Cytogenetic study of two *Solenanthus* Ledeb. species (Boraginaceae) in Iran

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Abstract

Chromosome number, meiotic behavior, and pollen viability were analyzed in 2 species of *genus Solenanthus*, *S. stamineus* (Desf.) Wettst. and *S. circinnatus* Ledeb, from Iran. This report is the first cytogenetic analysis of these species. All taxa are diploid and possess $2n = 2x = 24$ chromosome number, consistent with the proposed base number of $x = 12$. Although this taxon displayed regular bivalent pairing and chromosome segregation at meiosis, but some abnormalities were observed.

***Keywords*:** Boraginaceae, chromosome number, meiotic behavior, pollen viability, *Solenanthus*.

Introduction

The family Boraginaceae consists of 156 genera distributed throughout the tropical, subtropical and temperate regions (Al-Shehbaz, 1991; Ge-Ling, 1995). The genus *Solenanthus* belongs to tribe Cynoglosseae DC. and is mainly distributed in the north temperate regions, but centers of diversity are in the eastern Mediterranean area and western Asia (Al-Shehbaz, 1991). Morphologically, the genus is characterized by tubular corollas, long or short anthers, a style often exerted from the corolla. Nutlets dorsiventrally compressed, with dense glochids on abaxial margin (Riedl, 1967).

Materials and Methods

Cytogenetic

The chromosome number and meiotic behavior were analyzed in one population of *Solenanthus stamineus* and two populations of *S. circinnatus* which were collected from different regions within the natural geographical distribution of them during several excursions in Iran (table1). Fifteen flower buds 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% ethanol at 4 °C until used. Anthers were squashed and stained with 2% acetocarmine. All observations were photographed using an Olympus 3030 digital camera mounted on a BX-51 Olympus microscope.

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Pollen viability

Pollen stainability was considered as an indication of pollen viability. For this purpose pollen grains were first obtained from the flowers of herbarium specimen and then stained with acetocarmin/glycerin (1:1). Slides were stored at room temperature for 24-48 hours. The stainability was determined using samples of 1000 pollen grains per flower. Slides were examined and documented with an Olympus BX-51 photomicroscope.

Taxa	Herbarium number	Altitude (m)	Location	Date	Collector
S. circinnatus	35067	2144	Chaharmahal-e Bakhtiari, Gandoman toward Yasuj, Cheshmeh-Ali area	27.4.2011	Ranjbar & Almasi
S. circinnatus	33047	3700	Kohgiluyeh va Boyer-Ahmad, Eastern Dena, Gol mountain	28.4.2011	Ranjbar & Almasi
S. stamineus	35067	2250	Isfahan, Semirom, protected area of Hana	28.4.2012	Ranjbar & Almasi

Results

Chromosome number and meiotic behavior

All species analyzed by mitotic chromosome counting had a consistent number of $n = 12$ in pollen mother cells (PMCs). All taxa studied here displayed regular bivalent pairing and chromosome segregation at meiosis. However, some meiotic

abnormalities were observed. The meiotic irregularities observed

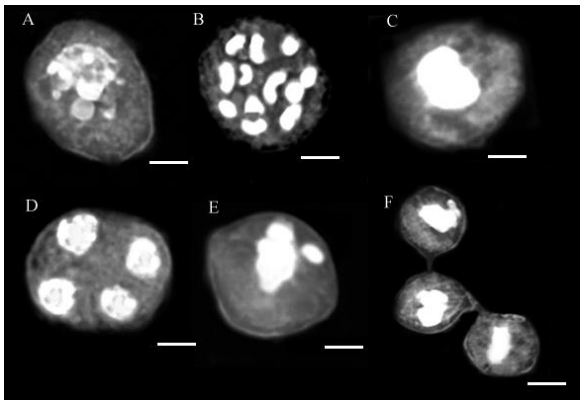

Figure 1. Representative meiotic cells in *S. circinnatus* 44 with *n* = 12. (A) Porophase, (B) Diakinesis, (C) Metaphase I, (D) telophase II, (E) Precocious migration to poles (F) Cytomixis in metaphase I. Scale bar = 3 μm.

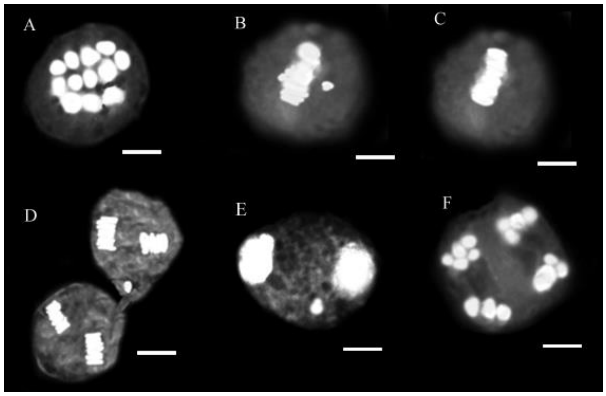

Figure 2. Representative meiotic cells in *S. circinnatus* 47 with n = 12. (A) Diakinesis, (B) Precocious migration to poles in metaphase I, (C) Metaphase I, (D) Cytomixis in telophase II, (E) Micronucleus in telophase I, (F) Anaphase II. Scale bar = 3 μm.

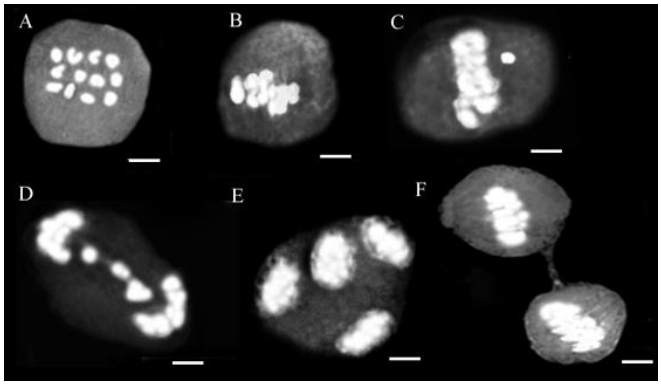

Figure 2. Representative meiotic cells in *S. stamineus* with *n* = 12. (A) Diakinesis, (B) Metaphase I, (C) Precocious migration to poles in metaphase I, (D) Laggard chromosome in Anaphase I, (E) Telophase I, (F) Cytomixis in metaphase I. Scale bar = 3 μm.

in different *Solenanthus* species included the occurrence of varied degree of precocious migration to poles, cytomixis and laggard chromosomes (table 2 and figures 1-3).

Cytomixis

The observation of cytomixis in metaphase I and telophase II stages of meiosis was one of irregularity in the studied genotypes. The phenomenon of cytomixis is characterized by the migration of chromatin/chromosomes between the proximate meiocytes through cytoplasmic channels or intercellular bridges. Though an infrequent cytological phenomenon, it has been reported to occur in a large array of plant species (Gottschalk, 1970; Cheng et al., 1975; Omara, 1976; Guochang, et al., 1987; Bedi et al., 1990; Bellucci et al., 2003). Cytoplasmic connections preexist between meiocytes in the form of plasmodesmata within the syncytium and then become severed as a result of insulation of meiocytes by the progressive deposition of callose (Heslop-Harrison, 1966). In some cases, however, the plasmodesmata still persist during meiosis and increase in size to generate cytomictic connections. These are termed as cytomictic channels and are large enough to permit the transfer of cytoplasmic organelle and in some cases chromatin material (Risueno et al, 1969; Lattoo et al., 2006; Ranjbar et al., 2011a).

Precocious migration to the poles and laggard chromosome

The most frequent abnormalities in the two meiotic divisions were those related to chromosome segregation, such as precocious migration to the poles during metaphase and laggards at anaphase (figure 1-3) that led to the formation of micronuclei at telophase. However, in this accession, only a few cells with micronuclei (1.7%) were detected in telophase I.

Micronucleus

Micronucleus is another abnormality that was found in *S. circinnatus* 47 (figure 2.) Chromosomes that produced micronuclei during meiosis were eliminated from microspores as microcytes. The micronucleus reached the microspore wall and formed a kind of bud, separated from the microspore. The eliminated microcytes gave origin to small and sterile pollen grains (Baptists-Giacomoelli et al., 2000; Ranjbar et al., 2009, 2010, 2011b).

Pollen viability

The results of the comparison between meiotic behavior and pollen viability showed the highest (99) and lowest (94) percentages of the stained pollens in *S. stamineus* and *S. circinnatus* 44, respectively. This result indicates that irregularities observed at meiosis probably have a direct relation with species fertility. The pollen viability of examined species are described in table 2 and illustrated in figure 4.

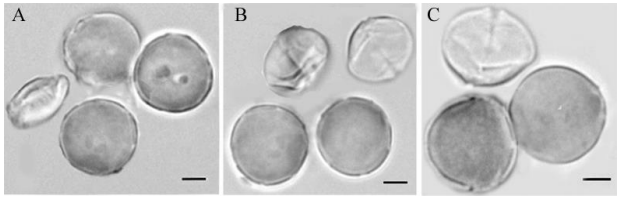

Figure 4. Pollen viability. (A) *S. stamineus*, (B) *S. circinnatus* 47, (C) *S. circinnatus* 44. Scale bar = 2 μm.

Table2. Characterization of meiotic behaviour and Pollen viability in one population of *S. stamineus* and two populations of *S. circinnatus* .

Meiotic characters	*S. circinnatus*	*S. circinnatus* 44	*S. circinnatus* 47
Cell number	480	431	290
D/MI	50	290	120
% D/MI	10	67	41
% Cytomixis	2	0	5
% Precocious migration to poles	3	8	6
AI/TI	220	65	10
% AI/TI	46	15	3.5
% Laggard chromosome	0	1	2
MII	80	0	20
% MII	16	0	7
AII/TII	130	76	140
% AII/TII	27	18	48
% Cytomixis	0	1	3
% micronucleus			1.7
% Laggard chromosome	0	0	2
x	12	12	12
% Pollen viability	99	98	94

Discussion

The most common chromosome number in tribe Cynoglosseae is $n = 12$ and has the lowest variation in contrast with the other tribes (Britton, 1951; Coppi et al., 2006).

Besides these, Coppi et al. (2006) also found evolution of new forms in this tribe seem to have involved minor chromosomal rearrangements with respect to tribe Boragineae and Lithospermeae, also in terms of changes in ploidy levels. There is a considerable difference in the size of the chromosomes between the genera of tribe Cynoglosseae (Britton, 1951). The relatively high base number $x = 12$ is possibly derived from lower ones in other tribes, such as $x = 6$ and this may support the traditional view that Cynoglosseae represent "the most highly specialized tribe in the family" (Johnston, 1924; Britton, 1951).

The present work confirmed that both species of *Solenanthus* are diploid with $2n = 2x = 24$ chromosomes, as reported in the literature. The meiosis is regular, with normal chromosome pairing, possibly existing chromosomes with complete and/or incomplete pairing. Many abnormalities were observed during the meiosis, as sticky chromosomes and irregular chromosome segregation.

According to Hartl and Jones (1998), mitotic and meiotic divisions in eukaryotic cells are rigorously controlled by checkpoint mechanisms intending to preserve the genome integrity. When at least one single chromosome does not present spindle fibers attached to the kinetochore during the metaphase, or when it is not aligned along the metaphase plate, specific proteins from the kinetochore signalize to delay the cellular division until the normal situation would be restored by proteins that act to maintain the genomic integrity during the cell cycle. Thus, proteins that control the repair mechanism during metaphase I and II could have been activated by the kinetochores of the delayed chromosomes, obstructing the elimination of those delayed chromosomes and the later formation of micronuclei have been observed in *S. circinnatus* 47.

The highest percentage of stained pollen grain (99%) was recorded for *S. stamineus*. This result is predictable based on meiotic behavior data and of the lowest percentages of irregularities in this population (table 2). In contrast, a lower percentage of pollen viability (94%) in population of *S. circinnatus* 47 can be explained by having high percent of precocious migration to the poles during metaphase and laggards at anaphase that led to the formation of micronuclei at telophase and could be provide small and sterile pollen grains.

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