

Cytotaxonomy study of four populations of *Astragalus anserinifolius* Boiss. of section *Malacothrix* Bunge from Iran

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Abstract

In this research, meiotic chromosome number and the behavior of four populations of *Astragalus anserinifolius* Boiss. of *Astragalus* sect. *Malacothrix* were studied. All wild populations were diploid and showed $2n=2x=16$ chromosome number, consistent with the proposed base number of $x=8$ from IPCN. Although all taxa displayed regular bivalent pairing and chromosome segregation at meiosis, some meiotic abnormalities included varied degrees of fragmented and sticky chromosomes in metaphase I, polynucleate and a variable number of laggards, forwarded chromosomes and bridges in anaphase I/telophase I, asynchronous nucleus and precocious chromosome migration in metaphase II and laggards, bridges and cytomixis in anaphase II/telophase II were observed.

Key words: *Astragalus* sect. *Malacothrix*, *A. anserinifolius*, chromosome number, meiotic abnormalities

Introduction

Astragalus L. (Fabaceae) is the most diverse genus in the southwest Asia (ca. 1000 spp). With more than 840 species, it is the largest genus in the flora of Iran and the most problematic group in the legume systematic (Lock and Simpson 1991; Yakovlev *et al.*, 1996; Ranjbar and Maassoumi, 1998; Ranjbar and Karamian, 2002, 2003; Ranjbar *et al.*, 2005, 2010a, 2010b, 2010c, 2010d, 2011). *Astragalus* sect. *Malacothrix*, with about 90 species in Iran, is one of the largest sections within the genus (Podlech *et al.*, 2010). Bunge (1868) placed the section together with seven other sections into subgenus *Hypoglottis*. All members of the subgenus share similar morphological characters such as dense capitates or spike like inflorescences. In recent Podlech's typification system (1982), this subgenus is no longer upheld, because of nearly continuous transitions to other groups of the genus. Most of the cytological studies in the genus have concentrated on the chromosome count (Aryavand, 1983; Maassoumi, 1987; Bader and Sherif, 2007; Sheidai *et al.*, 1996). The basic chromosome number ($x=8$) and the two ploidy levels ($2n=2x=16$, $2n=4x=32$) are present in the genus of the old world.

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Hence, investigations in different aspects can be useful to resolve taxonomic problems of this problematic group. This work follows previous studies conducted on leguminous fodder species in Iran (Ranjbar and Karamian, 2004; Ranjbar, 2007a, 2007b; Ranjbar *et al.*, 2009) and aims to: increase the knowledge about patterns of morphological variation, chromosome numbers and meiotic behaviour in the four populations of *A. anserinifolius* belonging to *A.* sect. *Malacothrix*. Such findings can be helpful in understanding the relationships between the chromosomal criteria and taxonomic delimitations.

Materials and methods

Morphology

This study is mainly based on field observations during several excursions in Iran and on herbarium materials. The field studies were carried out in different parts of Iran, primarily in central and southeastern Iran (Figure 1). All vouchers have been preserved in BASU, Hamedan, Iran. Also, several sheets were examined for each taxon from the following herbaria: W, P, TARI, BASU, Herbarium of Isfahan University and Herbarium of Research Centers of Natural Resources and Animal Affairs of Yazd and Hormozgan. The specimens studied morphologically are listed in Table 1 and used as operational taxonomic units (OTUs). A total of 14 quantitative characters related to vegetative and reproductive organs were studied in the 4 populations of *A. anserinifolius* (Table 2). Data were entered onto a computerized spreadsheet program, Microsoft Excel version 7. The spreadsheet was later transformed into a file format suitable for phenetic analysis. Principal component analysis (PCA) was performed using MVSP version 3.2 (Kovach, 1985-2004) and used to determine inherent or natural groupings.

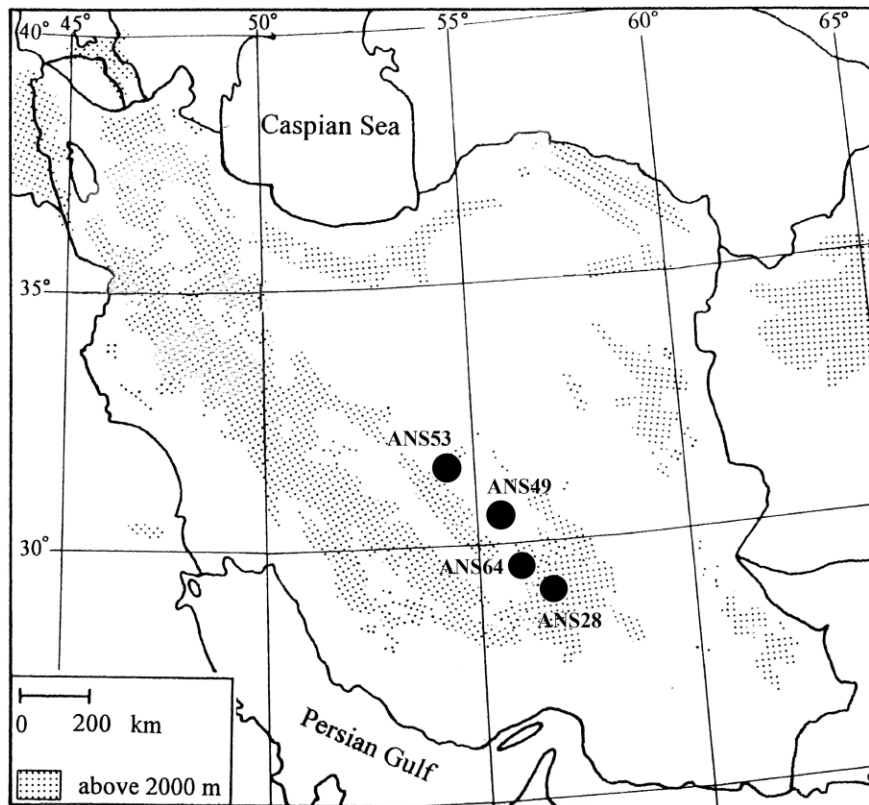


Figure 1. Distribution map of *A. anserinifolius* in Iran

Cytogenetics

Chromosome number and meiotic behavior were studied in 4 populations of *A. anserinifolius*. For each population, 15 flower buds from at least 2 plants at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6 : 3 : 2) for 24 h at room temperature and then stored in 70% alcohol at 4°C until used. Anthers were squashed and stained with 2% acetocarmine. All slides were made permanent by the Venetian turpentine. Photographs of chromosomes were taken on an Olympus BX-41 photomicroscope at an initial magnification of 1000 X. Chromosome counts was made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. Voucher specimens were kept at BASU, Hamedan, Iran (Table 1).

Table 1. Localities of the species used in this study

| Species | Voucher specimens | Locality | Altitude (m) | Collector name | Date | Abbreviation |
|--------------------------|-------------------|---|--------------|----------------------|-----------|--------------|
| <i>A. anserinifolius</i> | BASU 22653 | Yazd: Tang-e Chenar toward Mehriz, 15 km to Mehriz | 2018 | Ranjbar & Mahmoudian | 10.4.2010 | ANS53 |
| <i>A. anserinifolius</i> | BASU 22649 | Kerman: Anar toward Shahr-e Babak, 30 km after Anar, 60 km before Shahr-e Babak | 1982 | Ranjbar & Mahmoudian | 11.4.2010 | ANS49 |
| <i>A. anserinifolius</i> | BASU 22264 | Kerman: Sarcheshmeh toward Pariz, 5 km before Pariz | 2295 | Ranjbar & Mahmoudian | 11.4.2010 | ANS64 |
| <i>A. anserinifolius</i> | BASU 22528 | Kerman: Baft, Azad University of Baft | 2174 | Ranjbar & Mahmoudian | 13.4.2010 | ANS28 |

Table 2. Morphological characters of four populations of *A. anserinifolius* compared with its type specimen

| Morphological characters | ANS64 | ANS53 | ANS49 | ANS28 | ANS (Type) |
|--------------------------|-------|-------|-------|-------|------------|
| Plant height (cm) | 19 | 35 | 19 | 18 | 17.5 |
| Leaf length (cm) | 2.5 | 2.5 | 2.5 | 2.25 | 3 |
| Petiole length (cm) | 0.75 | 1 | 0.85 | 0.75 | 0.75 |
| Leaflet number | 9.5 | 6.5 | 10 | 11 | 11 |
| Leaflet length (mm) | 5.5 | 6.5 | 6 | 4.5 | 6 |
| Leaflet width (mm) | 2 | 2.5 | 2.5 | 1 | 4 |
| Stipule length (mm) | 4 | 4 | 4 | 4 | 2.5 |
| Peduncle length (cm) | 4 | 5.5 | 3.75 | 4.5 | 5 |
| Bract length (mm) | 3.5 | 5 | 3.5 | 2.5 | 4 |
| Calyx length (mm) | 13 | 14.5 | 16.5 | 12.5 | 13 |
| Calyx teeth length (mm) | 8 | 8 | 9.5 | 6.5 | 7 |
| Standard length (mm) | 12 | 12.5 | 13 | 11.5 | 15 |
| Standard width (mm) | 5 | 5 | 5.5 | 5 | 5 |
| Keel length (mm) | 9 | 9 | 10 | 8.5 | 13 |
| Wing length (mm) | 11.5 | 11 | 12.5 | 10 | 15 |

Results and discussion

Morphology

Results from morphological study showed an inter population variation within *A. anserinifolius*, so that its different populations were divided into two groups. The populations ANS-type, ANS28, ANS64 and ANS49 were placed in group 1 and the population ANS53 in group 2 (Figure 2). Group 2 with a single population (ANS53) was separated from the other populations by its higher plant height and lower number of leaflets (Table 2).

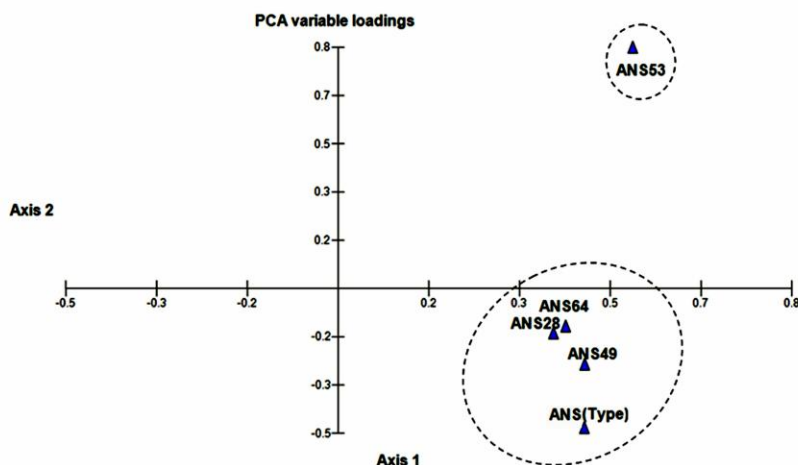


Figure 2. PCA analysis of different populations of *A. anserinifolius* based on morphological characters (abbreviations as listed in Table 1).

A. anserinifolius Boiss. 1843, Diagn. pl. orient., ser. 1, 2: 76 - *Malacothrix* - **Holotype**: [Iran] ad sinum Persicum, *P.M.R.* Aucher-Eloy 4410 (G-BOIS!; iso: BM, FI: foto MSB, G!, K!, LE, OXF, P!, W!) [1 sheet in W!: foto MSB *Aucher 4410*, 'erroneously ad Ispahan!'] (Figure 3).

Plants 11-24 cm tall, with developed stems. Stipules ca. 2-3 mm long, pilose, free from one another and the petiole. Leaves ca. 3 cm long, petiole 0.5-1 cm, both petiole and rachis covered with appressed or subpatent hairs. Leaflets 9-13 pairs, contiguous, complicate, obovate, ca. 6 mm long and 4 mm broad, both sides covered with appressed hairs. Inflorescence axillary, spherical or elliptic in flowering, elongated in fruit, flowers numerous, peduncle ca. 5 cm long, covered with short appressed hairs. Bracts linear, ca. 4 mm long, pilose. Pedicels ca. 1 mm long, pilose. Calyx campanulate or shortly tubular, at the base gibbous, ca. 10 mm long, covered with white hairs, rarely with some scattered black hairs, the teeth subulate, ca. 7 mm long. Corolla yellow or pale violet. Standard ca. 15 mm long, the limb elliptic, emarginated at apex. Wing-petals ca. 15 mm long, the limb narrowly elliptic, dilated toward the apex, round-tipped, at the base auriculate, equaling the claw. Keel ca. 10 mm long, the limb oblique-elliptic, equaling the claw. Ovary pilose, sessile. Pods ca. 9 mm long and 4 mm broad, covered with spreading long soft white hairs, bilocular.

Specimens seen

Kerman: Sarcheshmeh toward Pariz, 5 km before Pariz, 2295 m, 2010.4.11, Ranjbar & Mahmoudian 22264 (BASU); Sarcheshmeh toward Sirjan, After Pariz, Pasujan village, 2206 m, 2010.4.12, Ranjbar & Mahmoudian 22609 (BASU); Baft, Azad University of Baft, 2174 m, 2010.4.13, Ranjbar & Mahmoudian 22528 (BASU); Anar toward Shahr-e Babak, 30 km after Anar, 60 km before Shahr-e Babak, 1982 m, 2010.4.11, Ranjbar & Mahmoudian 22649 (BASU); 66 km to Sirjan, 35 km after Shahr-e Babak, 1816 m, Ranjbar & Mahmoudian 22601 (BASU).

Isfahan: 10 km SW Mourchekhort to Natanz, 1600 m, 2002.5.11, Rahiminejad, Sahebi & Ghaemmaghani 13224 (Isfahan University Herbarium); Isfahan University Campus, 4574 (Isfahan University Herbarium); Isfahan University Campus, Ahmad Zarrehabadi 6792 (Isfahan University Herbarium).

Yazd: Tang-e Chenar toward Mehriz, 15 km to Mehriz, 2018 m, 2010.4.10, Ranjbar & Mahmoudian 22653 (BASU); Bafgh, Bahabad, 1994.7.3, Jafarynejad & Javadian 404 (Animal & Natural Resources Research Center of Yazd).



Figure 3. Isotype of *A. anserinifolius* Boiss. (Aucher-Eloy 4410 P)

Cytogenetics

All the wild populations of *A. anserinifolius* studied here possessed $2n=2x=16$ chromosome number and showed regular bivalent pairing and chromosome segregation at meiosis. They were similar in life history, breeding system, ecology, and geographical distribution in Iran (Figure 1). However, some meiotic abnormalities were observed in different populations included the occurrence of varied degrees of sticky, fragmented and forward chromosomes in anaphase I, laggards and bridges in anaphase I to telophase II, asynchronism, precocious chromosome migration and cytomixis (Table 3; Figures 4-7).

Table 3. Number of pollen mother cells (PMCs) analyzed and percentage of PMCs meiotic behavior in different populations of *A. anserinifolius*

| Meiotic characters | ANS53 | ANS49 | ANS64 | ANS28 |
|-------------------------|-------|-------|-------|-------|
| Number of counted cells | 1573 | 660 | 1161 | 1424 |
| % D/MI | 17.16 | 21.81 | 15.15 | 18.53 |
| % Fragmented chromosome | 19.25 | 25.6 | 7.59 | 1.13 |
| % Bridge | 0.3 | 0 | 0 | 0 |
| % Chromosome stickiness | 18.14 | 39.58 | 17.61 | 0 |
| % Precocious migration | 10.74 | 7.63 | 4.54 | 0.37 |
| % AI/TI | 29.43 | 17.42 | 13.52 | 13.55 |
| % Laggard chromosome | 2.59 | 2.6 | 0.63 | 0 |
| % Bridge | 1.29 | 2.6 | 1.91 | 0 |
| % Forward chromosome | 0.64 | 1.73 | 0.63 | 0 |
| % Micronucleus | 0 | 0 | 0 | 0.37 |
| % Polynucleate | 0.43 | 0 | 3.18 | 5.69 |
| % Asynchronous nucleus | 0.64 | 0 | 0 | 0 |
| % Cytomixis | 0 | 0.86 | 0 | 0 |
| % MII | 8.77 | 13.78 | 5.25 | 8.14 |
| % Asynchronous nucleus | 48.55 | 15.38 | 21.31 | 43.1 |
| % Fragmented chromosome | 1.44 | 2.19 | 0 | 3.44 |
| % Bridge | 0.72 | 0 | 0 | 0 |
| % Precocious migration | 7.24 | 10.98 | 14.75 | 4.31 |
| % AII/TII | 44.62 | 46.96 | 66.06 | 100 |
| % Laggard chromosome | 0 | 0.32 | 1.17 | 0 |
| % Forward chromosome | 0 | 0 | 0 | 0 |
| % Bridge | 0.56 | 0 | 0.13 | 0 |
| % Precocious migration | 0 | 0 | 0 | 0 |
| % Multipolar cell | 0.14 | 0 | 0 | 0 |
| % Cytomixis | 0 | 0 | 0.13 | 0 |

Laggard, forward, sticky and fragmented chromosomes

Fragmented chromosomes, being unable to orient at the metaphase plate, were observed during metaphase I or metaphase II (Figures 4-I, J, R, 5-I, 6-H, 7-H). The highest frequencies of fragmented chromosomes of metaphase I and metaphase II cells were observed in populations ANS49 and ANS28, respectively. Laggard chromosomes were observed during anaphase I in populations ANS53, ANS49 and ANS64 (Figures 4-M; 5-K, L; 6-K); and during anaphase II in populations ANS49 and ANS64 (Figures 5-R; 6-P). According to Niclas and Ward (1994), non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers. Pagliarini (1990) reported that laggards may result from late chiasma terminalization (Souza *et al.*, 2006). These laggards might have degenerated or may have resulted in the formation of polyads particularly at the resting phase (Basi *et al.*, 2006). Forward chromosomes were seen during anaphase I in the populations ANS53, ANS49 and ANS64, among which ANS49 showed the highest frequency (Table 3). Sticky chromosomes were observed at diakinesis and metaphase I in the populations ANS53, ANS49 and ANS64 (Figures 4-J; 5-H, I; 6-I), among which ANS49 had the highest frequency. Chromosome stickiness might have been caused by genetic and environmental factors. However, several agents have been reported to cause chromosome stickiness (Pagliarini, 2000).

Chromosome bridges

Chromosome bridges resulting from stickiness were observed at anaphase I and anaphase II stages in the populations ANS53, ANS49 and ANS64 (Figures 4-N, S, U; 5-M; 6-L, Q).

The population ANS53 also showed chromosome bridges at metaphase II. The thickness of bridges observed and the number of the chromosomes involved in their formation varied among different meiocytes in different species.

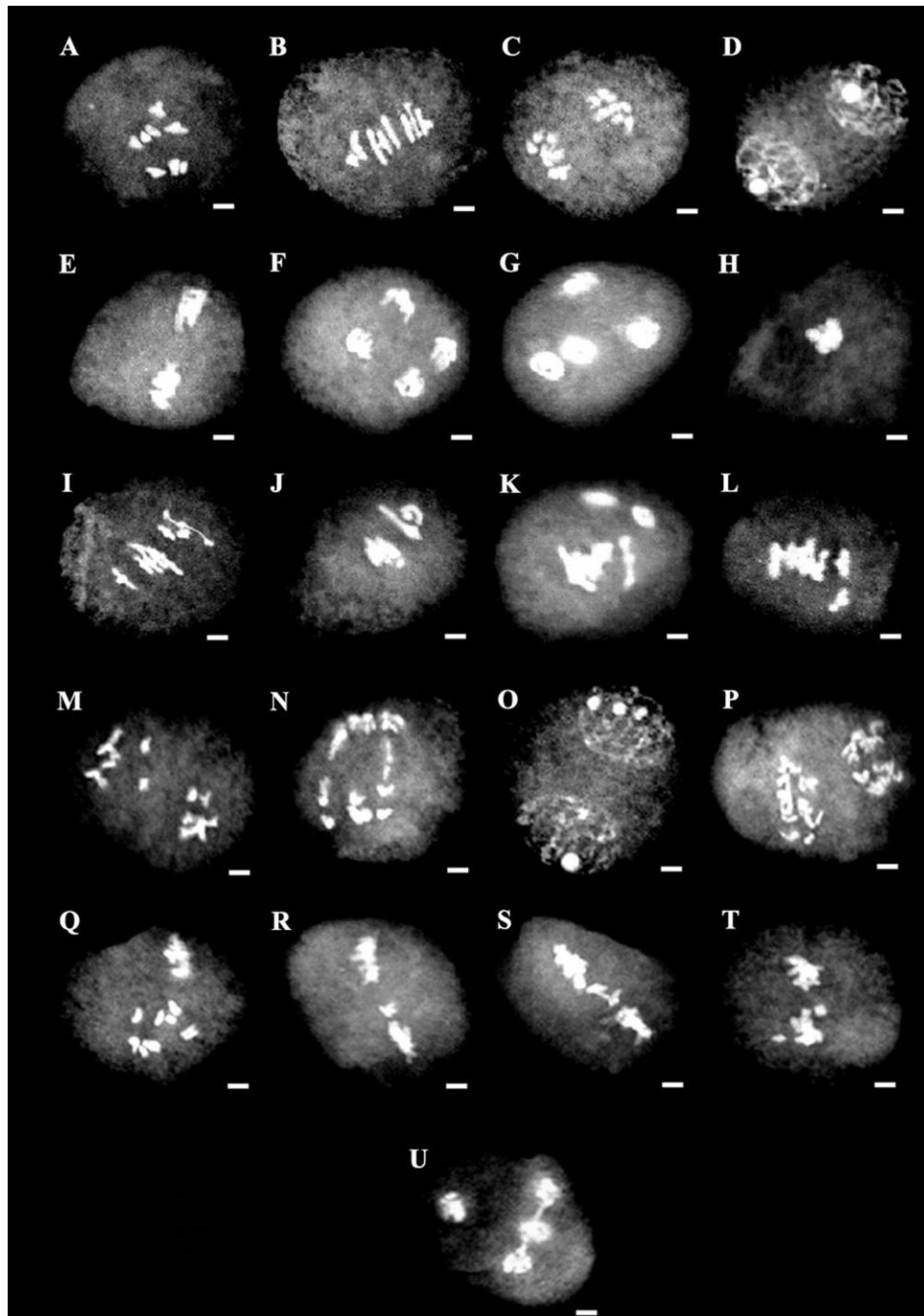


Figure 4. A-L) Representative meiotic cells in the population ANS53. A) Diakinesis; B) Metaphase I; C) Anaphase I; D) Telophase I; E) Metaphase II; F) Anaphase II; G) Telophase II; H) Sticky chromosomes in diakinesis; I) Fragmented chromosomes in metaphase I; J) Sticky and fragmented chromosomes in metaphase I; K-L) Precocious migration of chromosomes to the poles in metaphase I; M-U) Representative meiotic cells in the population ANS53. M) Laggards in anaphase I; N) Bridges in anaphase I; O) Trinucleate in telophase I; P-Q) Asynchronism in meiosis; R) Fragmented chromosome in metaphase II; S) Bridge in metaphase II plates; T) Precocious chromosome migrating to the poles; U) Bridge in late anaphase II. Scale bar: 3 μ m.

Cytomixis

The phenomenon of cytomixis consists in the migration of chromosome between meiocytes through cytoplasmic connection. cytomixis, which is principally a type of meiotic abnormality resulting in changes in gametic chromosome number through migration of chromosomes between adjacent PMCs, could be considered as a process of evolutionary significance in plant populations (Ghanima and Tallat, 2003; Ghaffari, 2006). The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are the effect of fixation (Gottschalk, 1970), mechanical injury (Sarvella, 1958), pathological conditions (Boback and Herich, 1978), temperature anomalies (Basavaiah and Murthy, 1987), polyploid level (Verma *et al.*, 1984), hybrid condition (Yen *et al.*, 1993), cell response as a consequence of pesticides and antibiotic dosages (Kumar and Sinha, 1991), abnormal genetic behavior due to treatment with a chemical mutagen (Kumar and Srivastava, 2001; Kumar and Sharma, 2002), crop culture condition (Pierozzi and Benatti, 1998), failure of cell wall formation during premeiotic mitosis (Kamra, 1960), and genetically controlled behavior (De Mantu and Sharma, 1983). This phenomenon was observed in the population ANS49 at telophase I (Figure 5-O) and in the population ANS64 at telophase II (Figure 6-R, Table 3).

Asynchronous nucleus

Asynchrony in nucleus was observed in all populations at metaphase II (Figures 4-P, Q; 5-P; 6-M, N; 7-K), among which the population ANS53 showed the highest frequency. Asynchronism was also seen in the population ANS53 at anaphase I (Table 3).

Precocious migration

Precocious migration of chromosomes to the poles was observed in all populations at metaphase I and II stages (Figures 4-K, L, T; 5-J, Q; 6-J, O; 7-L). The highest frequencies of such chromosome migrations at metaphase I and II were observed in the populations ANS53 and ANS64, respectively (Table 3).

Micronucleus

As a consequence of precocious migration of univalent, non-oriented bivalents and laggard chromosomes, some micronuclei were observed in telophase I only in the population ANS 28 (Figure 7-J).

Results obtained from PCA analysis based on cytogenetic data showed an inter population variation as well as morphological characters and resulted in dividing taxa into two groups but with different members in comparison to morphology. So that the populations ANS28, ANS64, ANS53 were divided in group 1 and the population ANS49 in group 2 (Figure 8). The population ANS49 is separated from other populations by high score in the formation of fragmented and sticky chromosomes at metaphase I (25.6% and 39.58%, respectively).

Results from PCA analysis of morphological characters represented variation only between different populations of *A. anserinifolius*. On the other hand, the responsible reasons were not strong enough to justify variation at interspecific level leading to a new species or even at intraspecific level for separating a new subspecies or a new variety. According to some previous works on the genera *Astragalus* and *Onobrychis* of the family Fabaceae (Ranjbar *et al.*, 2009, 2010b, 2011), the agreement between groupings resulted from morphological and meiotic analyses, occurred when the taxa demonstrate well inter/intraspecific variations in phenetic analysis. Quantitative/qualitative morphological

characters responsible for such variations affected meiotic behavior of the taxa, and thus, results from meiotic analysis support morphological groups well.

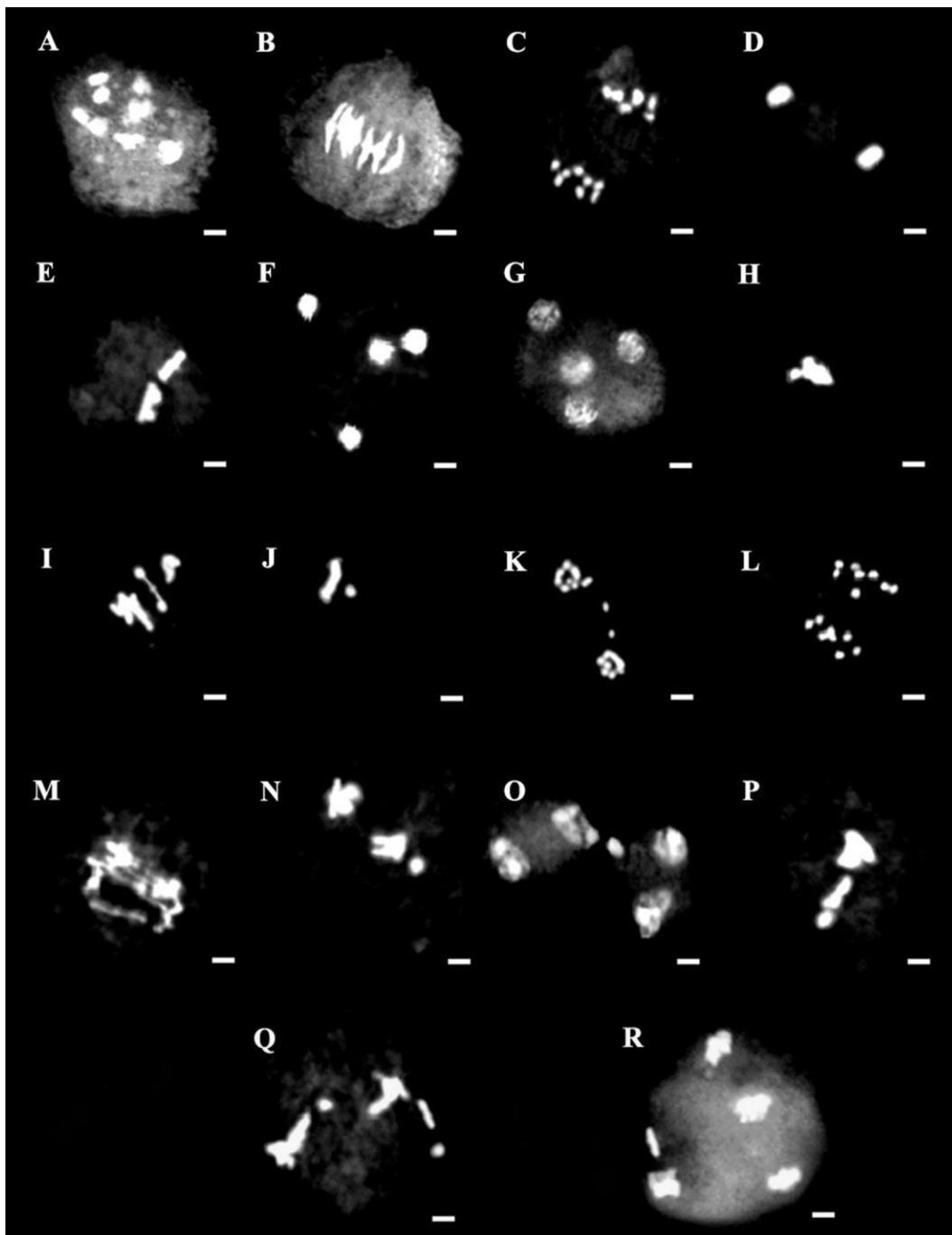


Figure 5. A-L) Representative meiotic cells in the population ANS49. A) Diakinesis; B) Metaphase I; C) Anaphase I; D) Telophase I; E) Metaphase II; F) Anaphase II; G) Telophase II; H) Sticky chromosomes in diakinesis; I) Fragmented and sticky chromosomes in metaphase I; J) Precocious chromosome migration in metaphase I; K-L) Laggard in anaphase I. M-R) Representative meiotic cells in the population ANS49. M) Bridges in Anaphase I; N) Forward chromosome migration in anaphase I; O) Cytomixis in telophase I; P) Asynchronous nucleus; Q) Precocious chromosome migration to the pole in metaphase II; R) Laggard in anaphase II. Scale bar: 3 μ m.

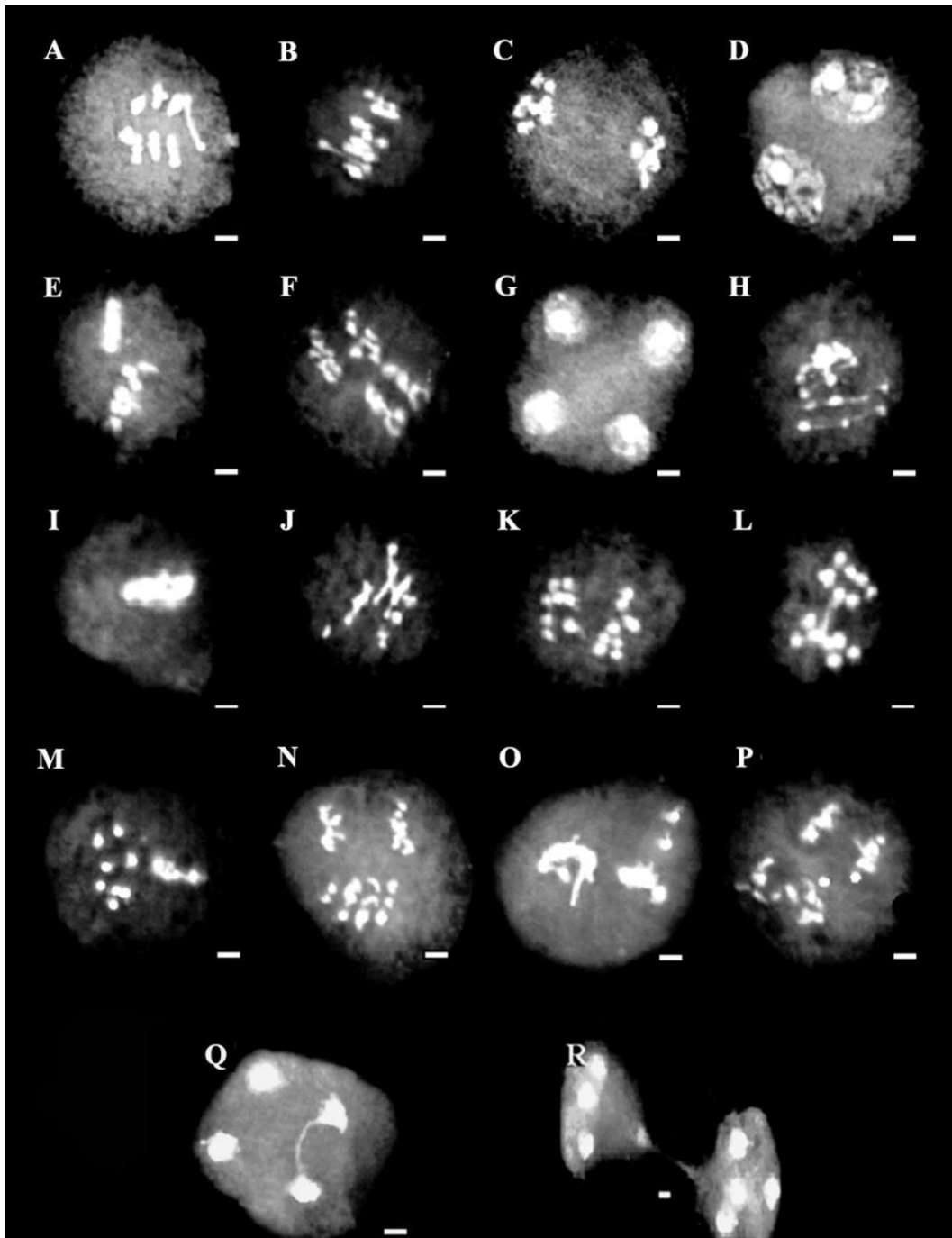


Figure 6. A-L) Representative meiotic cells in the population ANS64. A) Diakinesis; B) Metaphase I; C) Anaphase I; D) Telophase I; E) Metaphase II; F) Anaphase II; G) Telophase II; H) Fragmented chromosomes in metaphase I; I) Sticky chromosomes in metaphase I; J) Precocious chromosome migration to the poles in metaphase I; K) Laggard in anaphase I; L) Bridge in anaphase I. M-R) Representative meiotic cells in the population ANS64. M-N) Asynchronous nucleus; O) Precocious migration of chromosomes to the poles in metaphase II; P) Laggards in anaphase II; Q) Bridge in anaphase II; R) Cytomixis in telophase II. Scale bar: 3 μ m.

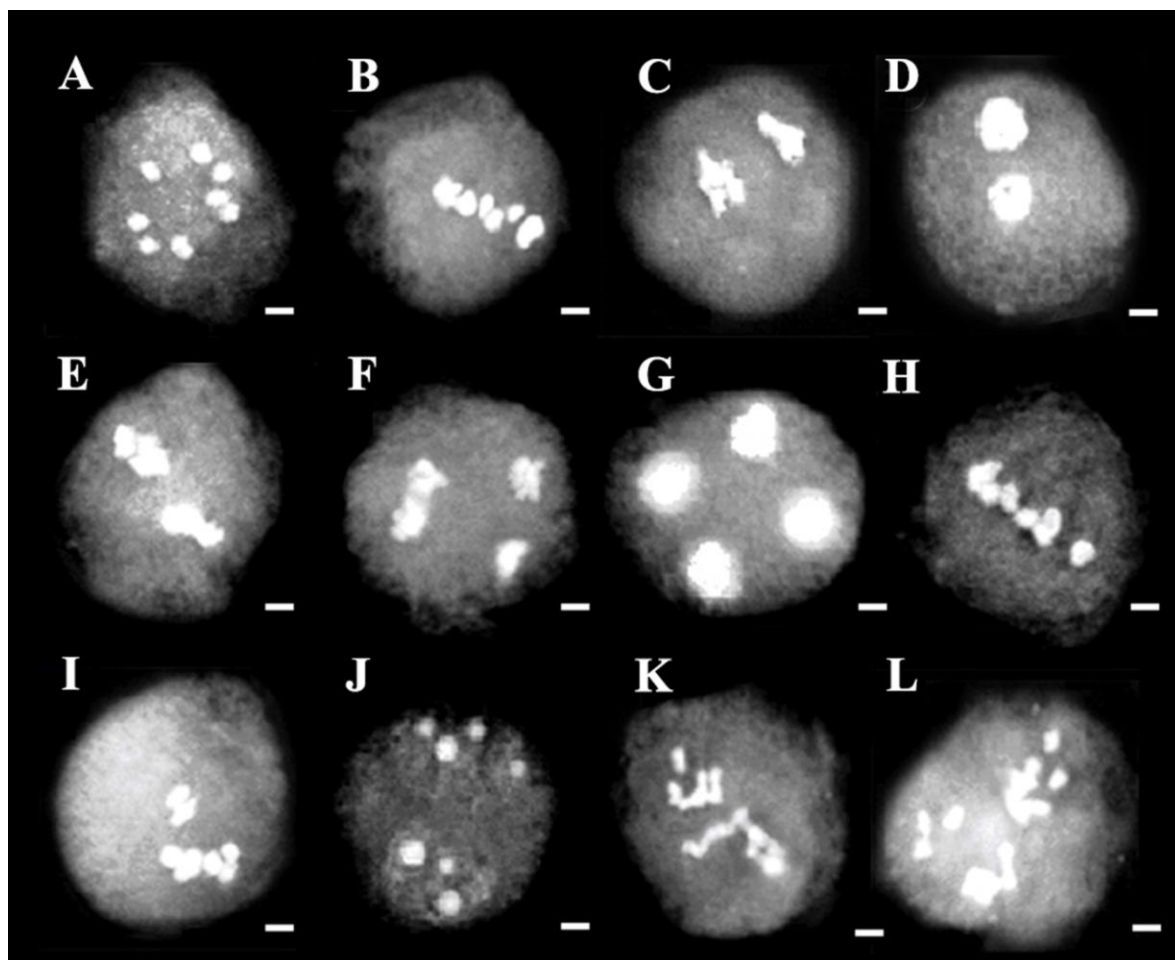


Figure 7. A-L. Representative meiotic cells in the population ANS28. A) Diakinesis; B) Metaphase I; C) Anaphase I; D) Telophase I; E) Metaphase II; F) Anaphase II; G) Telophase II; H) Fragmented chromosome in metaphase I; I) Precocious migration of chromosomes to the pole in metaphase I; J) Trinucleate in telophase I and one micronucleus; K) Asynchronous nucleus; L) Precocious migration of chromosomes in metaphase II. Scale bar: 3 μ m.

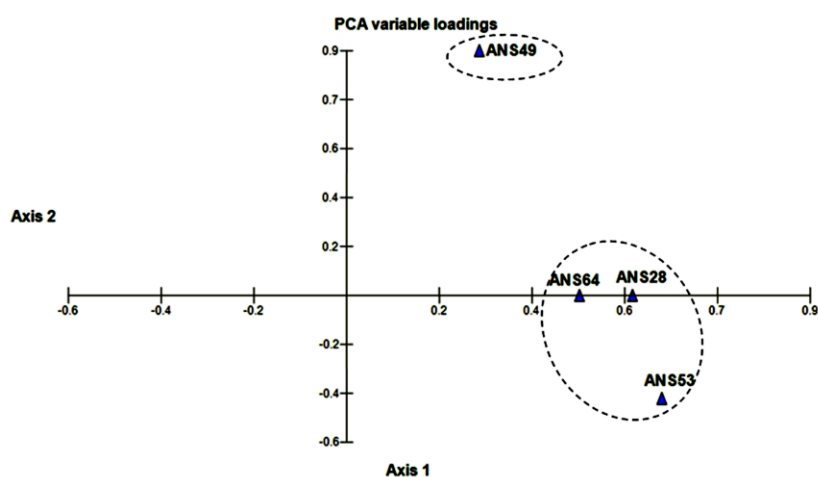


Figure 8. PCA analysis of different populations of *A. anserinifolius* based on cytogenetic data (abbreviations are as listed in Table 1).

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