

## Diploid-tetraploid mixoploidy in a new species of *Astragalus* (Fabaceae) from Iran

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*Astragalus gilvanensis* Ranjbar & Nouri *sp. nova* (Fabaceae) is described and illustrated. It belongs to *Astragalus* sect. *Inceni* and is endemic to Iran. Its morphological characters, meiotic chromosome number and meiotic behavior were studied. It is a mixoploid plant with the ploidy levels  $2n = 2x = 16$  and  $2n = 4x = 32$ , consistent with the proposed base number of  $x = 8$ . Although the species displayed regular bivalent pairing and chromosome segregation at meiosis, some meiotic abnormalities were observed. The meiotic irregularities included the occurrence of varied degrees of sticky chromosomes in diakinesis to metaphase, laggard chromosomes in anaphase, cytomixis in prophase to telophase, asynchronous nuclei, and binuclear cells.

### Introduction

In terms of species number, *Astragalus* (Fabaceae) may be the largest genus of vascular plants, represented by a total of ca. 2500 taxa (Lock & Simpson 1991, Mabberley 1997, Maassoumi 1998, Ranjbar & Karamian 2002, Ranjbar *et al.* 2011a, 2011b). *Astragalus* is well represented also in Iran, where the section *Inceni* is one of the largest, with about 70 species (Podlech & Maassoumi 2003, Ranjbar & Karamian 2003, Ranjbar *et al.* 2005, 2010d, Ranjbar 2007); the section comprises 120 species worldwide (Lock & Simpson 1991, Yakovlev *et al.* 1996). Due to their economic importance, legumes have attracted the attention of cytologists and more than 50% of their genera are cytologically known.

The basic chromosome number  $x = 8$  (see <http://www.tropicos.org/Project/IPCN>) and two

ploidy levels ( $2n = 2x = 16$ ,  $2n = 4x = 32$ ) have been reported for *Astragalus* in the Old World (Aryavand 1983, Maassoumi 1987, 1989, Sheidai *et al.* 1996, Sheidai *et al.* 2000, Bader & Sherif 2007). Studies on the impact of karyotypic data on the interspecific and phylogenetic relationships and also on meiotic behavior in the genus are still limited. For *A.* sect. *Inceni*, there are two reports on the chromosome count for a few species from Iran (Maassoumi 1987, Ranjbar *et al.* 2010d).

Hitherto mixoploidy has not been reported in this section. Mixoploidy is a condition in which the tissue is composed of cells with different ploidy levels. The origin of mixoploids may be either spontaneous or induced. However their spontaneous occurrence is rather an uncommon phenomenon. According to the hypothesis of Maletskii and Maletskaya (1996), plant tissue mixoploidy underlies gametophytic agamospermy, i.e. the presence of tetraploid cell admix-

tures among the bulk of diploid cells. The existence of mixoploidy has been demonstrated in many plants, especially in the Chenopodiaceae including the genus *Beta* (Gentcheff & Gustafsson 1939, D'Amato 1985, Carvalheira 2000). Reductional division of admixed tetraploid cells results in the formation of a diploid embryo sac with cells capable of embryogenesis. A perusal of the existing literature revealed that in the majority of the cases mixoploidy was confined mostly to somatic tissues, although there are some reports of its occurrence in germinal cells. Earlier investigators opined differently regarding the origin of mixoploidy and attributed it to the fusion of neighboring cells prior to preleptotene, assembling of chromosomes from different cells and defective cell wall formation.

The present study reports for the first time the occurrence of spontaneous mixoploidy in a new species that belongs to *A. sect. Incani* in Iran, and documents its cytomorphological details.

## Material and methods

### Morphology

The morphological study is mainly based on herbarium material. Several sheets were examined for each species, received on loan from W, WU, TARI, BASU, FMUH, Herbarium of Research Center of Natural Resources and Animal Affairs of Mashhad, Esfahan, Shiraz, Tabriz, Kerman and Zahedan. Moreover, during several excursions in Iran, many specimens of the species and some closely related taxa were studied in the field by the authors.

### Meiotic studies

The meiotic studies were made following the acetocarmine squash technique. Voucher specimens are deposited in BASU, Hamedan, Iran. Flower buds from randomly selected plants in the ideal stage for meiotic studies were collected and fixed in 96% ethanol, chloroform and propionic acid (6:3:2) for 24 hours at room temperature, and then washed and preserved in 70% ethanol at 4 °C until used. Microsporocytes

were prepared by squashing and stained with 2% acetocarmine. Chromosome number was determined for five plants per population during diakinesis. The meiotic chromosome association was evaluated in at least 20 cells in diakinesis and the meiotic behavior up to this phase was evaluated in more than 250 microsporocytes.

## Results and discussion

### *Astragalus gilvanensis* Ranjbar & Nouri, *sp. nova* (Figs. 1–4)

*Differt ab Astragalo askio foliis caulium principalium ad 52 cm (nec 10–30) longis, petiole 14.5–16.5 cm (nec 5–12) longo, foliolis 14–16 (nec 9–14) jugis, 7–26 × 0.7–5 mm (nec 10–40 × 3–20 mm), racemis 40–50 floris (nec 15–25 floris), calycibus 13–14 mm (nec 8–10 mm) longis, praecipue nigri-pilosae (nec albi-et nigri-pilosis), dentibus 2–3.5 mm (nec 1–2) longis, stipulis 18–26 × 7–10 mm (nec 7–15 × 6–12 mm).*

TYPE: NW Iran. Zanjan: 65 km to Zanjan, Gilvan to Tashvir, after Tashvir, Araschangan, 1287 m, 30.IV.2009 *Ranjbar & Nouri 18000* (holotype: BASU; isotype: TARI).

ETYMOLOGY: The epithet is derived from the Gilvan town in western Zanjan Province.

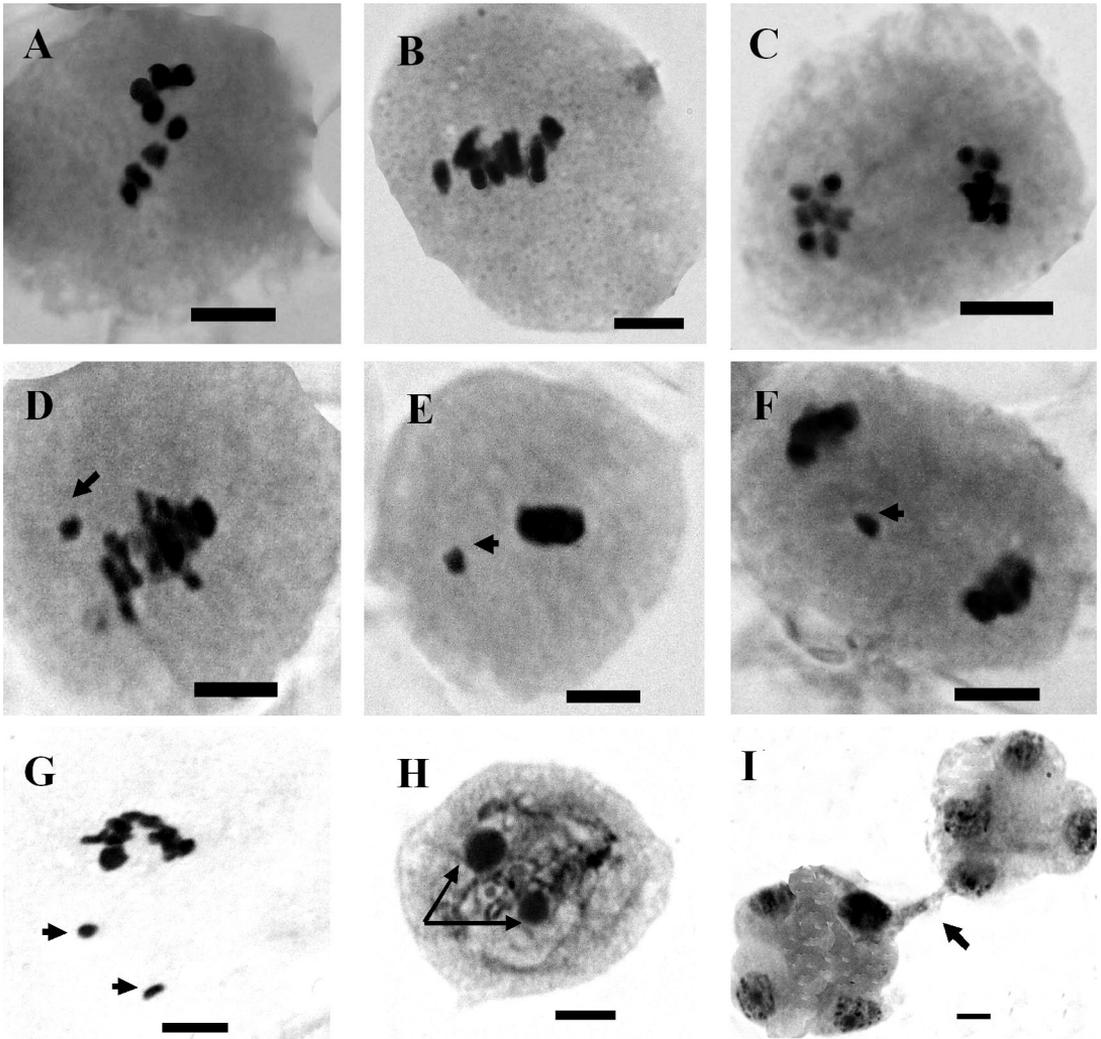
Plant acaulescent, 50–52 cm tall, with symmetrically medifixed appressed hairs 0.2–0.8 mm long. Caudex 3–5 mm in diameter, with few short branches, covered with remnants of old leaves. Stipules triangular to narrowly triangular-acuminate, 18–26 × 7–10 mm, adnate to the petiole for 7–10 mm, pilose. Leaves 30–36.5 cm long, petiole 14.5–16.5 cm long, striate like rachis, glabrous to rather sparsely hairy. Leaflets in 14–16 pairs, 7–26 × 0.7–5 mm, elliptic to lanceolate or linear, rounded to obtuse or acute at tip, mostly minutely mucronulate, loosely white-hairy on upper surface, loosely hairy on lower surface, both sides loosely spotted with minute purplish dots. Peduncle 19–29 cm long, 3–3.5 mm thick, finely striate, sparsely covered with white hairs. Raceme loosely 40–50-flowered, 24–30 cm long, often longer in fruit. Bracts membranaceous, linear-acute, 3–4 mm long, sparsely ciliate. Pedicels 1–2 mm long, flowers erect to spreading. Bracteoles whitish, linear, ca.



**Fig. 1.** *Astragalus gilvansensis* (holotype). Habit and habitat.



**Fig. 2.** *Astragalus gilvansensis* (holotype). Inflorescences.

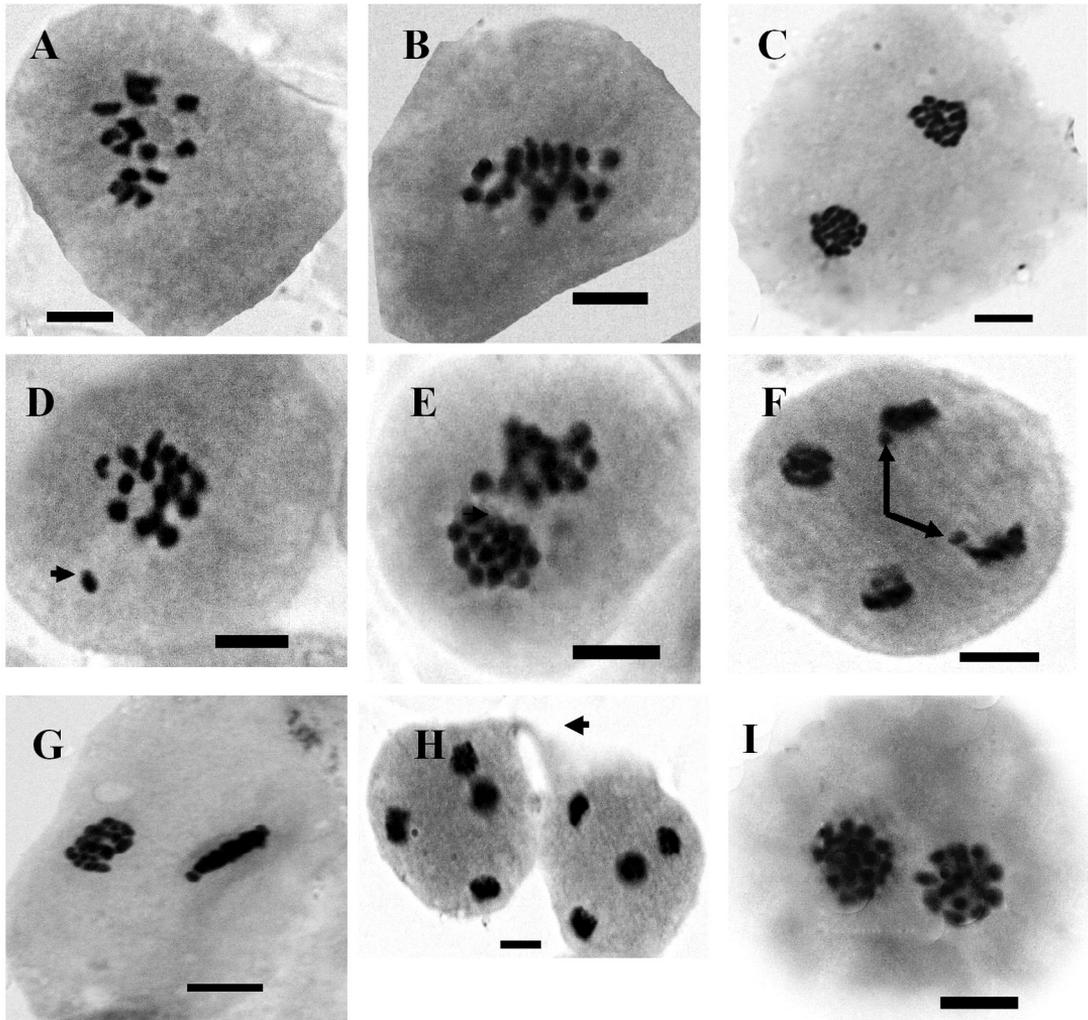


**Fig. 3.** Meiotic behavior of diploid cells in *Astragalus gilvanensis*. — **A:** Diakinesis. — **B:** Metaphase I. — **C:** Telophase I. — **D and E:** Metaphase I with fragmented chromosomes (arrows). — **F:** Anaphase I with laggard chromosome (arrow). — **G:** Diakinesis with fragmented chromosomes (arrows). — **H:** Binuclear cell (arrows). — **I:** Cytomixis (arrow). Scale bars = 6  $\mu\text{m}$ .

1.2 mm long, at base of calyx. Calyx 13–14 mm long, tubular, obliquely cut at orifice, loosely and mostly black-hairy, teeth narrowly triangular, acute to acuminate, 2–3.5 mm long. Corolla yellowish green. Standard 22.5–24 mm long, blade slightly to distinctly upcurved in upper part, 9.5–10 mm wide, obovate, minutely emarginate and attenuate at tip, basally gradually narrowed into a short cuneate claw. Wings 21.5–22 mm long, limbs obovate-spathulate, round-tipped, mostly minutely mucronulate, 11–11.5  $\times$  3–3.5 mm, auricle narrow, ca. 1 mm long, claw 10–11

mm long. Keel ca. 18 mm long, limbs obliquely obovate, with widely curved lower edge and slightly concave upper edge, acute-tipped, 7.5–8  $\times$  4–4.5 mm, auricle indistinct, claw 10–10.5 mm long. Stamen tube truncate at mouth. Ovary with a stipe 4–5 mm long, glabrous, 13–14-ovuled. Flowering and fruiting from April to May.

*Astragalus gilvanensis* is endemic to north-west Iran and occurs in the central Zanzan Province. It is known only from a single gathering collected in the steppe and clay zones south of the Alborz mountain. It is morphologically close



**Fig. 4.** Meiotic behavior of tetraploid cells in *Astragalus gilvanensis*. — **A:** Diakinesis. — **B:** Metaphase I. — **C:** Telophase I. — **D:** Diakinesis with fragmented chromosome (arrow). — **E:** Telophase II with fragmented chromosome. — **F:** Anaphase II with laggard chromosomes (arrows). — **G:** Asynchronous nuclei. — **H:** Cytomixis (arrow). — **I:** Telophase I. Scale bars = 6  $\mu$ m.

to *A. askius*, especially because of the similar type of indumentum, shape and color of flowers, but differs from it in the size and number of leaflets and flowers. In addition, the calyx and calyx teeth and the stipules in *A. gilvanensis* are clearly longer than in *A. askius*. There are also three other species morphologically close to *A. gilvanensis* (see Table 1).

#### Key to species of the *Astragalus askius* group

- |  |   |   |                           |
|--|---|---|---------------------------|
| 1. Pod erect to ascending or spreading .....             | 2 | and 30 mm, respectively .....   | <i>A. siahbishehensis</i> |
| 1. Pod pendulous .....                                   | 3 | 2. Leaflets elliptic to obovate; standard and pod ca. 23 and ca. 20 mm, respectively .....                    | <i>A. lacus-valashti</i>  |
| 2. Leaflets obovate to suborbicular; standard and pod 25 |   | 3. Calyx 13–14 mm long, calyx teeth 2–3.5 mm long, stipules 18–26 $\times$ 7–10 mm .....                      | <i>A. gilvanensis</i>     |
|  |   | 3. Calyx ca. 10 mm long, calyx teeth ca. 2 mm long, stipules ca. 10–15 $\times$ 2–5 mm .....                  | 4                         |
|  |   | 4. Leaflets linear-elliptic, stipe of pod ca. 10 mm long .....  |                           |
|  |   | .....   | <i>A. thionanthus</i>     |
|  |   | 4. Leaflets narrowly elliptic to ovate-elliptic or obovate; stipe of pod 9 mm long .....                      | 5                         |
|  |   | 5. Leaflets in 6–9 pairs, glabrous or sparsely covered with appressed hairs on midvein on lower surface ..... |                           |
|  |   | .....   | <i>A. subglaberrimus</i>  |
|  |   | 5. Leaflets in 9–14 pairs, sparsely to loosely covered with appressed hairs on both surfaces .....            | <i>A. askius</i>          |

## Meiotic studies

The PMCs with diploid and tetraploid chromosome numbers were intermixed, with the respective frequencies of 66.6% and 33.39%. Mixoploidy has rarely been observed in pollen mother cells in the same anther, ranging from diploidy to octaploidy, although this phenomenon is widely reported in somatic tissues (Nirmala & Rao 1996). Mixoploidy is often associated with the occurrence of polyploidy, hybridization, chemicals and, in some cases, it is genetically controlled. Mixoploidy is a cytogenetic event of great importance with practical and evolutionary implications. In higher plants lacking

sexual reproduction, mixoploidy is a potential force in evolution, while in sexually propagated species the chromosomal instability in reproductive tissue can produce gametes with variable chromosome numbers with the addition or loss of chromosomes and the formation of gametes that can produce aneuploids in subsequent generations, which may present low fertility due to meiotic irregularities.

In *A. gilvanensis*, different meiotic stages were found in anthers within the same flower (Figs. 3 and 4). A total of 1674 prophase (71.50%), 373 diakinesis/metaphase I (15.93%) and 12 anaphase I/telophase I (0.51%) for diploid cells, and 184 diakinesis/metaphase I

**Table 1.** A morphological comparison of *Astragalus gilvanensis* with the most similar taxa.

Taxon	<i>A. gilvanensis</i>	<i>A. askius</i>	<i>A. siahbishehensis</i>	<i>A. subglaberrimus</i>	<i>A. sabetii</i>
Plant height (cm)	50–52	30–95	30–35	30–40	30–40
Leaf length (cm)	30–36.5	10–30	10–25	12–30	10–18
Petiole length (cm)	14.5–16.5	5–12	6–10	4–9	4–7
Leaflet					
number of pairs	14–16	7–14	3–8	6–9	9–11
length (mm)	7–26	10–40	10–12	9–15	7–24
width (mm)	0.7–5	3–20	9–10	4–9	2–5
indumentum					
on upper surface	loosely hairy	glabrous to sparsely hairy	sparsely hairy	sparsely hairy	glabrous
on lower surface	very loosely hairy	sparsely hairy	loosely hairy	sparsely hairy	sparsely hairy
spots					
on upper surface	without spots	spotted	loosely spotted	spotted	spotted
on lower surface	without spots	spotted	loosely spotted	rarely spotted	without spots
Peduncle length (cm)	19–29	8–30	15–16	15–25	15–20
Number of flowers	40–50	15–25	?	15–26	ca. 35
Calyx					
length (mm)	13–14	8–10	9–10	8–9	8–9
indumentum	loosely and hairy	sparsely hairy	loosely and hairy	sparsely hairy	sparsely to densely hairy
Stipule					
length (mm)	18–26	7–15	6–9	7–10	ca. 8
width (mm)	7–10	?	2.5–5	?	ca. 2
shape (mm)	triangular	triangular	triangular	triangular	ovate
Calyx teeth length (mm)	2–3.5	1–2	1–2	0.5–1	1–2
Standard					
length (mm)	22.5–24	19–22	25–27	18	18
width (mm)	9.5–10	8–9	9–10	ca. 8	ca. 8
apex	rounded-retuse	emarginate	minutely emarginate	emarginate	rounded
Keel length (mm)	ca. 18	15–16	19–20	14	14
Wing					
length (mm)	21.5–22	17–19	22–23	15	16
claw length (mm)	10–11	9–10	?	ca. 8	ca. 8
Stipe of pod	4–5	ca. 4	4–5	ca. 4	ca. 2

(7.85%), 42 anaphase I/telophase I (1.79%) and five anaphase II/telophase II (0.21%) for tetraploid cells were analyzed. The meiotic irregularities included the occurrence of varied degrees of fragmented chromosomes in metaphase I, anaphase I and diakinesis, cytomixis in prophase, metaphase I and telophase II, asynchronous nuclei and binuclear cells.

## Cytomixis

Chromosome transfer from cell to cell through cytoplasmic connections, a phenomenon known as cytomixis, has been observed in many species in genera such as *Pilocarpus* (Pagliarini & Pereira 1992), *Centella* (Consolaro & Pagliarini 1995), *Brassica* (Souza & Pagliarini 1997) and *Zea* (Caetano-Pereira & Pagliarini 1997). The origin of cytomixis is not clear, but among the factors thought to cause it are the influence of genes, abnormal cell wall formation during premeiotic division, chemicals, pathological conditions, herbicides, radiation, temperature, mechanical injury, hybridization and polyploidy (Pagliarini 2000, Ranjbar *et al.* 2009, 2010a, 2010b, 2010c, 2010d). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originating from the pre-existing system of plasmodesmata formed within the tissues of the anther. The plasmodesmata become completely obstructed by the deposition of callose, forming conspicuous intermeiocyte connections or cytoplasmic channels that permit the transfer of chromosomes (Levan *et al.* 1964, Risueno *et al.* 1969, Diaz Lifante *et al.* 1992, Falistocco *et al.* 1995). Since cytomixis creates variation in the chromosome number of the gametes, it could be considered a mechanism of evolutionary significance. In *A. gilvanensis*, cytomixis was found in 79% and 20.47% of the diploid and tetraploid cells, respectively.

## Sticky and fragmented chromosomes

Sticky chromosomes were observed from the early stages of prophase to the final stages of meiosis. The number of sticky chromosomes

varied from two to many, often resulting in complete clumping of the chromosomes. Genetic and environmental factors (Nirmala & Rao 1996), as well as genotype–environment interaction (Baptista *et al.* 2000) have been implicated as reasons for chromosome stickiness in various plant species. In *A. gilvanensis*, sticky chromosomes were found in 13.16% of diakinesis, 16.15% of metaphase and 14.28% of anaphase I in the diploid cells. They were also observed in 5.64% of diakinesis and 30% of metaphase I in the tetraploid cells (Figs. 3 and 4).

## Laggard chromosomes

Laggards and non-oriented chromosomes may produce micronuclei, if they fail to reach the poles in time to be included in the main telophase nucleus (Koduru & Rao 1981, Utsunomiya *et al.* 2002), leading to the formation of micro-pollen and, probably, to gametes with unbalanced chromosome numbers (Mansuelli *et al.* 1995). Non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers (Nicklas & Ward 1994). It has been suggested that infertility in polyploids is not solely due to the production of aneuploid gametes formed by improper segregation of chromosomes during anaphase/telophase stages, the genetic factors may also bring about pollen sterility as evidenced in different tetraploid species (Hazarika & Rees 1967, Pagliarini 1990, 2000, Baptista-Giacomelli *et al.* 2000, Ranjbar *et al.* 2009, 2010a, 2011a). Laggard chromosomes were observed in anaphase I and II stages in the diploid and tetraploid cells of *A. gilvanensis* (Figs. 3 and 4).

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