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Cytogenetic study and pollen viability of four populations of *Trigonella spruneriana* Boiss. (Fabaceae) in Iran

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Abstract

In the present paper, the cytogenetic study including meiotic chromosome number and behavior along with pollen viability were performed in 4 populations of *Trigonella spruneriana* Boiss. This is the first cytogenetic report of the taxon. All populations are diploid and possess $2n = 2x = 16$ chromosome number, which is consistent with the proposed basic number of $x = 8$. In addition, some meiotic irregularities observed in different populations included chromosomes stickiness, B-chromosomes, chromosome bridges resulting from stickiness, the occurrence of laggard chromosomes, formation of micronuclei in tetrad cells and cytomixis. The highest and the lowest percentages of pollen viability were observed in populations SPR 658 and SPR 566, respectively.

***Keywords*:** chromosome number, Iran, meiotic behavior, pollen viability, *Trigonella spruneriana*

Introduction

The genus *Trigonella* L. is a member of the tribe Trifolieae of family Fabaceae, the second largest family of flowering plants in the world with 650 genera and 18000 species (Dangi et al., 2004). In Flora Iranica (Rechinger, 1984) the genus is represented by 58 annual and perennial species in 12 categories. *T. spruneriana* belongs to *T.* sect. *Cylindricae*. This category is represented with 3 annual members in Iran.

Several cytological investigations have been conducted in *Trigonella* (Singh and Roy, 1970; Singh and Singh, 1976; Agarwal and Gupta, 1983; Ahmad et al., 1999; Dundas et al., 2006; Aykut et al., 2009; Martin et al., 2008, 2011a, 2011b; Ranjbar et al., 2011). The number of mitotic chromosome was first reported by Kamari and Papatsou (1973) on an accession from Nisyros (Aegean Sea) of *T. balansae* Boiss. and Reuter, an annual pasture legume of Eurasian origin. Martin et al. (2010) analyzed karyotype of *T. spruneriana* and 9 species of the *T.* sect. *Cylindricae* in Turkey. There is only a few information about karyotype and phylogenetic relationships as well as meiotic behavior in the genus due to limited studies.

Basic information on the meiotic behavior and estimation of pollen viability are useful for germplasm characterization and identification of genetic variability, biodiversity, and evolutionary processes of the species (Palm-Ailva et al., 2004). Meiosis is a process which is controlled by genes (Gottschalk and Kaul, 1974; Golubovskaya, 1979). Although the meiocyte is a highly specialized cell capable of producing four haploid cells, mutations, hybridizations, environmental stress, endogamy and other factors may alter the constitution or the expression of genes that act during meiosis (Utsunomiya et al., 2002). The amount and quality of pollen produced by a flower is an important component of fitness. Pollen quality is often represented to pollen viability, i.e., the proportion of pollen grains that are viable (Stanley and Linskens, 1974; Heslop-Harrison et al., 1984). Because of its importance in fertilization and therefore production, several studies have focused on investigation of pollen viability (Asma, 2008).

Materials and Methods

Cytogenetics

The chromosome number and meiotic behavior were analyzed in 4 populations of *T. spruneriana* which were collected from different regions within the natural geographical distribution of *T. spruneriana* during several excursions in Iran (table 1). 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

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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-51 photomicroscope at initial magnification of X 1000. The chromosome number was counted from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

Pollen viability

Pollen stainability was considered as an indication of pollen viability. For this purpose pollen grains were first obtained from 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.

Table 1. Studied populations of *T. spruneriana* and their acronyms.

Taxa	Herbarium number	Altitude (m)	Location	Date	Collector name	Abbreviation
T. spruneriana	BASU 25667	1405	Azerbaijan Gharbi: Movana toward Salmas, 40 km to Salmas (37°.85'N, 44°.75' E)	19.5.2011	Ranjbar & Hajmoradi	SPR 667
T. spruneriana	BASU 23658	1100	Lorestan: Lorestan University (33°.50'N, 48°.45' E)	22.4.2009	Ranjbar & Hajmoradi	SPR 658
T. spruneriana	BASU 23689	1450	Lorestan: Dowrud, toward lake (33°.55'N, 49°E)	22.4.2009	Ranjbar & Hajmoradi	SPR 689
T. spruneriana	BASU 23566	1800	Kohgiluyeh and Boyer-Ahmad: Lordegan to Yasuj, 30 km before Pataveh (31°.75'N, 51°.25'E)	6.4.2010	Ranjbar & Hajmoradi	SPR 566

Results

Cytogenetics

With respect to meiotic chromosome number, meiotic stages, as well as abnormalities observed in each stage and data are presented in table 2. All populations are diploid and possess a chromosome number of $2n = 2x = 16$, which is consistent with the proposed basic number of $x = 8$. A total of 468 diakinesis/metaphases I (D/MI), 432 anaphase I/telophase I (AI/TI), 312 metaphase II (MII), and 1031 anaphase II/telophase II (AII/MII) cells were analyzed. Chromosomes stickiness, B-chromosomes, chromosome bridges resulting from stickiness, the occurrence of laggard chromosomes, formation of micronuclei in tetrad cells and cytomixis were meiotic irregularities, which observed in the above populations of *T. spruneriana.*

Pollen viability

The results of comparison between meiotic behaviour and pollen viability showed the highest (99.9) and lowest (16.2) percentages of the stained pollens in populations SPR 658 and SPR 566, 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 2.

Discussion

Meiosis is highly coherent and the process is genetically programmed which comprises of pairing homologous chromosomes, crossing over, reduced in chromosome number, and lacking of S period between the two divisions. Similar to any other biological process, all the sequential steps involved in meiosis are controlled by a large array

of genes (Ramana, 1974; Mok and Peloquin, 1975; Mok et al., 1976; Koduru and Rao, 1981; Falistocco et al., 1994). Mutation in any of these genes that govern micro or megasporogenesis from pre-meiotic to post meiotic events can lead to serious anomalies in the whole process resulting in the genetically aberrant end products having adverse impact on fertility and overall reproductive efficiency of the species (Lattoo et al., 2006). Furthermore, many abnormalities affecting plant fertility or causing total male sterility have been detected during the evaluation of meiotic behavior in some species.

Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000). Sticky chromosomes along with laggards were found in different stages such as D/MI (figures 1B and F) and MII (figures 1M and T). Chromosome bridges resulting from stickiness were observed in populations of SPR 689 and SPR 566 (figures 1H and K). The thickness of bridges and number of chromosomes involved in their formation are varied among different meiocytes. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao, 1996). The phenomenon of cytomixis consists of the migration of chromosome between meiocytes through cytoplasmic connection. Since cytomixis creates variation in the chromosome number of the gametes, it can be considered as an important mechanism of evolution (Ghaffari, 2006). The highest degree of cytomixis occurred in population of SPR 566 (figures 1O and P). There was another abnormality (asynchronous nucleus) in metaphase II (figure 1N) that was found in all studied populations except SPR 658 population.

Table2. Characterization of meiotic behaviour of pollen mother cells (PMCs) in 4 populations of *T. spruneriana*.

Meiotic characters	**SPR 566**	**SPR 667**	**SPR 689**	**SPR 658**
Cell number	449	631	636	244
D/MI	186	118	125	39
% D/MI	41.42	18.70	19.65	15.98
% Cytomixis	2.15	5.93	0	0
% B-chromosome	0	0.84	0	0
% Sticky and laggard chromosome	3.22	11.86	13.87	5.1
AI/TI	166	141	71	54
% AI/TI	36.97	22.34	11.16	22.13
% Sticky and laggard chromosome	0.6	0	4.21	0
% Bridge	0.6	0	1.4	0
% Micronucleus	0	2.12	0	0
% Cytomixis	0	0	1.4	0
MII	97	75	97	43
% MII	21.6	11.88	15.25	17.62
% Sticky and laggard chromosome	1.03	12	1.03	0
% Asynchronous nucleus	2.06	6.66	4.1	0
% Cytomixis	24.74	5.33	0	0
AII/TII	265	297	343	108
% AII/TII	59.02	47.06	53.93	44.26
% Micronucleus	0	0.33	0	0
% Cytomixis	7.79	0	0.2	4.32
% Asynchronous nucleus	0	0	0	0.92
% Pollen viability	16.2	94	96.5	99.9

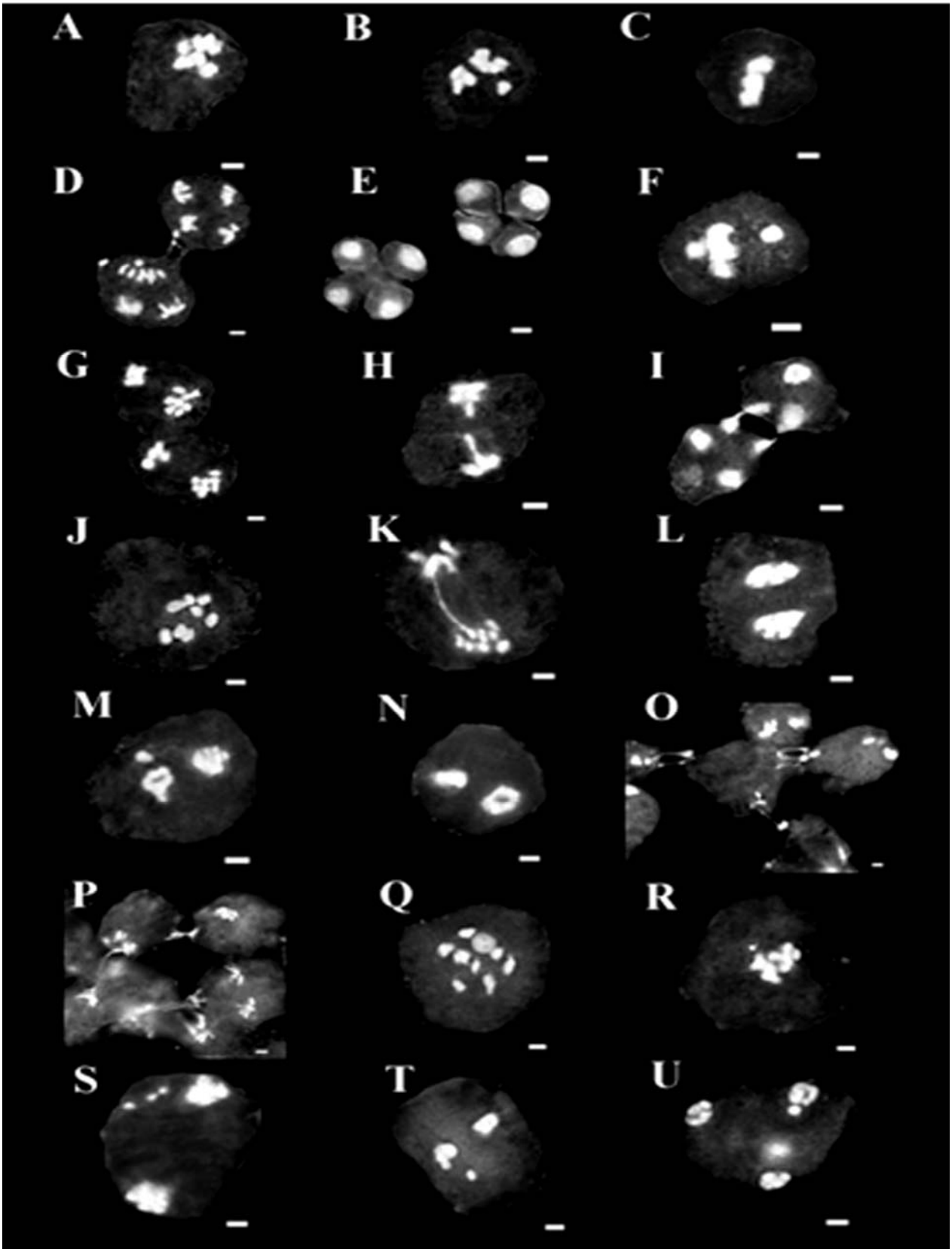

Figure 1. (A – E) Representative meiotic cells of population SPR 658. (A) Diakinesis, (B**)** Sticky chromosomes, (C) Metaphase I, (D) Cytomixis, (E) Tetrad, (F – I) Representative meiotic cells of population SPR 689, (F) Laggard chromosome, (G) Anaphase I, (H) Bridge, (I) Cytomixis, (J – P) Representative meiotic cells of population SPR 566, (J) Diakinesis, (K) Bridge, (L) Telophase I, (M) Laggard chromosome, (N) Asynchronous nucleus, (O) Cytomixis in metaphase II, (P) Cytomixis in anaphase II, (Q – U) Representative meiotic cells of population SPR 667, (Q) Diplotene, (R) B-chromosome, (S) Micronucleus, (T) Laggard chromosome, (U) Micronucleus. Scale bar = 10 µm.

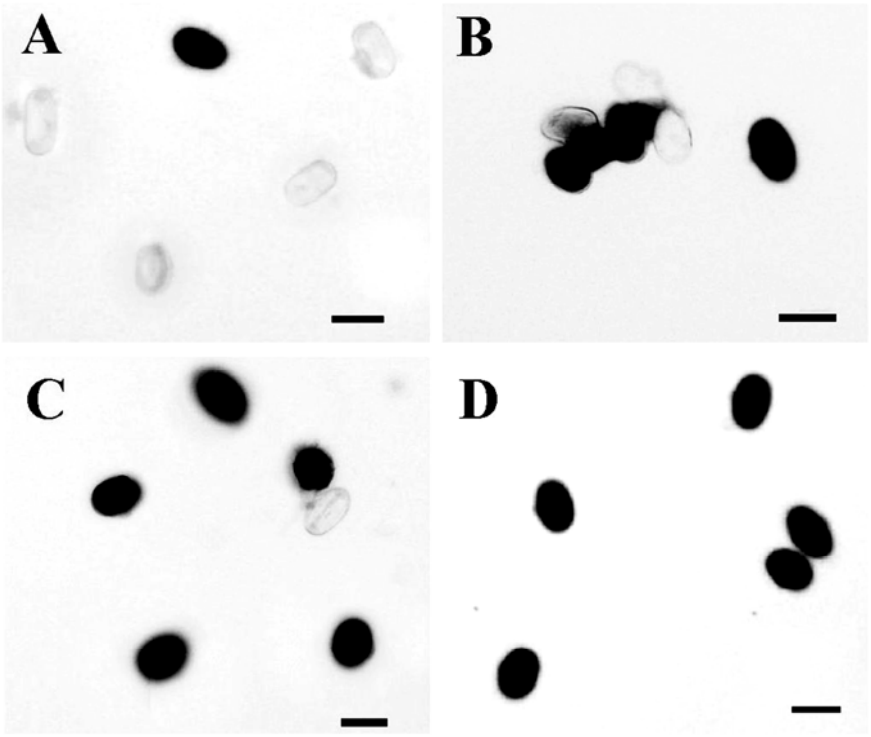

Figure 2. Pollen viability in different populations of *T. spruneriana*. (A) SPR 566, (B) SPR 667, (C) SPR 689, (D) SPR 658. Scale bars: 25 µm.

The highest degree of asynchronous nucleus was observed in population of SPR 667 (%6.6). B-chromosomes or extra chromosomes that occur in addition to the standard or A-chromosomes in some of the plants, are smaller than other chromosomes and do not form any synapsis with them. A large number of B-chromosomes have shown a negative effect on the growth and vigor of the plants, whereas in low numbers they may have some benefits for plant (Jones and Houben, 2003). B-chromosomes were only observed in population of SPR 667 (figure 1R). 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). Micronucleus was only seen in population of SPR 667 (figures 1S and U). The highest and lowest abnormalities were seen in populations of SPR 566 and SPR 658, respectively.

The high percentage of stained pollen grains (≥94%) was recorded for 3 populations of *T. spruneriana*. This result is predictable based on meiotic behavior data and of the low percentages of irregularities in these populations (table 3). So in populations that chromatin is translocated in their PMCs either does not occur or occurs at a very low frequency, meiotic abnormalities are almost negligible and most of the pollen grains are fertile.

In contrast, a low percentage of pollen viability (16.2%) in population of SPR 566 can be explained by having high percent of cytomixis. In this population a relatively high frequency of chromatin transfers in different stages of meiosis and consequently, low pollen viability (16.2%) was observed. So, it can be concluded that cytomixis affects the meiotic course considerably and results in reduced pollen viability. Lattoo et al. (2006) and Singhal and Kumar (2008) also showed that there is a direct relationship between occurrence of cytomixis and reduced pollen viability.

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