n = 4x = 32 was reported from India by Rani et al. (2014) in a general survey on angiosperms. The few deviating chromosome counts should be verified since polyploidy is relatively rare among the annual species of *Medicago*. Indeed, according to Small (2011), only *M. ovalis*, *M. rugosa* and *M. scutellata* are certainly tetraploid. Aneuploid forms were also reported for *M. ciliaris* (L.) Krocker by some authors. Early on, in 1956, Heyn found in a Middle East accession of *M. ciliaris* a diploid chromosome number 2n = 18 (2n = 16 + 2B). However, Clement (1962) and Lesins and Lesins (1979) are reluctant to accept this possibility and consider the "supernumerary chromosomes" as detached satellites, not countable as complete chromosomes. Later, Abdelguerfi and Guittonneau (1979) in an Algerian population and Kamari et al. (1996) in a Tunisian population again found this count of 2n = 18.

Analysis of meiotic chromosome behaviour in metaphase I is essential to formulate a breeding strategy. Our samples show exclusively bivalent associations. These chromosome pairings were a prerequisite for a balanced segregation (Da Ines et al. 2014). They also generate new combinations of parental alleles that increase genetic diversity (Naranjo 2012). The natural hybridizations reported within the complex *M. littoralis* - *M. truncatula* - *M. tornata* (Heyn 1963, Lesins and Lesins 1979) appear to be absent in the analysed *littoralis* population since meiosis seems regular. The low rate of meiotic anomalies could explain its morphological homogeneity and therefore, the studied population may correspond to the "pure" form of *M. littoralis*. On the other hand, although the studied *M. truncatula* population appears to be morphologically homogeneous, it showed numerous meiotic anomalies suggesting potential natural hybridization with some other related species, such as *M. littoralis* or *M. tornata*. The most common meiotic abnormality observed in all the studied samples is cytomixis. This is related to the migration of chromatin between meiocytes through cytoplasmic channels. This chromatin transfer is under genetic control but can be influenced by environmental conditions, as pointed out by Ranjbar et al. (2011a) in *Trigonella spruneriana* Boiss. The analysed samples show variable cytomixis rates. In *Astragalus*, thecytomixis rate was relatively low, varying from 0% to 3.82% (Ranjbar et al. 2011b). In contrast, this rate can reach 45% in *Lotus* (Sheidai and Jalilian 2006). In *Medicago* L., the few cytomictic studies reported have often dealt with polyploid taxa. The cytomixis rate recorded for the tetraploid species *M. sativa* reached 46% (Bellucci et al. 2003). Ranjbar et al. (2011b) reported that cytomixis in *Astragalus* seem to be more prevalent among polyploid taxa than in their diploid counterparts. In contrast, in Solanaceae, Singhal and Kumar (2008) have observed higher rates in diploids than in polyploids. In general, cytomictic activity is maximal during early prophase I due to the parallel transfer of trophic factors and signal molecules ensuring the synchronisation of meiosis (Mursalimov et al. 2013). In *M. sativa*, chromatin transfer is mostly detected in prophase I and rarely in later stages (Bellucci et al. 2003). In the analysed samples, the cytomixis was recorded from early prophase I to microspore stage. This report was similar to those observed in *Astragalus cyclophyllos* (Ranjbar et al. 2011b), *Lotus corniculatus* (Jeelani et al. 2012) and in *Nepeta govaniana* (Kaur and Singhal 2014). The chromatin transfer performed either by one or by several strands and by direct contact between two or more cells in all the analysed species is also reported by several authors for other taxa such as *Medicago sativa*, *Lotus corniculatus* and *Astragalus cyclophyllos* (Bellucci et al. 2003, Sheidai and Jalilian 2006, Ranjbar et al. 2011b). The chromatic bridges recorded in *M. truncatula*, *M. intertexta*, *M. ciliaris*, *M. polymorpha* and in *M. minima* samples have also been reported in some species such as *Onobrychis chorassanica* (Ranjbar et al. 2010), *Trigonella spruneriana* (Ranjbar et al. 2011a) and *Calamagrostis emodensis* (Saggoo and Kumari 2013) at variable frequencies. In the genus *Passiflora* (Passifloraceae), this rate can reach 26% (Souza and Pereira 2011). Chromatic bridges could be explained either by chromosomal rearrangement such as heterozygous paracentric inversions (Ruvalcaba-Ruiz and Rodríguez-Garay 2002, Elrod and Stansfield 2003) or by unrepaired double-strand breaks induced by *Spo11* enzyme (Horlow and Doutriaux 2003). The observed asynchronous divisions in *M. intertexta*, *M. polymorpha*, *M. laciniata* and in *M. minima* samples result from a mutation affecting the spindle "checkpoint" (Risso-Pascotto et al. 2003). In this case, only one cell of the dyad progresses in the cell cycle while the defective one stays blocked at metaphase II. The rate recorded for this irregularity was variable in the analysed samples. Variable rates are reported in many Leguminosae species. For reference, the rates vary from 0.41% to 0.79% in *Trigonella* and from 0

to 24.98% in *Astragalus* (Ranjbar et al. 2011a, Ranjbar and Jahanian 2013, Jahanian and Sarpoushi 2014). Micronuclei, the other anomaly recorded in our samples, would be caused by the condensation of the chromatin originating from cytomixis or fragments of chromatic bridges. These micronuclei can then be removed by cytokinesis or by budding producing thus sterile microcytes (Baptista-Giacomelli et al. 2000, Kiihl et al. 2011). A literature overview reveals that in angiosperms the micronuclei rate varies according to the ploidy level. Thus, in diploid taxa, it ranges from 0% in *Trigonella spruneriana* to 1.66% in *Astragalus ebenoides* (Ranjbar et al. 2011a, Ranjbar and Jahanian 2013). However, in tetraploid taxa, this rate varies from 0% in *Astragalus carmanicus* to 5.52% in *Vicia pallida* (Singhal and Kaur 2011, Jahanian and Sarpoushi 2014). In the octoploid *Alchornea triplinervia* (Euphorbiaceae) tree species, this rate can reach 9.61% (Godoy et al. 2012). The observed polyads in *M. truncatula* and in *M. intertexta* were the result of several mutations such as *ms28* and *ms17,* which affect the bipolar spindle formation (Albertsen and Phillips 1981, Golubovskaya and Distanova 1986). Another hypothesis, put forward by Caetano-Pereira and Pagliarini (2001), refers to the role of transposable elements. Spindle orientation abnormalities have been reported in several species: in *Agropyron cristatum*, *Carthamus tinctorius*, *Fuchsia*, *Thunbergia mysorensis*, *Aloysia lycioides*, *Zea mays* (Caetano-Pereira and Pagliarini 2001 and references therein). This abnormality is reported in many Leguminosae species at variable rates (e.g. 0.13% in *Onobrychis chorassanica*, 0.32% in *Astragalus carmanicus*, 10.98% in *Vicia pallida*) (Ranjbar et al. 2010, Singhal and Kaur 2011, Jahanian and Sarpoushi 2014).

The pollen viability showed an important intra- and interspecific variation. A significant variation in viability rate was reported within populations and among species in *Medicago* (Simon and Millington 1967, Lesins and Erac 1968, Singh and Lesins 1972). Sometimes, hybrids show rates similar to those of the parental taxa (Simon and Millington 1967). Our pollen samples also showed an important interspecific variation in size. There are no data in the literature about pollen grain size in *Medicago*. However in other species, some authors, such as Kumar et al. (2010), explain this pollen size heterogeneity by the presence of meiotic abnormalities.

In conclusion, the eight annual *Medicago* populations investigated in the Soummam Valley (Northeastern Algeria) showed regular meiosis with the presence of exclusively 8 bivalents in *M. truncatula* Gaertn., *M. littoralis* Rohde ex Lois., *M. intertexta* (L.) Miller, *M. ciliaris* (L.) Krocker, *M. arabica* (L.) Huds, *M. laciniata* (L.) Miller, *M. minima* (L.) and 7 bivalents in *M. polymorpha* L. at metaphase I revealing the diploid nature of these species. The observed meiotic abnormalities related to cytomixis, multipolar cells, micronucleus, asynchronous divisions and chromatic bridges seem to be correlated with pollen size and pollen viability. These meiotic anomalies would be potential mechanisms at the origin of the genetic variability in natural populations of the *Medicago* L. genus. *Medicago* species, especially annuals because of their potential source of germplasm for alfalfa breeding programs, can be used to valorize, improve and increase the crop production.

Acknowledgement

The authors greatly thank Mr. Hocine ABBACI, Assistant Professor at the University of Bejaia, for his helpful and valuable assistance in using image processing software.

References

Abdelguerfi, A., Abdelguerfi-Berrekia, R., Guittonneau, G. G., 1988a: Contribution à l'étude des espèces spontanées du genre *Medicago* L. en Algérie. III. Dénombrement chromosomique chez 35 populations de 17 espèces. Annales de l'Institut national agronomique - El Harrach 12, 342–354.

Abdelguerfi, A., Chapot, J.Y., Conesa, A.P., Roseau, R., 1988b: Contribution à l'étude des espèces spontanées du genre *Medicago* L. en Algérie. 1. Répartition des espèces en fonction des facteurs du milieu. Annales de l'Institut national agronomique – El Harrach 12, 304–328.

Abdelguerfi, A., Guittonneau, G. G., 1979: IOPB chromosome number reports LXIV, Taxon 28, 392–393.

Albertsen, M. C., Phillips, R. L., 1981: Developmental cytology of 13 genetic male sterile loci in maize. Canadian Journal of Genetics and Cytology 23, 195–208.

Amouri, A.A., Fyad-Lameche, F. Z., 2012: Analyse comparative de la tolérance à la salinité du gamétophyte mâle et du sporophyte chez *Medicago* au stade germination. Acta Botanica Malacitana 37, 93–102.

Baptista-Giacomelli, F. R., Pagliarini, M. S., Almeida, J. L., 2000: Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). Cytologia 65, 371–378.

Bellucci, M., Roscini. C., Mariani, A., 2003: Cytomixis in pollen mother cells of *Medicago sativa* L. Journal of Heredity 94, 512–516.

Caetano-Pereira, C. M., Pagliarini, M. S., 2001: A new meiotic abnormality in *Zea mays*: multiple spindles associated with abnormal cytokinesis in both divisions. Genome 44, 865–871.

Cannon, S. B., Sterck, L., Rombauts, S., Sato, S., Cheung, F., Gouzy, J., Wang, X., Mudge, J., Vasdewani, J., Schiex, T., Spannagl, M., Monaghan, E., Nicholson, C., Humphray, S. J., Schoof, H., Mayer, K. F., Rogers, J., Quétier, F., Oldroyd, G. E., Debellé, F., Cook, D. R., Retzel, E. F., Roe, B. A., Town, C. D., Tabata, S., Van de Peer, Y., Young, N. D., 2006: Legume genome evolution viewed through the *Medicago truncatula* and *Lotus japonicus* genomes. Proceedings of the National Academy of Sciences, USA 103, 14959–14964.

Cheriet, D., Ouartsi, A., Chekireb, D., Babaarbi, S., 2014: Phenotypic and symbiotic characterization of rhizobia isolated from *Medicago ciliaris* L. growing in Zerizer from Algeria. African Journal of Microbiology Research 8, 1763–1778.

Classen, D., Nozzolillo, C., Small, E., 1982: A phenolic–taxometric study of *Medicago* (Leguminosae). Canadian Journal of Botany 60, 2477–2495.

Clement, W. M., 1962: Chromosome numbers and taxonomic relationships in *Medicago*. Crop Science 2, 25–28.

Da Ines, O., Gallego, M. E., White, C. I., 2014: Recombination-independent mechanisms and pairing of homologous chromosomes during meiosis in plants. Molecular Plant 7, 492–501.

de Sousa, F., Bertrand, Y. J. K., Pfeil, B. E., 2016: Patterns of phylogenetic incongruence in *Medicago* found among six loci. Plant Systematics and Evolution 302, 493–513.

Elrod, S., Stansfield, W., 2003: Génétique. 4ième Edition. Ediscience international. Paris.

Fyad-Lameche, F. Z., Iantcheva A., Siljak-Yakovlev, S., Brown S. C., 2016: Chromosome number, genome size, seed storage protein profile and competence for direct somatic embryo formation in Algerian annual *Medicago* species. Plant Cell Tissue and Organ Culture 124, 531–540.

Godoy, S. M., Pereira, A. R. A., Romagnolo, M. B., Risso-Pascotto, C., 2012: Meiotic behavior during microsporogenesis of *Alchornea triplinervia* (Sprengel) Müller Argoviensis. Ciência Rural 42, 1027–1032.

Golubovskaya, I. N., Distanova, E. E., 1986: Mapping mei-gene ms43 by B–A translocation stocks. Genetica 22, 1173–1180.

Heyn, C. C., 1963: The annual species of *Medicago*. Scripta Hierosolymitana 12. Publications of the Hebrew University, Jerusalem.

Horlow, C., Doutriaux, M. P., 2003: Les mécanismes moléculaires de la méiose chez les plantes. Médecine/Sciences 19, 717–23.

Hu, D., Li, F. F., Liu, J., Sun, Y. X., Li, X. W., Yan, J., Li, J. Q., 2014: Systematic positions of *Medicago edgeworthii* and *M. archiducis-nicolai* (Leguminosae) inferred from plastid trnk/matk, nuclear GA3ox1 and ITS sequences. Pakistan Journal of Botany 46, 775–778

Humphries, C. J., Muitay, B. G., Bocquet, G., Vasudevan, K. N., 1978: Chromosome numbers of phanerogams from Morocco and Algeria. Botaniska Notiser 131, 391–404.

Jahanian, S., Sarpoushi, M. R., 2014: Study of meiotic behavior and chromosome number of 3 populations of *A. carmanicus* of the *Astragalus* sect. *Microphysa* Bunge. in Iran. International Research Journal of Applied and Basic Sciences 8, 2294–2299.

Jeelani, S. M., Kumari, S., Gupta, R. C., 2012: Male meiosis in *Lotus corniculatus* L. Plant Systematics and Evolution 298, 1977–1985.

Jurzysta, M., Burda, S., Oleszek, W., Ploszynski, M., Small, E., Nozzolillo, C., 1992: Chemical composition of seed saponins as a guide to the classification of *Medicago* species. Canadian Journal of Botany 70, 1384–1387.

Kamari, G., Felber, F., Garbari, F., 1996: Mediterranean chromosome number reports – 6. Flora Mediterranea 6, 223–337.

Kaur, M., Singhal, V. K., 2014: First report of cytomixis and meiotic abnormalities in *Nepeta govaniana* from Solang Valley, Kullu District, Himachal Pradesh. Cytologia 79, 227–233.

Kiihl, P. R. P., Pereira, A. R. A., Mataroli de Godoy, S., Stenzel, N. M. C., Risso-Pascotto, C., 2011: Chromosome stickiness during meiotic behavior analysis of *Passiflora serrato-digitata* L. (Passifloraceae). Ciência Rural 41, 1018–1023.

Kumar, P, Singhal, V.K., 2011: Chromosome number, male meiosis and pollen fertility in selected Angiosperms of the cold deserts of Lahaul-Spiti and adjoining areas (Himachal Pradesh, India). Plant Systematics and Evolution 297, 271–297.

Kumar, P., Singhal, V. K., Kaur, D., Kaur, S., 2010: Cytomixis and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*. Biologia Plantarum 54, 181–184.

Lesins, K., Erac, A., 1968: Relationship of taxa in the genus *Medicago* as revealed by hybridization. III. *M. ashersoniana* x *M. laciniata*. Canadian Journal of Genetics and Cytology 10, 777–781.

Lesins, K., Lesins, I., 1979: Genus *Medicago* (Leguminosae). A taxogenetic study . Dr. W. Junk bv Publishers. The Hague Boston London.

Mariani, A., Pupilli, F., Calderini, O., 1996: Cytological and molecular analysis of annual species of the genus *Medicago*. Canadian Journal of Botany 74, 299–307

Maureira-Butler, I. J., Pfeil, B. E., Muangprom, A., Osborn, T. C., Doyle, J. J., 2008: The reticulate history of *Medicago* (Fabaceae). Systematic Biology 57, 466–482.

Medoukali, I., Bellil, I., Khelifi, D., 2015: Morphological and isozyme variation in natural populations of the genus *Medicago* L. prospected in Northern Algeria. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 43, 86–95.

Mertens, T. R., Hammersmith, R. L., 1998: Genetics Laboratory Investigations 11 ed. Laboratory manual Prentice Hall, New Jersey.

Moulai, D., Fyad-Lameche, F. Z., 2017: Comparison of genetic diversity among *Medicago ciliaris* populations prospected from highly salted region (Oran Great Sebkha) using morphological pod descriptors of IBPGR and SDS-PAGE markers. International Journal of Biosciences 11, 27–38.

Mursalimov, S. R., Sidorchuk, Y. V., Deineko, E. V., 2013: New insights into cytomixis: specific cellular features and prevalence in higher plants. Planta 238, 415–423.

Naranjo, T., 2012: Finding the correct partner : The meiotic courtship. Scientifica 2012, 1–14.

Olivieri, I., Gouyon, P.H., Prosperi, M., 1991: Life-cycles of sape mediterranean invaders. In: Groves, R.H., di Castri, F. (eds.), Biogeography of Mediterranean invasions, 145–157. Cambridge University Press.

Prosperi, J. M., Guy, P., Génier, G., Angevain, M., 1995: Les luzernes ou le genre *Medicago*. In: Prosperi, J. M., Guy, P., Balfourier, F., (eds.), Ressources génétiques des plantes fourragères et à gazon, 131-168. BRG-INRA, Paris.

Rani, S., Gupta, R.C., Kumari, S., 2012. Meiotic studies in some polypetalous species from District Kangra (Himachal Pradesh), India. Cytologia 77, 197–209.

Rani, S., Kumari, S., Gupta, R. C., Chahota, R. K., 2014: Cytological studies of Angiosperms (174 species) from District Kangra, Himachal Pradesh (India). Plant Systematics and Evolution 300, 851–862.

Ranjbar, M., Hajmoradi, Z., Karamian, R., 2011a: Cytogenetic study and pollen viability of four populations of *Trigonella spruneriana* Boiss. (Fabaceae) in Iran. Journal of Cell and Molecular Research 3, 19–24.

Ranjbar, M., Jahanian, S., 2013: Cytotaxonomic study of *Astragalus* Sect. *Megalocystis* Bunge (Fabaceae) in Iran. Cytologia 78, 181–193.

Ranjbar, M., Karamian, R., Hajmoradi, F., 2010: Chromosome number and meiotic behaviour of two populations of *Onobrychis chorassanica* Bunge (O. sect. *Hymenobrychis*) in Iran. Journal of Cell and Molecular Research 2, 49–55

Ranjbar, M., Karamian, R., Nouri, S., 2011b: Impact of cytomixis on meiosis in *Astragalus cyclophyllos* Beck (Fabaceae). Caryologia 64, 256–264

Rasband, W. S., 1997-2016: ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/

Refoufi, A., 1988: Etude comparée de la tolérance au chlorure de sodium de quelques espèces annuelles du genre *Medicago* L. Annales de l'Institut national agronomique – El Harrach 12, 356–373.

Risso-Pascotto, C., Pagliarini, M. S., Valle, C. B., 2003: A mutation in the spindle checkpoint arresting meiosis II in *Brachiaria ruziziensis*. Genome 46, 724–728.

Ruvalcaba-Ruiz, D., Rodríguez-Garay, B., 2002: Aberrant meiotic behavior in *Agave tequilana* Weber var. azul. BMC Plant Biology 2, 10–14.

Sadeghian, S., Hesamzadeh Hejazi, S. M., 2014: Cytogenetic studies in some species of *Medicago* L. in Iran. IUFS Journal of Biology 73, 21–30.

Saggoo, M. I. S., Kumari, K., 2013: Male meiosis in reed grass *Calamagrostis emodensis* Griseb. from cold desert region of Western Himalayas. Chromosome Botany 8, 97–101.

Schlarbaum, S. E., Small. E., Johnson. L. B., 1984: Karyotypic evolution, morphological variability and phylogeny in Medicago sect. *intertextae*. Plant Systematics and Evolution 145, 203–222.

Sheidai, M., Jalilian, N., 2006: Caryology of some Iranian species and populations of *Lotus* L. Caryologia 59, 37–42.

Simon, J. P., 1965: Relationship in annual species of *Medicago*. II. Interspecific crosses between *M. tornata* (L.) Mill. and *M. littoralis* Rhode. Australian Journal of Agricultural Research 16, 51–60.

Simon, J. P., Millington, A. J., 1967: Relationship in annual species of *Medicago*. III. The complex *M. littoralis* Rhode-*M. truncatula* Gaertn. Australian Journal of Botany 15, 35–73.

Simon, J. P., Simon, A., 1965: Relationship in annual species of *Medicago*. I. Number and morphology of chromosomes Australian Journal of Agricultural Research 16, 13–50.

Singh, S. M., Lesins, K., 1972: Relationship of taxa in the genus *Medicago* as revealed by hybridization. VI. *M. laciniata* x *M. sauvagei*. Canadian Journal of Genetics and Cytology 14, 823–828.

Singhal, V. K., Kaur, D., 2011: Cytomixis and Associated Abnormalities during male meiosis in a new tetraploid cytotype of *Vicia pallida* Turcz. (Fabaceae). Cytologia 76, 387–393.

Singhal, V. K., Kumar, P., 2008: Cytomixis during microsporogenesis in the diploid and tetraploid cytotypes of *Withania somnifera* (L.) Dunal, 1852 (Solanaceae). Comparative Cytogenetics 2, 85–92.

Small, E., 2011: Alfalfa and relatives: Evolution and classification of *Medicago*. NRC Research Press, Ottawa, Ontario, Canada.

Souza, M. M., Pereira, T. N. S., 2011: Meiotic behavior in wild and domesticated species of *Passiflora*. Revista Brasileira de Botanica 34, 63–72.

StatSoft Inc., 2008: STATISTICA (Data Analysis Software System), Version 8.0. http://www.statsoft.com.

Steele, K. P., Ickert-Bond, S. M., Zarre, S., Wojciechowski, M. F., 2010: Phylogeny and character evolution in *Medicago* (Leguminosae): evidence from analyses of plastid *trnK*/*matK* and nuclear *GA3OX1* sequences. American Journal of Botany 97, 1142–1155.

Vogt, R., Aparicio, A., 1999: Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. Bocconea 11, 117–169.