CYTOTAXONOMIC STUDY OF SIX POPULATION FROM GENUS ASTRAGALUS SECTION MICROPHISA IN IRAN

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ABSTRACT

The genus Astragalus is the largest vascular plant on earth, which includes annual and perennial species and has 245 sections. In this study, the Astragalus genus from the Fabaceae family was studied in terms of the behavior of the chromosomes during meiosis, the chromosomal base number and the ploidy level in the stages of meiosis. The results show the presence of chromosomal abnormalities such as chromosomal separation or adhesion, and cytomixy. By studying 6 populations of Microphysa species A. fragiferus Bung, A. callistachys Buhse, A. lurorum Bornm, A. cephalanthus DC, A. reuterianus Boiss and A. submitis Boiss & Hohen, the results show all are diploid (2n=2x=16) and their basic chromosomal number is x=8.

Keywords: Genus Astragalus. L, chromosome number, ploidy, abnormality

INTRODUCTION

Genus Astragalus L. is one of the largest genera of flowering plants with 2500 to 3000 species (Roofgar et al., 2019) and a wide global distribution, nearly 1000 of which are distributed in the Iranica flora (Ranjber et al., 2011). The habitat of this plant in Iran can be seen from an average height of 1100 meters in the Iranian plateau and lowlands and at lower altitudes in the mountains, and it is specific to the steppe, semi-arid and dry mountainous regions of Iran (Mahmodian et al., 2011). This genus belongs to the Fabaceae family, which is placed together with 19 other genera in the Galegeae Bronn Torrey & Gray genus (Qharamani et al., 2000). In all floras written based on the

monograph (Singh, G. 2001), and Flora Sharq (1872), who have assigned subgenus divisions for this genus, some changes have been made in this subgenus and they have been reduced from 8 or 9 numbers to two numbers (Guldin et al., 1987). But based on molecular studies on new world Astragalus species (Wojciechowski et al., 2003) and also the achievements of molecular research on many species belonging to the ancient world (Kazimpoor et al., 2003) it does not approve any subgenus divisions in the genus and considers it artificial (massuomi. 2002). Iran, having about 750 species of this genus, of which 400 species are exclusive, is considered one of the important centers of speciation and species diversity (Ranjbar et al., 2014).

In racial research, cytogenetic studies are one of the primary measures. Knowing the chromosome number is effective in choosing racial methods. Determination of the ploidy level is also obtained from chromosomal number. It has a significant role in crossbreeding, identification and classification of plants in the new system as part of new taxonomy. A great diversity in chromosomes, the constant number of chromosomes in individuals of the same species are the useful indicators for taxonomic purposes. On the other hand, the study of chromosomal structure and their behavior is important in phylogeny and genus architecture.

In case of chromosomal number in Astragalus species, the available sources confirm the chromosomal number of some species (Javadi et al., 2006). Studies show that the basic chromosomal number in all populations of Astragalus L. are equal to 8 and the populations are placed in two ploidy levels including diploid 2n=2x=16 and tetraploid 2n=4x=32. The types of chromosomes among populations are metacentric and submetacentric. A. pseudocyclopyllus, A. ebenoides, A. stevenianus and A. jodostachys species were tetraploid, respectively and other populations were diploid (Ghulamzadah et al., 2019). All studied species, like most species of Astragalus genus, show the basic chromosome number of x=8, which were three ploidy levels (4x, 2x, and 6x), and 16, 32, and 48 chromosomes, respectively. Therefore, there is a big difference between the number of chromosomes in Hymenostegis, Astragalus and Oxytropis sections in terms of ploidy level (Bagheri et al., 2022). The diversity of these plants in the Mediterranean region and West Asia includes annual or prennial plants, mostly flowering plants, and rarely thorny shrubs with simple long hairs, which are cultivated as fodder. Also, there is the report of chromosomal numbers x=7 and x=8 and three ploidy levels 2n=2x=14, 2n=4x=28, 2n=8x=56 and 2n=2x=16, 2n=4x=32 in this genus (Ranjbar et al., 2010). Cytological data and chromosomal numbers based on the base number x=8 are found in most of the studied species, a diploid number of 2n=2x=16 is recorded in most species, while a tetraploid number of 2n=4x=32 is only

recorded in one sample of A. vegetus99. The chromosomal number of x=8, has been reported in the vast majority of the genus Astragalus (Ranjbar et al., 2013). Chromosome number of 6 exclusive and rare species of Astragalus genus also shows the basic chromosomal number of x=8 for all species which A. assadabadensis, A. cardochrom, A. kirrindicus, A. nervistipulus species were diploid and A. trachyacanthos was tetraploid. The basic chromosomal number report of these species is from Rhacophorus section (Jalilian et al., 2022). Variations and genome size showed that the genome size values among the studied species are slightly different at the ploidy level and most of the species and close groups are almost constant. Among the species related to Astragalus genus, there is a relatively strong correlation between the genome and the number of chromosomes. In the Hymenostegis section, the difference and the size of the genome seems to be very small (Bagheri et al., 2022).

This study aims to find the behavior of the chromosomes during meiosis, the chromosomal base number and the ploidy level in the stages of meiosis in six populations of genus Astragalus sect. Microphisa in Iran.

RESEARCH METHODOLOGY

In this research, collected samples from natural habitats and herbarium samples were examined.

Collecting the studied species for the study of meiosis

Meiosis is a major evolutionary event that culminates in the reduction of the number of chromosomes. The coordinated and normal course of meiosis causes gametes with viability. Cytological events of gametogenesis are controlled by a wide range of genes. Mutation in these genes causes abnormality with adverse effects on fertility (Pagliarini, M. 2000). Meiosis in higher plants includes two stages of specialized cell division that is necessary for the production of gametes or gamete-producing cells. Pairing of homologous chromosomes in the meiosis stage ensures chromosome separation in the next stage (which causes the genome to be haploid). Synapsis of homologous chromosomes and meiotic recombination takes place during meiotic prophase (the first division of meiosis) and reduction division is a prerequisite (Bass et al., 2003).

Study process

In order to study chromosomes, buds were collected from different species of Astragalus genus in different regions. In order to achieve all stages of meiosis, sampling was done from different buds in different sizes and at different times. In this study, pollen mother

cells were used in the anthers of the stamens. Chromosomes were stained by standard Stokarman method. Before staining, the following steps were performed on cytological materials (unripe flowers).The young buds were placed for 24-48 hours in Pinar's fixative solution which consists of 6 volumes of pure ethyl alcohol, 3 volumes of chloroform and 2 volumes of propionic acid. Cells are killed and fixed in this solution. After 24-48 hours, the buds were removed from the fixing solution and washed with distilled water in order to eliminate the effects of Pinar. Then the buds were placed in 70% ethanol solution. Due to the smallness of the buds, a loop was used to separate the anthers. After separating the anthers and placing them on the slide, 1-2 drops of Stokarman's dye were poured on them and they were completely beaten with an aluminum rod in order to release the pollen mother cells. A coverslip was placed on the sample and then it was gently heated on the alcohol lamp (by alternately heating the coverslip, the nuclei of the cells are better stained). Then the slide was placed between several layers of drying paper and by applying pressure on the paper and the slide, the excess color was remove, and the microsporocytes were spread evenly. Thus, it became possible to observe the chromosomes in different stages of meiosis. Since most of the microsporocytes obtained from one flower often show one stage of meiotic division, if a slide is prepared from the anthers of several flowers, different stages of division can be observed. Finally, the divided cells were examined by an Olympus BX-51 optical microscope with a 100x magnification lens, and the obtained images were photographed by an Olympus DP25 digital camera. To prepare the color, 45 ml of pure acetic acid was heated in an Erlenmeyer flask to boiling point, then the container was removed from the heat and 2 grams of Carmen powder was slowly added to it. While boiling the solution again, the powder was completely dissolved in the acid using a magnetic stirrer. After cooling the solution to 50°C, 55 ml of distilled water was added to it and the stirring continued for one hour. After cooling, the dye was filtered and stored in a dark glass container in the refrigerator. On one side of the prepared slide, 2-3 drops of Venetian turpentine glue were placed and on the other side, 1-2 drops of Stokarman paint were placed. Then by placing a folded sheet of tissue paper in the place of the paint, the paint was gradually removed from under the slide and the glue was replaced. After the glue was completely replaced, the permanent slide was dried for two days and then its excess glue was removed by the paper smeared with xylol.

RESULTS

Astragalus fragiferus Bunge species

Chromosome study on population of Astragalus fragiferus Bunge species showed that this population is diploid (2n=2x=16) and its chromosomal number is x=8. Different stages of meiotic division and chromosomal behavior were observed in this population, including different degrees of diakinesis, metaphase I, metaphase II, metaphase I, telophase I, telophase II, cytomixy and the end of metaphase I (Figure 1). Cytomixy is the migration of chromatin components between adjacent meiotic cells through cytoplasmic junctions originating from the plasmodesmata pre-structures in the anther tissue is called cytomixy. Plasmodesmata are normally completely blocked by callose deposition, but in some cases they remain during meiosis and enlarge to form connections between meiotic cells or cytomeric channels that allow chromosomes to pass through. It seems that cytomixy is of little evolutionary importance, but this phenomenon leads to the creation of aneuploid plants or unreduced gametes (in terms of chromosome number) (Shaidaye et al., 2007). Cytomixy has been reported in different plants and mostly in the pollen mother cells, but the available information shows that cytomixy occurs in the meristem cells of the root tip, stem, Hodgdon's wall cells and other cells as well which this is not specific to pollen mother cells (Shaidaye et al., 2001). Sometimes chromosome migration may be done by dissolving the cell wall between adjacent cells, which in this case forms a syncytium (Shaidaye et al., 2003).

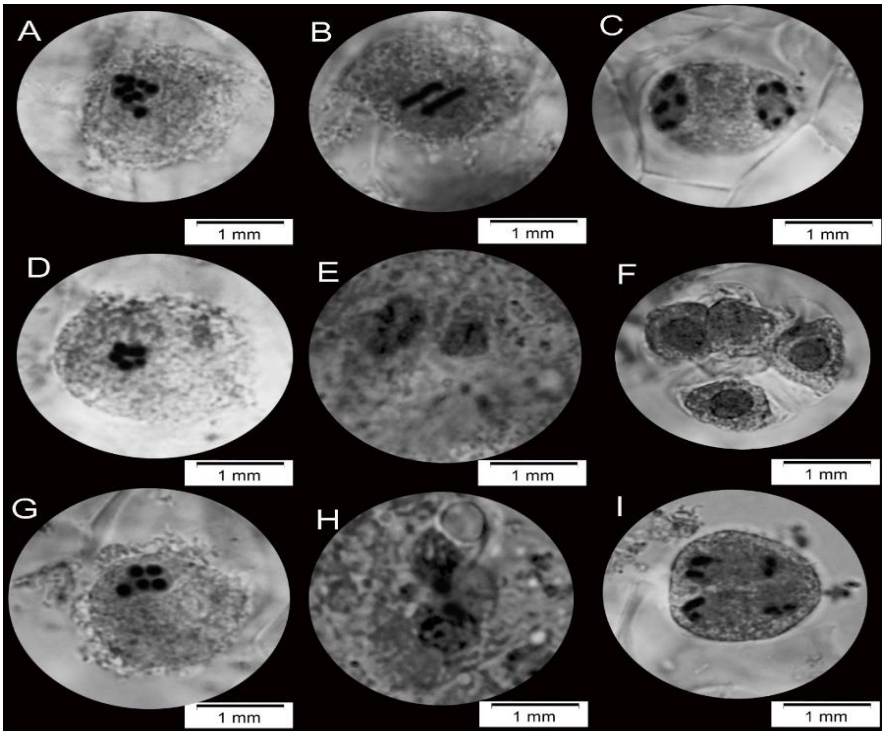

Figure 1- Stages of meiosis in the population of *Astragalus fragiferus* (234731).

A. diakinase, B. metaphase I, C. metaphase II, D. metaphase I, E. telophase I, F. telophase II, G. diakinase, H. cytomixy, I. end of metaphase I.

Astragalus callistachys Buhse species

The chromosomal study on Astragalus callistachys Buhse species population showed different stages of meiosis including diakinesis, metaphase II, anaphase I, telophase I, telophase II, and the end of anaphase I. Chromosomal abnormalities including chromosomal adhesion and separation were observed in this species. The adhesion of chromosomes to each other is caused by the formation of sticky ends between two or more chromosomes and the formation of sticky bridges in anaphase (Eraj et al., 2003). Genetic and environmental factors are considered as the cause of chromosome adhesion. Due to the difference in cells that show adhesion, it is suggested that genome-environment interaction is the main cause of this phenomenon (Shaidaye et al., 2007). This species is diploid 2n=2x=16 and its chromosomal number is x=8 (Figure 2).

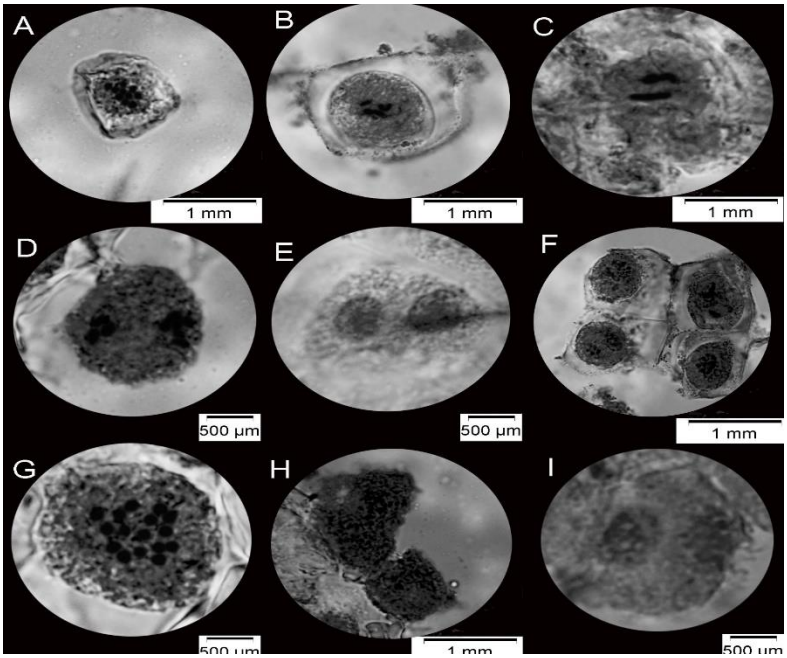

Figure 2- Stages of meiosis in the population of *Astragalus callistachys* Buhse (43934).

A. Diakinesis, B. Metaphase II, C. Anaphase I, D. Chromosome adhesion, E. Telophase I, F. Telophase II, G. Separation, H. Cytomixy, I. Anaphase II.

Astragalus lurorum Bornm species

Chromosome study on this population showed that this population is diploid (2n=2x=16) and its chromosomal number is x=8. Different stages of meiosis division in this population including different degrees of diakinesis, metaphase I, Lagarde in metaphase I, telophase I, telophase II and chromosomal abnormalities including chromosomal adhesion, leading chromosome in metaphase I, and cytomixy were observed in this species (Figure 3).

Astragalu reuterianus Boiss species

Chromosome study on this population showed that this population is diploid (2n=2x=16) and its chromosome number is x=8. Different stages of meiosis including different degrees of diakinesis, metaphase I, metaphase II, anaphase II, telophase I, telophase II, and chromosomal abnormalities including chromosomal separation and cytomixy were observed (Figure 4).

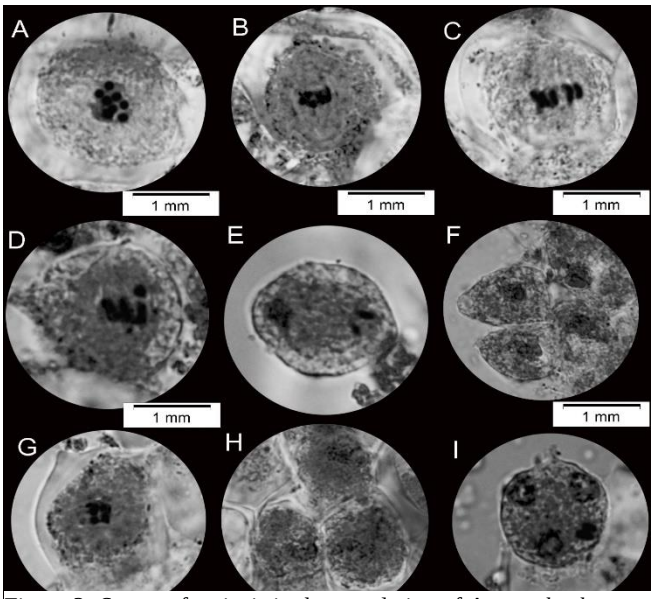

Figure 3- Stages of meiosis in the population of *Astragalus lurorum* Bornm (43935)

A. Diakinase, B. Metaphase I, C. Lagarde in Metaphase I, D. Chromosome leading in Metaphase I, E. Anaphase I, F. Telophase II, G. Chromosome adhesion, H. Cytomixy, I. Telophase II.

Astragalus cephalanthus DC species

Chromosome study on this population showed that this population is diploid (2n=2x=16) and its chromosome number is x=8. Different stages of meiosis including different degrees of diakinesis, metaphase I, metaphase I, telophase I, telophase II, anaphase II and chromosomal abnormalities including chromosomal adhesion, and cytomixy were observed (Figure 5).

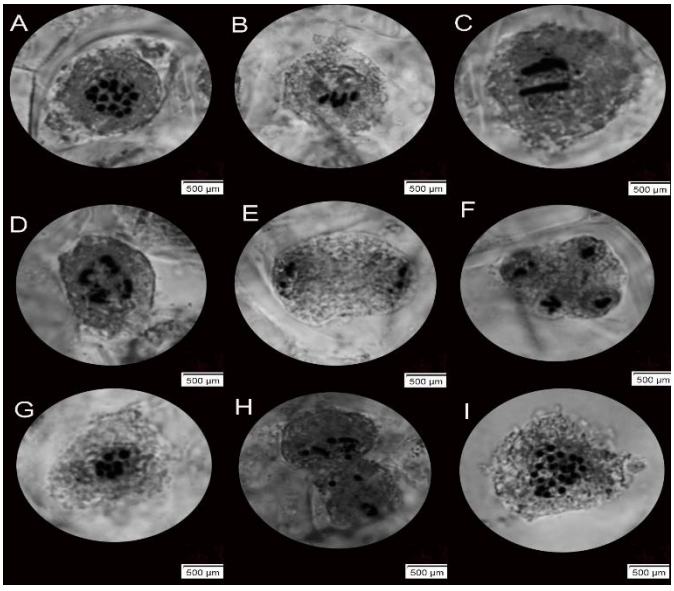

Figure 4- Stages of meiosis in the species population *A. reuterianus* Boiss (22979)

A. diakinesis, B. metaphase I, C. metaphase II, D. anaphase II, E. telophase I, F. telophase II, G. separation, H. cytomixy, I. diakinesis.

***A.* submitis Boiss. & Hohen Species**

B.

Chromosome study on this population showed that this population is diploid (2n=2x=16) and its chromosome number is x=8. Different stages of meiosis including different degrees of diakinesis, metaphase I, telophase I, telophase II and anaphase II and chromosomal abnormalities including chromosomal adhesion, leading chromosome in metaphase I, chromosomal separation and cytomixy were observed (Figure 6).

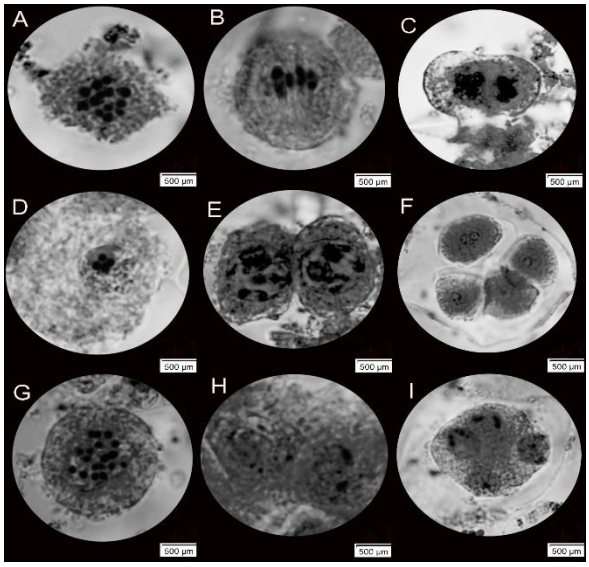

Figure 5- Phases of meiosis in *Astragalus cephalanthus* DC (2397) population

A. diakinesis, B. metaphase I, C. telophase I, D. adhesion in diakinesis, E. telophase I, F. telophase II, G. diakinase, H. cytomixy, I. anaphase I.

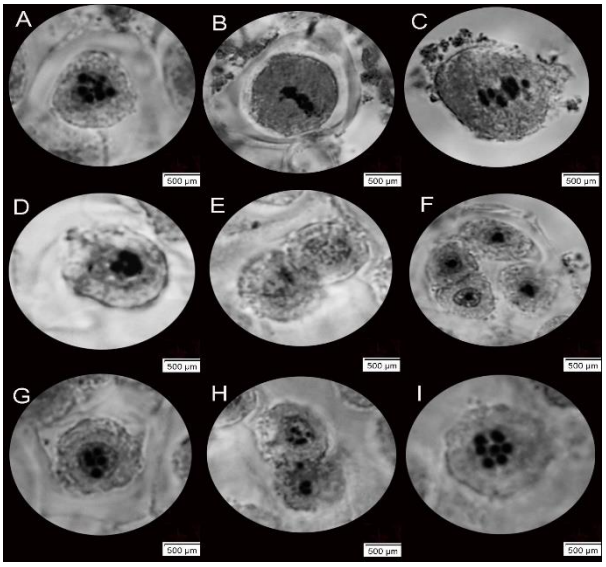

Figure 6- Phases of meiosis in the population of *Astragalus submitis* Boiss. & Hohen (21560).

A. Diakinesis, B. Metaphase I, C. Chromosome advancing in Metaphase I, D. Adhesion in Diakinesis, E. Telophase I, F. Telophase II, G. Separation in Diakinesis, H. Cytomixy, I. Diakinesis.

DISCUSSION AND CONCLUSION

This study is conducted on 6 populations of A. fragiferus Bunge, A. chllistachys Buhse, A. lurorum Bornum, A. reuterianuss Boiss, A. cephalanthus DC, and A. submitis Boiss. & Hohen in Iran. The findings showed that they are diploid (2n=2x=16) and their chromosome number is x=8. Most of the cytological studies in this genus are focused on counting the chromosome number. This species has a base chromosome number of x=8 with two ploidy levels of 2n=4x=32 and 2n=2x=16 (Ranjbar et al., 2010) which are the same with this study. Meiosis study on different populations of A. macrostachys showed that three populations of A. macrostachys, A. macrostachys and A. macrostachys have chromosomal number of 2n=16 and they are diploid, while the only population A. macrostachys is tetraploid (Karamian et al., 2012). The results of the chromosomal study of A. aznabjurtieus, A. cancellatus and A. elegans species by Javadi et al. (2019) showed that all three species had a basic chromosomal number of x=8 and showed a tetraploid state of 2n= 4x=32. Also, two pairs of satellite chromosomes were identified in all three species. This study showed the different behavior of 6 populations of Astragalus L. genus in meiosis. Also, the findings showed some abnormalities such as cytomixy, chromosomal adhesion and separation. Study of chromosomal number in all these 6 populations are reported for the first time in Iran.

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