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# First karyotype analysis, physical rDNA mapping, and genome size assessment in 4 North African *Astragalus* taxa (Fabaceae)

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**Abstract:** Four taxa of Algerian *Astragalus* L. were studied for their chromosome number, karyotype features, and genome size. Fluorochrome banding was done for detection of GC-rich DNA regions, fluorescence in situ hybridization (FISH) for physical mapping of 35S and 5S rRNA genes, and flow cytometry for nuclear DNA content. All the taxa present the same chromosome number ($2n = 2x = 16$) and single 35S and 5S rDNA loci, but their distributions on chromosomes are different. The GC heterochromatin pattern was different among studied taxa and an unusually high number of chromomycin-positive bands were observed in *A. pseudotrigonus* Batt. & Trab. The genome size differed between species, ranging from 2C = 1.39 pg in *A. cruciatus* Link. to 2C = 2.71 pg in *A. armatus* subsp. *tragacanthoides* (Desf.) Maire. No difference in nuclear DNA amount was detected between the 2 subspecies of *A. armatus*. Although *Astragalus* is a large genus comprising some 3000 species, such morphometric and molecular cytogenetic karyotype analyses, with genome sizes, are particularly scarce therein. Therefore, published genome sizes have also been compiled into one table.

**Key words:** *Astragalus*, DNA content, fluorescence in situ hybridization, fluorochrome banding, rDNA mapping

## 1. Introduction

The genus *Astragalus* L. (Fabaceae) is one of the largest genera of vascular plants in the world, comprising about 3000 species (Scherson et al., 2008) grouped in 150 sections (Podlech, 1986). The genus is especially abundant in both the Old World (around 2400 species) and the New World (500 species) (Zarre and Azani, 2013). The geographic centers of origin and diversification of the genus *Astragalus* are the steppes and mountains from southwestern to south-central Asia and the Himalayan Plateau (Wojciechowski, 2005). In Algeria, the genus is represented by 40–45 species (Quézel and Santa, 1962), belonging to 18 sections and distributed in different phytogeographical regions.

Earlier karyotaxonomic investigations of *Astragalus* species have been restricted to chromosome number determination. Senn (1938) established that the most common basic chromosome number of Old World species is $x = 8$. Later studies confirmed Senn's report and demonstrated that $x = 11$, 12, 13, 14, and 15 were common for the New World species (Kazempour Osaloo et al., 2005 and references therein). Many cytological studies of *Astragalus* have since been performed throughout the world (Manandhar and Sakya, 2004; Badr and Sharawy, 2007; Martin et al., 2008; Kazem et al., 2010; Abdel Samad et al., 2014).

Despite the botanical and economic importance of the genus, investigations employing molecular cytogenetic techniques are scarce. There is one paper on chromosome fluorescence in situ hybridization (FISH) mapping (Kim et al., 2006) and reports on *Astragalus* genome size concerning Balkan and Lebanese florae (Siljak-Yakovlev et al., 2010; Temsch et al., 2010: Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014; Vallès et al., 2014).

In order to determine karyotype features and genome size, we investigated 3 endemic taxa from North Africa:

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*A. armatus* subsp. *tragacanthoides* (Desf.) Maire and *A. armatus* subsp. *numidicus* (Coss. & Dur.) Maire from section *Poterion* Bunge, and *A. pseudotrigonus* Batt. & Trab from section *Astragalus* Boiss. One widespread species, *A. cruciatus* Link. from section *Annulares* DC., was also sampled from different geographic localities of Algeria (Agerer-Kirchhof, 1976; Tietz, 1988; Podlech, 1994).

In order to increase the knowledge of the 4 studied North African *Astragalus* taxa and their relationships, the main objectives of our study were a) to construct the karyotypes of these taxa, b) to assess the number and distribution of their GC-rich DNA regions, c) to determine their 5S and 35S rRNA gene patterns, and d) to assess their nuclear DNA contents.

## 2. Materials and methods

### 2.1. Plant material

Eight populations belonging to 4 taxa of the genus *Astragalus* were included in this study. Plants were collected from different localities in the semiarid and Saharan bioclimatic zone of Algeria as shown in Table 1 and Figure 1. The vouchers were deposited at the herbarium of the Laboratory of Genetics, Biochemistry, and Plant Biotechnologies, Faculty of Natural Sciences, University of Constantine 1, Constantine, Algeria. Taxonomic identification was assessed following Quézel and Santa (1962).

### 2.2. Karyotype analysis

The seeds were germinated on moist filter paper in petri dishes at room temperature (18–24 °C) for 4 days for all species. Root tips of about 0.5–1 cm in length were excised between 0800 and 1100 hours, pretreated with 8-hydroxyquinoline (0.002 M) for 3 h at 16 °C, and fixed in freshly prepared absolute ethanol-acetic acid (v/v, 3:1) solution. Fixed root tips were kept at 4 °C in the first fixative for a few days or for several months in 70% ethanol, until use.

For morphometrical analysis, meristems were hydrolyzed in 1 N HCl at 60 °C for 8 min, stained, and squashed in a drop of aceto-orcein. After freezing at –80 °C for 24 h, cover slips were removed; preparations were air-dried for at least 24 h and then mounted in DPX. Chromosome counts were made on well-spread metaphase plates from a minimum of 20 seeds per population. Karyotype was determined by examining 5 metaphase plates obtained from different individuals. Determination of centromere position and chromosome type was done according to Levan et al. (1964). The mean total length (TL) of chromosomes, the arms ratio (r = long arm / short arm), the centromeric index [Ci = 100 × short arm / (long + short arm)], the total length of diploid chromosome complement (ΣTL), asymmetry index [Ai% = (Σ long arms / Σ total chromosome lengths) × 100, according to Arano and Saito (1980)], and symmetry class according to Stebbins (1971) were calculated.

### 2.3. Chromosome preparation for FISH and fluorochrome banding

A slightly modified air drying technique (Geber and Schweizer, 1987) was used for chromosome preparation. Root tips were rinsed in 0.01 M citrate buffer (pH 4.7) for 20 min and then softened in an enzymatic mixture containing 3% cellulase R10 (Yakult Honsha Corporation, Tokyo, Japan), 1% pectolyase Y23 (Seishin Corporation, Tokyo, Japan) and 4% hemicellulase (Sigma Chemical, Saint-Quentin Fallavier, France). The digestion was done in a moist chamber at 37 °C for 10–20 min. The obtained protoplasts were dropped on a clean slide and kept for different staining techniques.

### 2.4. Fluorochrome banding

For detection of GC-rich DNA regions, chromomycin $A_3$ (CMA; Sigma, Germany) was used according to Schweizer (1976) with minor modification as described by Siljak-Yakovlev et al. (2002). The slides were incubated in McIlvaine buffer (pH 7, with 5 mM $MgSO_4$) for 15 min

**Table 1.** Geographical origins and bioclimatic characteristics of studied *Astragalus* populations.

| Taxon | Section | Locality | Latitude and longitude | Altitude (m) | Rainfall (mm) | Bioclimatic zone |
|---|---|---|---|---|---|---|
| *A. armatus* subsp. *tragacanthoides* | *Poterion* Bunge | El Kantara | 35°32′57″N, 6°04′74″E | 1150 | 300–600 | Semiarid |
| | | Tamarin | 35°51′54″N, 6°29′38″E | 800 | 300–600 | Semiarid |
| | | Oum Tiour | 35°18′10″N, 5°48′49″E | 730 | 300–600 | Semiarid |
| *A. armatus* subsp. *numidicus* | *Poterion* Bunge | Oued Hamla | 35°19′22″N, 5°50′22″E | 838 | 300–600 | Semiarid |
| | | Sbakh | 34°13′56″N, 5°40′15″E | 85 | 300–600 | Semiarid |
| *A. cruciatus* | *Annulares* DC. | El Robah | 33°17′15″N, 6°55′01″E | 90 | 100 | Saharan |
| | | El Oued | 33°41′55″N, 6°41′08″E | 30 | 100 | Saharan |
| *A. pseudotrigonus* | *Astragalus* Boiss. | Tamanrasset | 23°41′20″N, 5°12′09″E | 980 | 100 | Saharan |

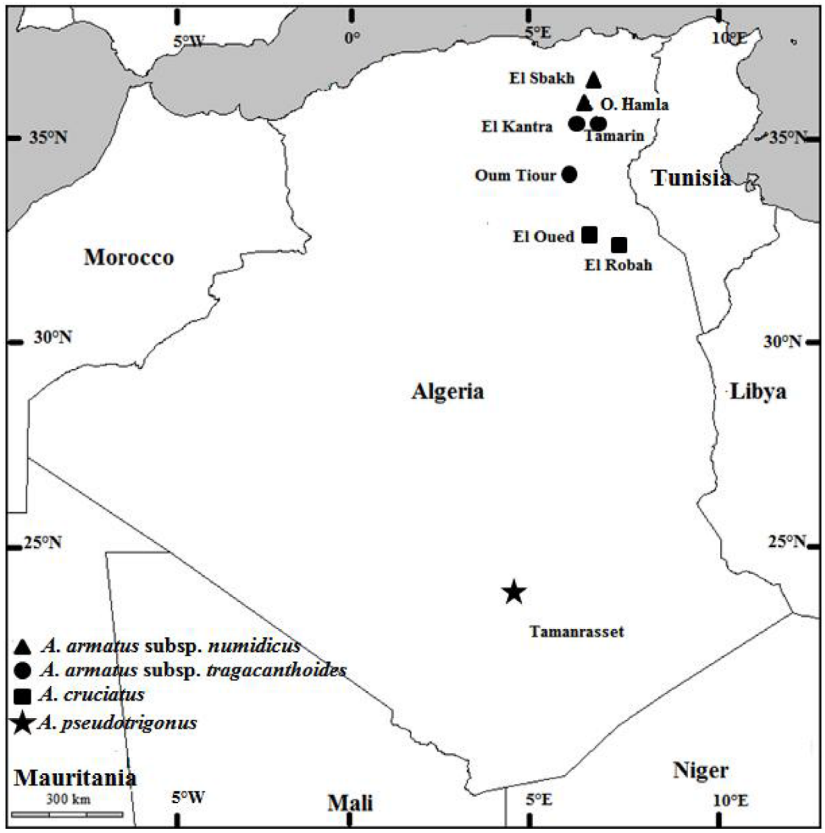


**Figure 1.** Geographical origin of the 8 Algerian populations studied corresponding to 4 *Astragalus* species.

and stained with $CMA_3$ (0.2 mg/mL in the same buffer) for 1 h in the dark. The slides were then rinsed in the same buffer (without $MgSO_4$), counterstained with methyl green (0.5% in McIlvaine buffer, pH 5.5) for 7 min, and finally rinsed in McIlvaine buffer (pH 5.5). The slides were mounted in Citifluor AF1 anti-fade agent (Agar Scientific, Stansted, UK).

**2.5. Fluorescence in situ hybridization**

A double FISH experiment was carried out with 2 DNA probes according to the protocol of Heslop-Harrison et al. (1991) with slight modifications. The probe 35S rDNA is a clone of a 4-kb *Eco*RI fragment, including 35S rDNA from *Arabidopsis thaliana* (L.) Heynh. labeled by nick translation with direct Cy3 (Amersham, Courtaboeuf, France). The pTa 794 probe is a 410-bp *Bam*HI fragment of the 5S rDNA from *Triticum vulgare* Vill. labeled with digoxigenin-11-dUTP (Roche Diagnostics, Meylan, France) after PCR amplification using universal M13 primers and revealed by antidigoxigenin-fluorescein (Roche Diagnostics). Slides were counterstained and mounted in Vectashield medium (Vector Laboratories, Peterborough, UK) with 4′,6-diamidino-2-phenylindole (DAPI). Chromosome plates were observed using an epifluorescence Zeiss Axiophot microscope with different combinations of excitation and emission filter sets (01, 07, 15, and triple 25). The acquisition and treatment of images were performed using a highly sensitive CCD camera (RETIGA 2000R, Princeton Instruments, Evry, France) and an image analyzer (MetaVue, Evry, France).

**2.6. Nuclear DNA content assessment by flow cytometry**

The total nuclear DNA was assessed by flow cytometry according to Marie and Brown (1993). *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg) was used as internal standard for all investigated *Astragalus* taxa. At least 5 individuals per population were measured. The leaves of the sample and the internal standard were chopped together using a razor blade in a plastic petri dish in 600 µL of Gif nuclear buffer, namely an autoclaved stock of 45 mM $MgCl_2$, 30 mM sodium citrate, 60 mM 4-morpholinepropane sulfonate (pH 7), 1% polyvinyl pyrrolidone (~10,000 Mr, P6755, Sigma Aldrich, France), 0.1% (w/v) Triton X-100, and 10 mM sodium metabisulfite supplemented with RNAse (2.5 U/mL, Roche). The suspension was passed through a 50-µm mesh nylon filter. The nuclei were stained with 50 µg/mL propidium iodide, a DNA intercalating dye, and analyzed after 5 min at 4 °C. DNA content of 5000–

10,000 stained nuclei was determined for each sample using a CyFlow cytometer with a 532-nm, 30-mW laser (CyFlow SL3, Partec, Münster, Germany).

The 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of *Astragalus* specimens and the internal standard.

## 3. Results

### 3.1. Karyotype analysis

In all studied taxa, mitotic metaphases showed both the same diploid ($2n = 2x = 16$) and basic ($x = 8$) chromosome number. Karyomorphometric data concerning the karyotypes are presented in Table 2.

The karyotype of *Astragalus armatus* subsp. *tragacanthoides* presents 7 metacentric chromosome pairs and 1 submetacentric pair (Table 2; Figures 2 and 3). Chromosome length ranged from 3.80 ± 0.50 µm to 5.47 ± 0.43 µm, with the longest total chromosome length (73.22 ± 1.59 µm) among the studied taxa. The karyotype symmetry was type 1A according to Stebbins (1971), and the asymmetry index (58.48%) according to Arano and Saito (1980) was the lowest among these taxa. A secondary constriction was clearly visible at the intercalary position of the long arm of chromosome pair 1.

In *A. armatus* subsp. *numidicus*, the karyotype also possesses 7 metacentric chromosome pairs and 1 submetacentric chromosome pair (Table 2; Figures 2 and 3). Chromosome length ranged from 3.87 ± 0.10 µm to 5.92 ± 0.25 µm, and total chromosome length was 72.44 ± 2.80 µm. Karyotype symmetry was type 1A and the asymmetry index 59.05%. An intercalary secondary constriction was observed on the long arm of chromosome pair 1.

In *A. cruciatus*, the karyotype showed 2 metacentric, 4 submetacentric, and 2 subtelocentric chromosome pairs (Table 2; Figures 2 and 3). Chromosome length ranged from 3.45 ± 0.95 µm to 5.51 ± 0.55 µm. The total chromosome length was 72.64 ± 2.09 µm. The karyotype symmetry was type 2A and the asymmetry index 67.54%. A secondary constriction was observed adjacent to the centromere on the long arm of chromosome pair 4.

In *A. pseudotrigonus*, the karyotype presented 1 metacentric satellite pair and 3 submetacentric and 4 subtelocentric chromosome pairs (Table 2; Figures 2 and 3). Chromosome sizes ranged from 2.30 ± 0.04 µm to 6.98 ± 0.11 µm. Total chromosome length was the smallest among studied taxa (70.86 ± 3.60 µm). The karyotype symmetry type and the asymmetry index were respectively 2B and 76.43%.

### 3.2. Physical mapping of heterochromatin and rRNA genes

The number and distribution of GC-rich heterochromatic bands ($CMA^+$) and 5S and 35S rDNA loci are given in Table 3 and Figure 3. The patterns obtained by CMA staining showed the strongest GC-rich heterochromatic bands on both sides of the secondary constriction on chromosome pair 1 in *A. armatus* subsp. *tragacanthoides* and *A. armatus* subsp. *numidicus* and on pair 4 in *A. cruciatus* (Figure 3). One supplementary weak $CMA^+$ signal was localized on pair 8 of *A. armatus* subsp. *tragacanthoides* and pair 6 of *A. armatus* subsp. *numidicus* adjacent to the centromere on short arms. In *A. pseudotrigonus*, several bright $CMA^+$ bands were detected: 1 in telomeric region of chromosome pair 1; 8 in paracentromeric regions of chromosome pairs 1, 3, 4, 5, 6, and 7; 10 in intercalary positions on chromosome pairs 1, 2, 3, 4, 5, and 6; and, finally, 2 on the short arm and satellite of the smallest chromosome pair (Figure 3). Numerous thin $CMA^+$ bands were also distributed across the chromosome set in different positions.

In *A. armatus* subsp. *tragacanthoides* and *A. armatus* subsp. *numidicus*, FISH revealed 2 strong 35S rDNA signals situated at each side of the secondary constriction in chromosome pair 1 colocalized with $CMA^+$ bands, and 2 thin 5S rDNA signals localized on chromosome pair 6 in the paracentromeric region of short arm (Figure 3). In *A. armatus* subsp. *numidicus*, the 5S rDNA signals were colocalized with $CMA^+$ bands (Figure 3). In *A. cruciatus*, 35S and 5S rDNA signals were situated on chromosome pair 4. The 35S rDNA signals were situated at each side of the secondary constriction and colocalized with $CMA^+$ bands, while the 5S rDNA signals were situated in the telomeric region of the short arm (Figure 3). In *A. pseudotrigonus*, 2 35S rDNA signals were colocalized with $CMA^+$ bands on the satellites and short arm of the smallest chromosome pair. The weak 5S rDNA signals were located between 2 strong $CMA^+$ bands in the subtelomeric region of the long arm of chromosome pair 1 (Figure 3).

### 3.3. Genome size

Nuclear DNA content ranged from 2C = 1.39 pg in *A. cruciatus* to 2.71 pg in *A. armatus* subsp. *tragacanthoides*, which presented a 1.95-fold genome increment at the same ploidy level. No significant differences were found between the 2 subspecies of *A. armatus*. *Astragalus pseudotrigonus* possessed an intermediary genome size of 1.55 pg (Table 3).

## 4. Discussion

### 4.1. Chromosome number and karyotype features

The diploid chromosome number of $2n = 16$ found in these taxa agrees with data previously reported for numerous *Astragalus* taxa (Badr and Sharawy, 2007; Kazem et al., 2010; Abdel Samad et al., 2014).

Chromosomes were medium-sized and their length ranged from 2.30 to 6.98 µm, in contrast with the majority of *Astragalus* species, which have smaller chromosomes (<4 µm) (Badr and Sharawy, 2007; Martin et al., 2008; Kazem et al., 2010). This is certainly one reason for

**Table 2.** Morphometric data for the chromosome sets of the 4 *Astragalus* taxa.

| Taxon | Chp | L (µm) (±SD) | S (µm) (±SD) | TL (µm) (±SD) | r | Ci | Ct | ΣTL (±SD) | Ai% | SyC |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. armatus* subsp. *tragacanthoides* | 1 | 3.29 (0.20) | 2.18 (0.30) | 5.47 (0.43) | 1.51 | 39.85 | m* | 73.22 (1.59) | 58.48 | 1A |
| | 2 | 2.90 (0.40) | 2.10 (0.10) | 5.00 (0.46) | 1.38 | 42.00 | m | | | |
| | 3 | 2.75 (0.20) | 1.90 (0.30) | 4.65 (0.46) | 1.44 | 40.86 | m | | | |
| | 4 | 2.58 (0.10) | 2.04 (0.40) | 4.62 (0.60) | 1.26 | 44.16 | m | | | |
| | 5 | 2.64 (0.10) | 1.90 (0.30) | 4.54 (0.31) | 1.38 | 41.85 | m | | | |
| | 6 | 2.29 (0.20) | 2.14 (0.20) | 4.43 (0.34) | 1.07 | 48.31 | m | | | |
| | 7 | 2.45 (0.30) | 1.65 (0.10) | 4.10 (0.27) | 1.48 | 40.24 | m | | | |
| | 8 | 2.51 (0.40) | 1.29 (0.30) | 3.80 (0.50) | 1.94 | 33.95 | sm | | | |
| *A. armatus* subsp. *numidicus* | 1 | 3.43 (0.30) | 2.49 (0.20) | 5.92 (0.25) | 1.37 | 42.06 | m* | 72.44 (2.80) | 59.05 | 1A |
| | 2 | 3.09 (0.20) | 1.64 (0.10) | 4.73 (0.35) | 1.88 | 34.67 | sm | | | |
| | 3 | 2.62 (0.30) | 1.96 (0.3) | 4.58 (0.30) | 1.33 | 42.79 | m | | | |
| | 4 | 2.76 (0.20) | 1.73 (0.20) | 4.49 (0.29) | 1.59 | 38.53 | m | | | |
| | 5 | 2.50 (0.20) | 1.91 (0.20) | 4.41 (0.20) | 1.31 | 43.31 | m | | | |
| | 6 | 2.49 (0.20) | 1.80 (0.10) | 4.29 (0.21) | 1.38 | 41.95 | m | | | |
| | 7 | 2.43 (0.30) | 1.50 (0.20) | 3.93 (0.15) | 1.62 | 38.16 | m | | | |
| | 8 | 2.07 (0.10) | 1.80 (0.00) | 3.87 (0.10) | 1.15 | 46.51 | m | | | |
| *A. cruciatus* | 1 | 3.60 (0.30) | 1.91 (0.10) | 5.51 (0.55) | 1.88 | 34.66 | sm | 72.64 (2.09) | 67.54 | 2A |
| | 2 | 3.65 (0.60) | 1.46 (0.30) | 5.11 (0.10) | 2.50 | 28.57 | sm | | | |
| | 3 | 3.40 (0.40) | 1.46 (0.20) | 4.86 (0.70) | 2.32 | 30.04 | sm | | | |
| | 4 | 2.45 (0.30) | 2.18 (0.40) | 4.63 (0.15) | 1.12 | 47.08 | m* | | | |
| | 5 | 3.52 (0.20) | 0.98 (0.30) | 4.50 (0.10) | 3.59 | 21.77 | st | | | |
| | 6 | 2.90 (0.30) | 1.43 (0.40) | 4.33 (0.10) | 2.02 | 33.02 | sm | | | |
| | 7 | 2.29 (0.40) | 1.64 (0.30) | 3.93 (0.61) | 1.39 | 41.73 | m | | | |
| | 8 | 2.72 (0.30) | 0.73 (0.10) | 3.45 (0.95) | 3.72 | 21.15 | st | | | |
| *A. pseudotrigonus* | 1 | 5.81 (0.05) | 1.17 (0.02) | 6.98 (0.11) | 4.96 | 16.76 | st | 70.86 (3.60) | 76.43 | 2B |
| | 2 | 5.00 (0.03) | 0.99 (0.04) | 5.99 (0.06) | 5.05 | 16.53 | st | | | |
| | 3 | 3.51 (0.04) | 1.23 (0.03) | 4.74 (0.10) | 2.85 | 25.95 | sm | | | |
| | 4 | 3.19 (0.04) | 0.94 (0.02) | 4.13 (0.02) | 3.39 | 22.76 | st | | | |
| | 5 | 2.76 (0.10) | 1.12 (0.04) | 3.88 (0.12) | 2.46 | 28.86 | sm | | | |
| | 6 | 2.90 (0.06) | 0.95 (0.03) | 3.85 (0.07) | 3.05 | 24.67 | st | | | |
| | 7 | 2.61 (0.05) | 0.95 (0.03) | 3.56 (0.07) | 2.75 | 26.68 | sm | | | |
| | 8 | 1.30 (0.04) | 1.00 (0.02) | 2.30 (0.04) | 1.30 | 43.48 | m-sat | | | |

Chp- chromosome pair, L- long arm, S- short arm, TL- total chromosome length, r- arm ratio, Ci- centromeric index, and Ct- chromosome type according to Levan et al. (1964); ΣTL- total length of diploid chromosome complement and Ai%- index of karyotype asymmetry according to Arano and Saito (1980); SyC- symmetry class according to Stebbins (1971); sat- satellite; *- presence of secondary constriction; SD- standard deviation.

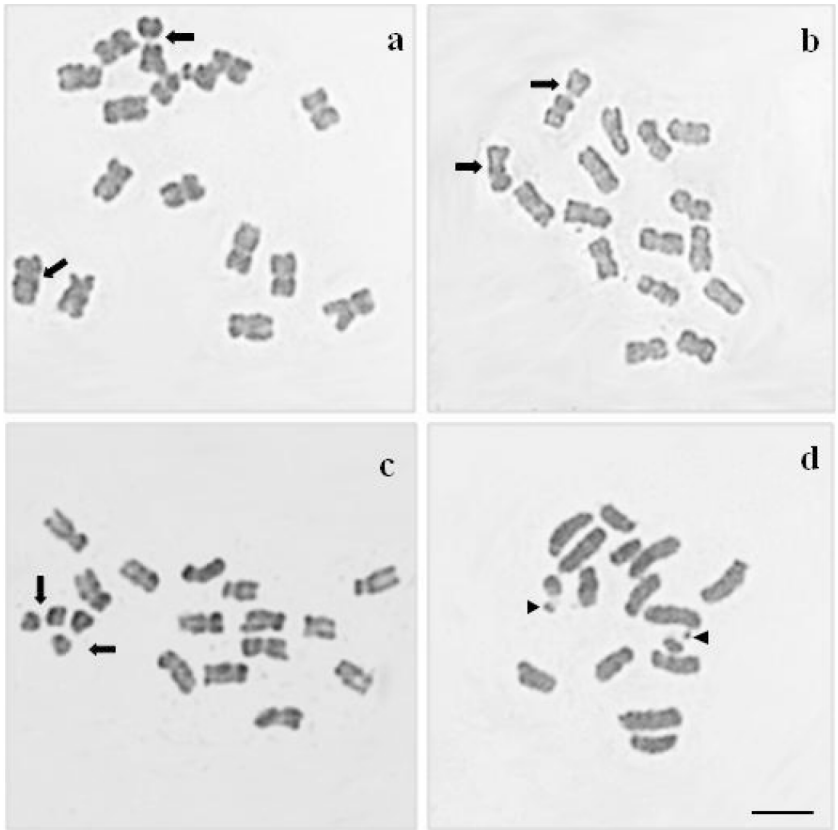


**Figure 2.** Metaphase plates of 4 *Astragalus* taxa: (a) *Astragalus armatus* subsp. *tragacanthoides*, (b) *A. armatus* subsp. *numidicus*, (c) *A. cruciatus*, (d) *A. pseudotrigonus*. Arrows indicate position of intercalary secondary constrictions. Arrowheads indicate satellites in d. Scale bar = 4 µm.

the paucity of karyotype descriptions for *Astragalus* species. In this study satellites have been observed in *A. pseudotrigonus*. It is possible that more satellites exist, but they are not always detectable, depending on the degree of chromatin condensation. Previous reports indicate the presence of satellites in the species belonging to other sections. Namely, in *A. stella* L. of section *Sesamei* DC., Badr and Sharawy (2007) found a single chromosome pair with satellites. Martin et al. (2008) reported 2 satellite chromosome pairs in *A. nezaketae* A. Duran & Aytaç of section *Incani* DC., while Manandhar and Sakya (2004) observed 3 chromosome pairs bearing satellites in *A. chlorostachys* Lindl. of section *Chlorostachys* Bunge.

Secondary constrictions were detected in *A. armatus* subsp. *numidicus*, *A. armatus* subsp. *tragacanthoides*, and *A. cruciatus*. Otherwise, only Ma et al. (1984) have reported the existence of intercalary secondary constrictions in 2 species from section *Cenantrum* Bunge: *A. membranaceus* (Fisch.) Bunge and *A. mongholicus* Bunge.

The values of asymmetry indices (Arano and Saito, 1980) and Stebbins' classes (1971) give indications about the chromosome evolution. *Astragalus armatus* subsp. *tragacanthoides* and *A. armatus* subsp. *numidicus* are characterized by very symmetrical karyotypes with a predominance of metacentric chromosomes, which seems to be a common trait in the genus (Manandhar and Sakya, 2004; Badr and Sharawy, 2007; Martin et al., 2008). On the other hand, *A. cruciatus* and especially *A. pseudotrigonus* possess asymmetrical karyotypes with predominance of submetacentric and subtelocentric chromosomes. Stebbins (1971) indicated that a group of angiosperms with a more asymmetrical karyotype could be derived from a rather symmetrical group. Following this idea, *A. cruciatus* and *A. pseudotrigonus* could be considered as more derived species than *A. armatus* subsp. *tragacanthoides* and *A. armatus* subsp. *numidicus*.

### 4.2. rDNA and heterochromatin patterns

To our knowledge, this is the first report describing GC-rich DNA regions by fluorochrome banding in this genus. The heterochromatin patterns of the studied taxa showed variation in number, intensity, and position of CMA bands along the chromosomes. *Astragalus pseudotrigonus* presented an unusually high number of CMA bands with a distribution across all chromosomes

**Table 3**. GC-rich DNA regions, rDNA sites, and DNA content in 4 *Astragalus* taxa.

| Taxa | $CMA^+$ bands | 35S rDNA locus | 5S rDNA locus | 2C DNA in pg (±SD) |
|---|---|---|---|---|
| *A. armatus* subsp. *tragacanthoides* | 2 p, 2 SC | 1 SC | 1 p | 2.71 (0.06) |
| *A. armatus* subsp. *numidicus* | 2 p, 2 SC | 1 SC | 1 p | 2.68 (0.06) |
| *A. cruciatus* | 2 SC | 1 SC | 1 t | 1.39 (0.02) |
| *A. pseudotrigonus* | 16 p, 20 i, 4 t, 2sat | 1sat+t | 1 st | 1.55 (0.01) |

sat- satellite; sat+t- both sides of secondary constriction; SC- secondary constriction; t- telomeric; st- subtelomeric; i- interstitial; p- para/pericentromeric; SD- standard deviation.

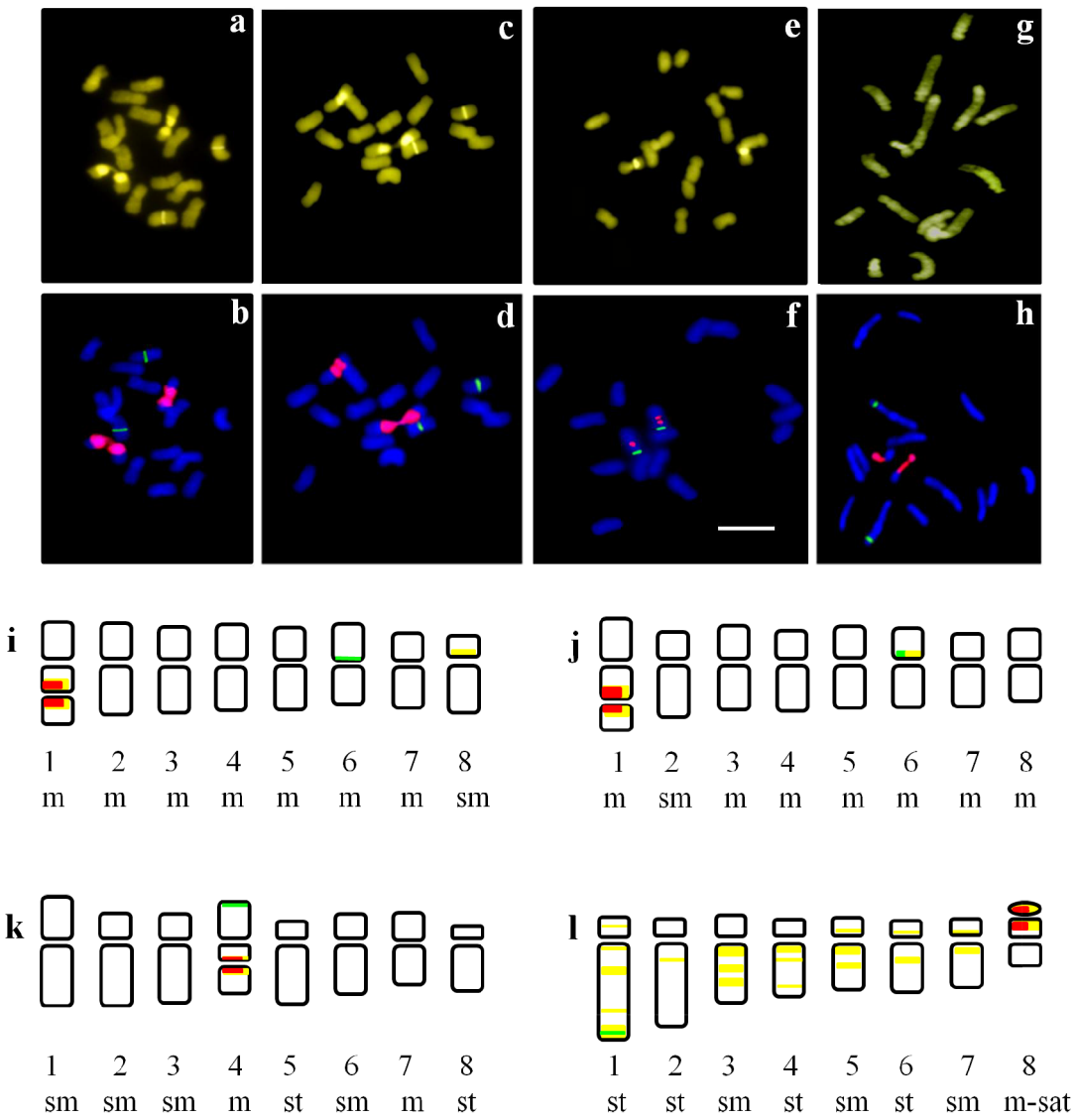


**Figure 3.** Karyotypes and idiograms of 4 *Astragalus* taxa. Chromomycin staining (yellow), in situ hybridization with 5S (green) and 35S (red) rDNA probes on DAPI (blue), and idiograms: *Astragalus armatus* subsp. *tragacanthoides* (a, b, i), *A. armatus* subsp. *numidicus* (c, d, j), *A. cruciatus* (e, f, k), and *A. pseudotrigonus* (g, h, l). Scale bar = 4 µm.

**Table 4.** Compilation of genome size values for the *Astragalus* species studied here and those retrieved from the literature.

| Taxon | Section | Origin | (2*n*) | 2C (pg) | 1C (pg) | 1C (Mbp) | Sources |
|---|---|---|---|---|---|---|---|
| *A. angulosus* DC. | *Incani* | Lebanon | N/A | 2.12 | 1.06 | 1037 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. angustifolius* Lam. | *Tragacantha* | Lebanon | 16 | 1.4 | 0.7 | 685 | Abdel Samad et al., 2014 |
| *A. angustifolius* Lam. subsp. *angustifolius* | *Tragacantha* | Lebanon | 16 | 1.56 | 0.78 | 763 | Abdel Samad et al., 2014 |
| *A. angustifolius* Lam. subsp. *biokovoënsis* Kusan | *Tragacantha* | Croatia | 16 | 1.45 | 0.73 | 714 | Siljak-Yakovlev et al., 2010 |
| *A. armatus* subsp. *numidicus* (Coss. & Dur.) Maire | *Poterion* | Algeria | 16 | 2.68 | 1.34 | 1311 | Present study |
| *A. armatus* subsp. *tragacanthoides* (Desf.)Maire | *Poterion* | Algeria | 16 | 2.71 | 1.36 | 1325 | Present study |
| *A. baalbekensis* Bornm. | *Rhacophorus* | Lebanon | N/A | 4.15 | 2.08 | 2029 | Abdel Samad et al.. 2014 |
| *A. barbatus* Lam. | *Stereothrix* | Lebanon | N/A | 1.9 | 0.95 | 929 | Abdel Samad et al.. 2014 |
| *A. berytheus* Boiss. | *Platyglottis* | Lebanon | 16 | 2.81 | 1.41 | 1374 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. bethlehemiticus* Boiss. | *Rhacophorus* | Lebanon | N/A | 4.5 | 2.25 | 2201 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. cedreti* Boiss. | *Dasyphyllium* | Lebanon | N/A | 4.99 | 2.5 | 2440 | Abdel Samad et al., 2014 |
| *A. cephalotes* Banks & Solander | *Macrophyllium* | Lebanon | N/A | 2.12 | 1.06 | 1037 | Abdel Samad et al., 2014 |
| *A. coluteoides* Willd. | *Anthylloidei* | Lebanon | 16 | 2.17 | 1.09 | 1061 | Abdel Samad et al., 2014 |
| *A. cruciatus* Link. | *Annulares* | Algeria | 16 | 1.39 | 0.7 | 680 | Present study |
| *A. cruentiflorus* Boiss. | *Rhacophorus* | Lebanon | N/A | 4.58 | 2.29 | 2240 | Abdel Samad et al., 2014 |
| *A. dictyocarpus* Boiss. | *Incani* | Lebanon | N/A | 1.71 | 0.86 | 836 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. drusorum* var. *maroniticus* Boiss. & Blanche | *Pterophorus* | Lebanon | 16 | 2.15 | 1.08 | 1051 | Abdel Samad et al., 2014 |
| *A. echinops* Boiss. | *Alopecuroidei* | Lebanon | N/A | 2.38 | 1.19 | 1164 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. ehdenensis* Mout. | *Dissitiflori* | Lebanon | 16 | 3.13 | 1.57 | 1531 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. ehrenbergii* Bunge | *Alopecuroidei* | Lebanon | 16 | 2.16 | 1.08 | 1056 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. emarginatus* Labill. | *Dasyphyllium* | Lebanon | 16 | 4.73 | 2.37 | 2313 | Abdel Samad et al., 2014 |
| *A. epiglottis* L. | *Epiglottis* | Lebanon | 16 | 0.92 | 0.46 | 450 | Abdel Samad et al., 2014 |
| *A. glycyphyllos* L. | *Glycyphyllos* | Slovenia | 16 | 1.5 | 0.75 | 734 | Temsch et al., 2010 |
| *A. gomboeformis* Pomel | *Astragalus* | Algeria | 16 | 1.66 | 0.83 | 812 | Our unpublished data |
| *A. gombo* Coss. & Dur. | *Astragalus* | Algeria | 16 | 1.56 | 0.78 | 763 | Our unpublished data |
| *A. gyzensis* Delile | *Annulares* | Algeria | 48 | 2.46 | 1.23 | 1203 | Our unpublished data |
| *A. gummifer* Labill. | *Rhacophorus* | Lebanon | 16 | 2 | 1 | 978 | Abdel Samad et al., 2014 |
| *A. hirsutissimus* DC. | *Dasyphyllium* | Lebanon | 16 | 3.97 | 1.99 | 1941 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. illyricus* Bernh. [*A. monspessulanus* L. subsp. *illyricus* (Bernh.) Chater] | *Incani* | Bosnia and Herzegovina | N/A | 2.11 | 1.055 | 1030 | Vallès et al., 2014 |
| *A. kurnet-es saudae* Eig | *Hololeuce* | Lebanon | N/A | 6.29 | 3.145 | 3076 | Abdel Samad et al., 2014 |
| *A. lepidanthus* Boiss. | *Rhacophorus* | Lebanon | N/A | 4.38 | 2.19 | 2142 | Abdel Samad et al., 2014 |

**Table 4.** (Continued).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *A. lusitanicus subsp. orientalis* Chater & Meikle | *Erophaca* | Lebanon | 16 | 1.78 | 0.89 | 870 | Abdel Samad et al., 2014 |
| *A. macrocarpus* DC. | *Astragalus* | Lebanon | N/A | 2.51 | 1.26 | 1227 | Abdel Samad et al., 2014 |
| *A. oleaefolius* DC. | *Macrophyllium* | Lebanon | 96 | 12.87 | 6.44 | 6293 | Abdel Samad et al., 2014 |
| *A. onobrychis* L. | *Onobrychoidei* | Bosnia and Herzegovina | 16 | 1.66 | 0.83 | 812 | Pustahija et al., 2013 |
| *A. pinetorum* Boiss. | *Caprini* | Lebanon | 16 | 2.02 | 1.01 | 988 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. pseudotrigonus* Batt. & Trab. | *Astragalus* | Algeria | 16 | 1.55 | 0.78 | 758 | Present study |
| *A. psilodontius* Boiss. | *Rhacophorus* | Lebanon | N/A | 2.83 | 1.415 | 1384 | Abdel Samad et al., 2014 |
| *A. schizopterus* Boiss. | *Incani* | Lebanon | 16 | 2.42 | 1.21 | 1183 | Bou Dagher-Kharrat et al., 2013; Abdel Samad et al., 2014 |
| *A. sofarensis* (J. Thiebaut) Podl. | *Macrophyllium* | Lebanon | 16 | 10.46 | 5.23 | 5115 | Bou Dagher-Kharrat et al., 2013 |
| *A. trichopterus* Boiss. | *Caprini* | Lebanon | N/A | 1.97 | 0.99 | 963 | Abdel Samad et al., 2014 |
| *A. vesicarius* L. subsp. *carniolicus* (A.Kerner) Chater | *Dissitiflori* | Croatia | 16 | 2.29 | 1.15 | 1125 | Siljak-Yakovlev et al., 2010 |
| *A. trifoliolatus* Boiss. | *Onobrychoidei* | Lebanon | N/A | 1.68 | 0.84 | 822 | Abdel Samad et al., 2014 |
| *A. zachlensis* Bunge | *Rhacophorus* | Lebanon | 28 | 4.5 | 2.25 | 2201 | Abdel Samad et al., 2014 |

1 pg = 978 Mbp according to Doležel et al. (2003).

at paracentromeric, intercalary, subtelomeric, and satellite positions, while the 3 remaining taxa possessed only 2 or 4 CMA bands (mainly colocalized with rDNA). Several authors pointed out the existence of relationships between heterochromatin (number of bands) and environmental parameters such as type of habitat, altitude, and longitude (Vosa and Stergianou, 1990; Bogunic et al., 2007). Such numerous CMA bands in *A. pseudotrigonus* could reflect an adaptation to high mountain environments under dry conditions. These variations can be interpreted as a response to selection pressures in ecological conditions where growing period is short, as shown by Vosa and Stergianou (1990). Heterochromatin variations could also be attributed to other mechanisms like tandem duplications (Greilhuber and Speta, 1976) or transposition events (Schubert and Wobus, 1985).

All these species present 2 signals (1 locus) for each of the 35S and 5S FISH probes. All 35S rDNA signals corresponded to bright and large $CMA^+$ bands. This seems to be a general feature, observed in numerous genera such as *Cedrus* Mill., *Picea* Mill., *Solanum* L., and *Hydrangea* L. (Bou Dagher-Kharrat et al., 2001; Siljak-Yakovlev et al., 2002; Srebniak et al., 2002; Mortreau et al., 2010) where GC-rich bands are linked to nucleolar organizing regions, where 35S rDNA is located (Siljak-Yakovlev et al., 2002).

The 5S rDNA sites were finer and more varied, distributed in paracentromeric, subtelomeric, or telomeric chromosome regions, either on the same chromosome as that bearing the 35S locus (*A. cruciatus*) or on separate chromosomes as for the other 3 taxa. In *A. armatus* subsp. *numidicus* and *A. pseudotrigonus*, 5S rDNA loci were adjacent to GC-rich regions. This type of colocalization has also been reported for some other genera: *Lilium* L. (Muratović et al., 2010) and *Coffea* L. (Hamon et al., 2009). The intensity of 5S signals was weaker in *A. cruciatus* and *A. pseudotrigonus* than in the 2 subspecies of *A. armatus*, suggesting differences in gene copy numbers (Maluszynska and Heslop-Harrison, 1991).

### 4.3. Nuclear DNA amount variation

The 2C DNA values found in the 4 studied taxa vary from 1.39 pg to 2.71 pg. According to the classification of Leitch et al. (1998), our investigated taxa have "very small" 1C values (<1.4 pg).

To date, less than 2% of all *Astragalus* species have been assessed for DNA amount (Siljak-Yakovlev et al., 2010; Temsch et al., 2010; Bou Dagher-Kharrat et al., 2013; Pustahija et al., 2013; Abdel Samad et al., 2014; Vallès et al., 2014), including the current 4 studied taxa plus 3 other taxa (unpublished data). Table 4 compiles all data and demonstrates that 2C values ranged from 0.92 pg for diploid *A. epiglottis* L. of section *Epiglottis* Bunge to 13.25 pg for highly polyploid (12*x*) *A. oleaefolius* DC. of section *Macrophyllium* Boiss. (Abdel Samad et al., 2014).

Comparison of values indicates variability of DNA amount within the same section, independently of geographical origin. For example, *A. pseudotrigonus*,

which belongs to the same section as *A. macrocarpus* DC., has a smaller genome, but there are no data about ploidy level for this species. This value is also very close to *A. angustifolius* Lam. of section *Tragacantha* Bunge (2C= 1.55 pg).

Variation of 2C DNA in a genus could result from 2 factors: genomic increase, caused by retrotransposition, and genomic decrease, induced by recombination and deletion (Piegu et al., 2006). Other mechanisms appear to have a small impact on genome size differences among closely related species. These include variation in intron size (Deutsch and Long, 1999), expansion of tandemly repeated DNA sequences (Morgante et al., 2002), accumulation of pseudogenes (Zhang, 2003), and transfer of organellar DNA to the nucleus (Adams and Palmer, 2003). Among our results, we observe interspecific but not intraspecific differences; the 2 subspecies of *A. armatus* possess the same 2C DNA value.

Some authors reported significant correlations between DNA content and environmental conditions (Lysák et al., 1999); others, on the contrary, detected no significant correlations (Mortreau et al., 2010). In our case, the sampling does not allow us to confirm or refute these hypotheses.

The present study provides novel cytogenetic data about *A. armatus* subsp. *tragacanthoides*, *A. armatus* subsp. *numidicus*, *A. cruciatus*, and *A. pseudotrigonus*, and shows variations in the morphometric data concerning their karyotypes, in the number and position of GC-rich DNA regions and in the physical mapping of 5S and 35S rRNA genes. Interspecific differences in genome size could be related to the geographical origin of studied taxa and populations. Further studies are planned, especially for species that grow in other geographical regions.

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