Karyotype traits in *Grindelia squarrosa* (Pursh) Dunal (Asteraceae), an invasive plant in Romania

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Abstract

The description of the karyotype features and idiogram in *Grindelia squarrosa* (Pursh) Dunal (Asteraceae), an invasive plant in Romania, are reported here for the first time. The diploid chromosome number is 2n = 2x = 12, in agreement with the data published for the other species of the genus. The karyomorphological data show that the complements of the studied genotypes have small chromosomes (mean chromosome length is $\bar{X} \pm SE = 2.56 \pm 0.10$ µm, and mean length of haploid complements is $\bar{X} \pm SE = 15.33 \pm 0.69$ µm, with a range of variability comprised between 12.87–17.51 µm). The karyotypes are made up of six pairs of metacentric and submetacentric chromosomes, with an identical formula of the diploid complement: KF = 2n = 12 = 8m+ 2sm + 2sm-SAT. Satellites are located on the short arms of the chromosomes of pair III. The karyotypes show a relatively high level of intra-specific uniformity as well as similar symmetry patterns (R = 1.29–1.53; TF% = 38.78–41.57%; AsI% = 54.54–57.61%; A1 = 0.24–0.32; A2 = 0.08–0.16), belonging to 1A and 2A classes of symmetry. The small size of the chromosomes, the presence of only two chromosome morphometric types, and the preponderance of metacentrics confer a relatively high degree of symmetry to the karyotypes studied.

Key words: Grindelia squarrosa, invasive plant, karyotype, mitotic chromosomes, satellites, asymmetry indexes.

Introduction

Grindelia Willd. is a genus with ca. 45 species in North America and 26 species in South America, comprising annual, biennial, perennial forbs or subshrubs widely distributed in xerophytic and halophytic areas (Baeza and Schrader, 2005; Deble and Oliveira-Deble, 2010). In 1804, *Grindelia* seeds from Mexico were brought to Europe (Royal Gardens – Madrid, Spain) and cultivated as *Aster spathulatus* Hort.; afterwards they were distributed to other botanical gardens (Steyermark, 1937).

The curlycup gumweed – *Grindelia squarrosa* (Pursh) Dunal (Brummitt and Powell, 1992), a common weed originating in the central prairies of North America is now largely spread over Eastern, Central and Western Europe (Russia, the Ukraine, Republic of Moldova, Estonia, Lithuania, Czech Republic, Belgium, Sweden, Latvia, Ireland) (Sirbu and Oprea, 2008). Although the species is most common in the lower elevations of plains and foothills, it was met, too, at 3000 m altitude in Colorado and New Mexico. In Northern Utah, it occurs in Tony Grove Canyon (2000 m) in the Cache National Forest, also on disturbed ground throughout the valleys and in the adjacent Wasatch Mountains at elevations of at least 2200 m (McDonough, 1975; Walsh, 1993). Reported for the first time in the flora of Romania in 1998 (Sirbu and Oprea, 1998), *G. squarrosa* can be considered an invasive alien plant in this country. Probably, the plant came into Romania by accident from the former USSR, carried across by goods or passenger trains. It was first identified in the ruderal areas on the sidetracks of the Socola-Iasi railway station. Meanwhile it invaded anthropic habitats both in the North-East and the South of the Moldavian Region of Romania (Sirbu and Oprea, 2011). At present, *G. squarrosa* is considered as being fully naturalized in Romania, it having an evident invasive tendency. Although in Romania the invasion of this species into agricultural crops or native plant communities has not occurred yet, this trend is not excluded in the future, given its behaviour in the neighbouring countries (Sirbu and Oprea, 2008, 2011). In the Ukraine and the Republic of Moldova, *G. squarrosa* is considered as a very aggressive plant, while for Spain it is noted as potentially invasive (Sanz Elorza *et al.,* 2001).

The invasive alien species, including curlycup gumweed, are seen as a major threat to the native biodiversity, ecosystem structure and conservation of the protected areas, thus causing damages to agriculture, forestry, fisheries and other human activities, and threatening human health (Stinson *et al.,* 2006). Except the aspect of its invasiveness – sometimes seen as harmful – *G. squarrosa* is one of the only two officinal plants in the genus *Grindelia* (the other is *G. robusta*) (Ghedira *et al.,* 2010). The chemical profile, represented by diterpenes (grindelic acid and its methylesthers), flavonoids (quercetin, kempferol), tannins (5.3%), vitamin P, resin, phenolic acids, and essential oils (0.3–0.5%), confers numerous medical and pharmaceutical valences to plant extracts and bio-preparations. Because of this complex chemical constitution, the extracts are valuable as stimulants, sedatives, astringents, purgatives, emetics, diuretics, antiseptics, and disinfectants and are used in the treatment of bronchial spasm, whooping cough, asthma, and rashes caused by poison ivy (*Toxicodendron radicans* L. Kunze), while the tinctures are useful for bladder and urethra infections (Johnson and Nichols, 1970; Bare, 1979).

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Curlycup gumweed is used as an ornamental plant due to the intense yellow colour of its flowers as well as of their persistence over a long period of time, even in poor and dry soils. Because of its deep and extensive root system and of its high ability to survive and grow under adverse conditions, curlycup gumweed is utilized for the rehabilitation of disturbed sites (http://www.fs.fed.us/database/feis/plants/forb/grisqu/all.html). The plants are unpalatable to livestock (the unpleasant taste is given by tannins, volatile oils, a bitter saponin, and resins) and they can readily absorb selenium from the soil, for which reason their presence is considered undesirable by many farmers (BARE, 1979).

In Romania, *G. squarrosa* is a biennial plant, with a deep and palar root (SIRBU and OPREA, 2008, 2011). In the first year of vegetation, it forms a rosette, and the following year many branched erect stems develop; the branches carry numerous yellow flower heads. At the flower base there are shiny, sticky bracts, curved downward (hence the name *squarrosa*). The leaves are alternate and oblong, tooth-edged, gland-dotted, and gummy. The fruits are pseudoachenes, without thistledown, and show a marked heteromorphism (SIRBU and OPREA, 2011). The plant shows a high reproductive capacity in Romania, counting up to 76,000 achenes per individual per year. The disc and ray achenes are morphologically distinct in this species, and they differ in germination rate (MCDONOUGH, 1975; SIRBU *et al.,* 2011) (*Figure 1*).

Since the taxonomic relationships in *Grindelia* genus have not yet been clarified completely, they are revised permanently (TADEY *et al.,* 2009; DEBLE and OLIVEIRA-DEBLE, 2010), this process giving reasons for getting thoroughly into chromosome analysis, by both classical and molecular methods. To reach a full impact, it is essential that the new exciting molecular findings should be fully integrated with the traditional cytogenetical data in order to clarify the taxonomy of *Grindelia* (STACE, 2000).

There are relatively few cytogenetical works on the *Grindelia* genus. This is unfortunate since both the chromosome numbers and the karyotype traits are important criteria in establishing the evolutionary patterns as well as in plant systematics. Nothing has been published on the karyomorphology of *G. squarrosa*.

Considering the invasive character of *G. squarrosa*, its pharmacological valences and the quasi-absence of data on its cytogenetics, the knowledge of detailed karyotype patterns in this species becomes a necessity, since only the diploid chromosome number is available in literature (PINKAVA and KEIL, 1977; LANE and HARTMAN, 1996). The deciphering of the genetic constitution is an essential prerequisite in establishing the species structure and polymorphism, its geographical distribution, systematics and evolution within the genus, at inter- and intra-specific levels.

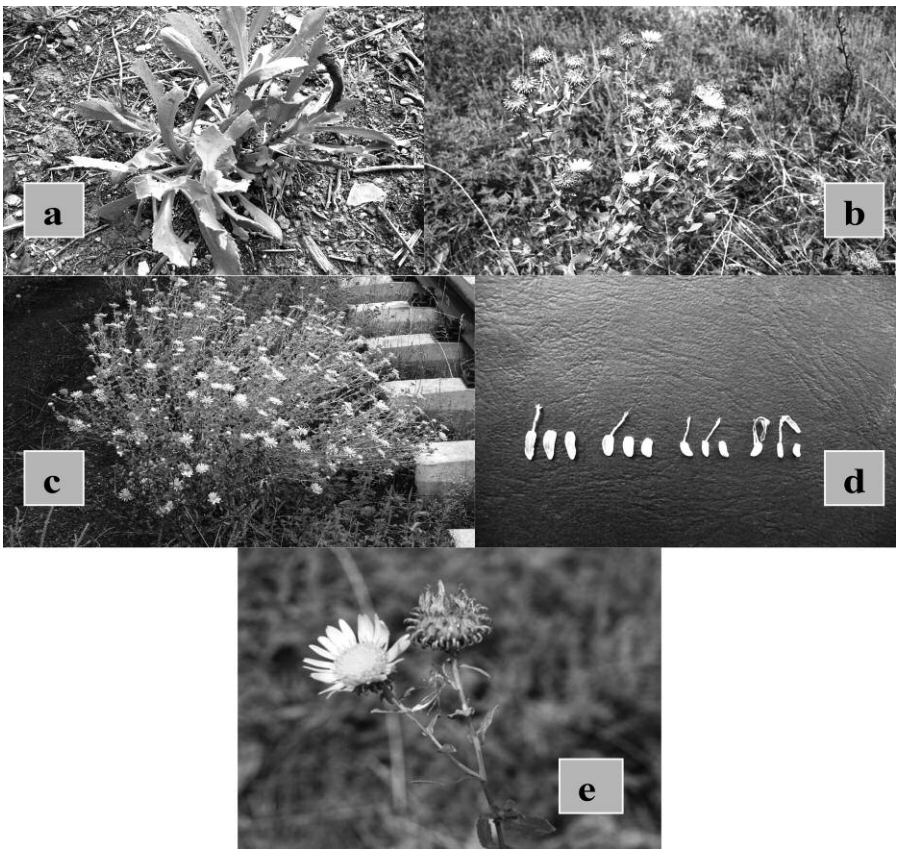

Figure 1. – *Grindelia squarrosa* (Pursh) Dunal: a, b, c – different phenophases; d. achenes; e. flower (photographer: SIRBU, 2011).

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Because *G. squarrosa* has not been studied yet from a karyomorphological point of view and because it is necessary to know whether the invasive character and the new habitat conditions of Romania have induced some changes at the level of the genetic material, the purposes of this paper are: (1) to establish the chromosome diploid number, (2) to detail the morphological traits of somatic chromosomes, (3) to construct the karyotypes, and (4) to construct the idiogram, providing thus valuable supplementary data on the chromosome constitution of *Grindelia* genus.

Material and Methods

Plant material

The cytogenetic investigations have been carried out on root tips of seedlings obtained by germinating seeds of *G. squarrosa* (Pursh) Dunal plants, collected from naturally growing populations from the Iasi – Socola area (N 47° 08' 23.63", E 27° 37' 02.14", Alt. 40 m.s.l.). Voucher specimens have been deposited in the Herbarium of the University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania (Herbar IASI 17915 – 17922).

Chromosome preparation

The germination took place at 22 °C, in the dark, on water-moistened filter paper in Petri glass dishes. At 10–15 mm in length, the root tips were pre-treated with 8-hydroxyquinoline (0.002 mol/L), for 4 h and were fixed in an ethanol-acetic acid mixture (3:l) for 24 h at room temperature. The samples were stored in 70% alcohol, at 4 °C, until required. For cytogenetic analysis, the root tips were hydrolyzed in 50% hydrochloric acid for 6 minutes and then stained in a modified carbol fuchsin solution (Gamborg and Wetter, 1975). The squashed preparations were produced in 45% glacial acetic acid. The microscopic investigation was carried out by using a Nikon Eclipse 600 microscope and the metaphases with well-spread chromosomes were photographed with a Cool Pix Nikon digital camera, 1600 x 1200 dpi, 100x objective. The chromosome metric determinations, the organization of chromosomes in karyotypes and the diagrammatic representation of chromosome traits in idiogram were carried out by using an Adobe Photoshop CS-3 software package.

Biometrics

Our chromosome measurements included the absolute length of individual chromosomes (CL), long arm length (L), short arm length (S), arm ratio, r (r = L/S), centromeric index, CI (CI = 100 x S/CL), length of the haploid complement (LHC), and the relative length of each chromosome (CL/LHC x 100). For each parameter were calculated the mean ($\bar{X}$), the standard error of the mean (SE), and the standard deviation (SD). $\bar{X} \pm$ SE is represented graphically. The chromosome designation followed the terminology recommended by Levan *et al.* (1964), while homology was assigned according to similarities in length, morphology, and centromere position, on the basis on CI and r values, respectively. In karyotypes, the chromosome pairs (I–VI) have been grouped in the descending order of their length. Average data for each karyotype – included in tables – are the results of the metric determinations realized on five metaphases/genotype.

Karyotype asymmetry

To evaluate the karyotype asymmetry, the following indexes were analyzed: TF%, AsI%, A1 and A2.

AsI% index (synonymous with *AsK%*) (Arano and Saito, 1980; Paszko, 2006) represents the ratio between the sum of long arm lengths of individual chromosomes and the haploid complement length: AsI% = (Σlong arms/haploid complement length) x 100.

The total form percent (TF%) (Huziwara, 1962) is expressed by the ratio between the total sum of short arm lengths of individual chromosomes and the haploid complement length: TF% = (Σshort arms/haploid complement length) x 100.

The intra-chromosomal asymmetry index (A_1) and the inter-chromosomal asymmetry index (A_2) were calculated according to Romero Zarco (1986) and Paszko (2006):

$A_1 = 1 - [\Sigma(b/B)/n]$, where b and B are the mean lengths of short and long arms of each pair of homologues, and n is the number of homologues. It measures the average position of the centromere in karyotype and ranges from 0 (completely symmetrical) to 1 (completely asymmetrical).

$A_2 = S_{CL}/X_{CL}$, where S_{CL} is the standard deviation of chromosome length and X_{CL} is the mean chromosome length for each genotype. It is a coefficient used to appreciate the heterogeneity of the chromosome length. Both indices (A_1, A_2) are independent of the chromosome number and size.

Stebbins's classification (1971), based on the frequency of chromosomes with arm ratio (r) higher than 2 and on the ratio between the lengths of the longest and the shortest chromosomes in the complement (R), was employed to establish the karyotype symmetry classes. The asymmetry increases from type 1 to type 4 (as the proportion of chromosomes with r > 2 increases) and from type A to type C (in relation to the size ratio between the largest and smallest chromosomes).

Idiograms of haploid complements were drawn by considering the mean values calculated for the analyzed karyotypes.

Results

The chromosome complements in the studied genotypes of *G. squarrosa* displayed a somatic diploid number of 12 small-sized chromosomes (*Table 1*), grouped in six pairs of homologues (I–VI) after the metric determinations of the cytogenetic parameters. The sizes of individual chromosomes vary between 3.44 µm (the longest chromosome pair of Gsq-1 genotype) and 1.81 µm (the shortest chromosome pair of Gsq-6 genotype), with a mean chromosome length/haploid complement ranging from 2.91 ± 0.10 µm (Gsq-1) to 2.17 ± 0.09 µm (Gsq-6). The mean value of the length of the haploid complements of the seven karyotypes is 15.33 ± 0.69 µm, with

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Table 1. – Karyotype features in *G. squarrosa* studied genotypes (all karyotypes show satellite to chromosome pair III).

genotype	KF	LHC (µm) $\bar{x}$ ±SE	CL (µm) $\bar{x}$ ±SE	CL (µm) Range	CI $\bar{x}$ ±SE	R	AsI %	TF %	A_1	A_2	SKC
Gsq-1	8m+2sm+2sm-SAT	17.51±0.26	2.91±0.11	2.62-3.50	41.42±3.48	1.31	54.88	41.57	0.24	0.10	2A
Gsq-1	8m+2sm+2sm-SAT	12.87±0.30	2.21±0.11	1.79-2.76	38.91±2.99	1.46	57.57	38.92	0.32	0.13	2A
Gsq-3	8m+2sm+2sm-SAT	16.94±0.23	2.82±0.13	2.19-3.22	39.05±3.07	1.34	57.61	39.07	0.31	0.11	2A
Gsq-4	8m+2sm+2sm-SAT	16.32±0.53	2.72±0.18	2.18-3.40	38.45±3.40	1.53	57.59	38.78	0.32	0.16	2A
Gsq-5	8m+2sm+2sm-SAT	14.75±0.17	2.45±0.08	2.04-2.73	41.26±3.59	1.29	54.54	41.24	0.24	0.08	2A
Gsq-6	8m+2sm+2sm-SAT	13.06±0.14	2.17±0.09	1.78-2.56	39.45±3.06	1.38	55.85	39.63	0.30	0.11	1A
Gsq-7	8m+2sm+2sm-SAT	15.88±0.30	2.64±0.10	2.25-3.09	41.95±3.47	1.34	54.72	41.30	0.25	0.09	2A

KF = karyotype formula, LHC = length of haploid complement, CL = chromosome length, CI = centromeric index, R = longest/shortest chromosome pair, AsI % = asymmetry index, TF % = total form percent, A_1 = intrachromosomal asymmetry index, A_2 = interchromosomal asymmetry index, SKC = Stebbins' karyotype classification; $\bar{X} \pm SE$ = mean ± standard error of the mean.

variations between 12.87 µm (Gsq-2) and 17.51 µm (Gsq-1). The arm ratio average values calculated per each genotype show variation from 1.14 ± 0.18 (Gsq-1) to 1.60 ± 0.20 (Gsq-4) with limits of variability ranging from r = 1.04 (Gsq-1) to 2.30 (Gsq-3). Two satellite-bearing chromosomes have been identified in all studied karyotypes. The secondary constrictions delimiting satellite bodies are present on the short arms of the submetacentric chromosomes of pair III. The mean size of the satellites is $\bar{X} \pm SE = 0.57 \pm 0.02$ µm. The small size of chromosomes and the high level of chromosome condensation sometimes hindered the identification of the structural details concerning the secondary constriction position and the presence of satellites.

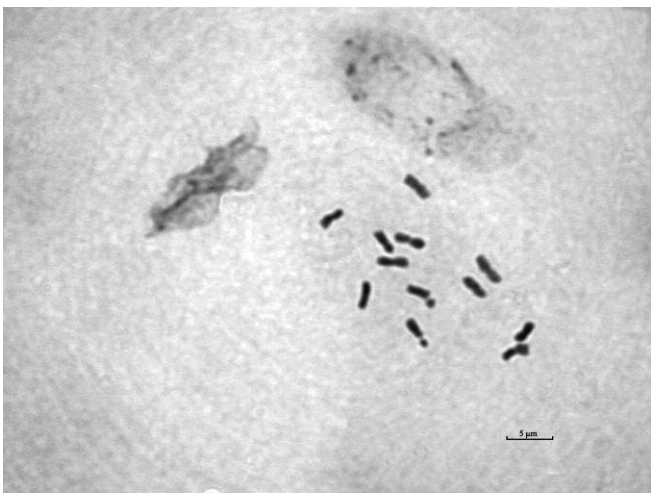

Figure 2. – Metaphase from *G. squarrosa* (2n = 12) – Gsq-1 genotype. Scale bar = 5 µm.

The karyotype formula of the diploid complements is identical: KF = 2n = 12 = 8m + 2sm + 2sm-SAT. If we consider the values of arm ratios and centromeric indexes, all the karyotypes have exclusively metacentric (m) and submetacentric (sm) chromosomes, the metacentrics being numerically prevalent (66.66%). Because of the karyotypes' resemblance, only metaphase and karyotype of Gsq-1 genotype are given here as photos (*Figures 2*, *3*).

As to the symmetry/asymmetry degree, considering that the numerical predominance of m and sm chromosomes of approximately the same size defines a symmetrical karyotype, the detailed analysis of the *G. squarrosa* karyotypes shows a relatively high level of intraspecific uniformity for all measured variables. The average centromeric index is $\bar{X} \pm SE = 40.07 \pm 0.53$ (limits of variability: 38.45–41.95), R ranges from 1.29 to 1.53, the values of AsI% are comprised between 54.54 and 57.61%, while TF% varies from 38.78 to 41.57%. The intra- and inter-chromosomal asymmetry indexes are also similar between genotypes and express the slight karyotype asymmetry. Thus, A_2 index indicates that there is little variation among the chromosome sizes in the studied karyotypes, while the data for A_1 index show no sharp inter-karyotype differences of chromosome arms.

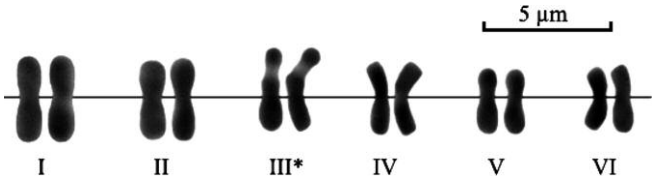

Figure 3. – Karyotype of *G. squarrosa* – Gsq-1 genotype (* satellite position). Scale bar = 5 µm.

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Table 2. – Average values of karyotype parameters of the seven *G. squarrosa* genotypes, calculated for idiogram construction (LHC = 15.33 ± 0.69 µm).

pair	type	total length (µm)			long arm (µm)			short arm (µm)			arm ratio		centromeric index		relative length (%)	
		$\bar{X}$ ±SE	range	SD	$\bar{X}$ ±SE	range	SD	$\bar{X}$ ±SE	Range	SD	$\bar{X}$ ±SE	SD	$\bar{X}$ ±SE	SD	$\bar{X}$ ±SE	SD
I	m	2.98±0.13	2.44–3.50	0.36	1.63±0.07	1.35–1.91	0.20	1.34±0.08	0.99-1.67	0.22	1.23±0.07	0.20	45.06±1.42	3.76	19.58±0.78	2.07
II	m	2.73±0.13	2.21–3.32	0.36	1.49±0.07	1.21-1.80	0.19	1.24±0.06	0.91-1.55	0.18	1.21±0.03	0.08	45.03±0.76	2.03	17.95±0.87	2.30
III	sm	2.61±0.11	2.15–3.04	0.31	1.39±0.07	1.05–1.79	0.21	0.64±0.03*	0.53-0.82	0.08	2.14±0.04	0.11	25.08±0.17	0.46	17.21±0.88	2.32
IV	m	2.48±0.10	2.00–2.79	0.29	1.42±0.08	1.11–1.87	0.23	1.06±0.05	0.80-1.34	0.20	1.39±0.14	0.39	42.69±2.38	6.32	16.32±0.70	1.86
V	m	2.33±0.10	1.93–2.72	0.27	1.31±0.07	1.00-1.64	0.19	1.02±0.06	0.68-1.34	0.18	1.33±0.12	0.33	43.58±2.15	5.68	15.35±0.63	1.68
VI	m	2.17±0.11	1.78–2.67	0.29	1.33±0.08	1.08–1.72	0.22	0.83±0.03	0.66-0.97	0.10	1.61±0.08	0.22	38.50±1.34	3.54	14.20±0.59	1.56

$\bar{X}$ ± SE = mean ± standard error of the mean; SD = standard deviation; * = satellite location

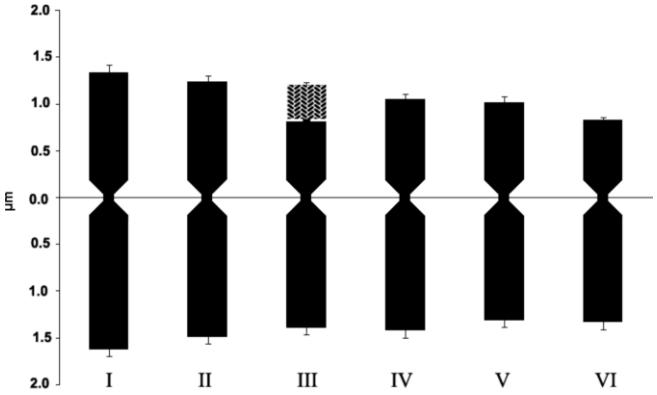

Figure 4. – Idiogram of haploid complement of *G. squarrosa*. Bars represent the standard errors of the mean length of long and short arms. Chromosome pairs are marked with Roman fonts.

If we consider the two parameters used by STEBBINS (1971) in order to appreciate the karyotype symmetry, we can see that six of the investigated genotypes (Gsq-1 – Gsq-5, Gsq-7) have chromosomes with R < 2, varying from 1.29 to 1.53, whereas the proportion of chromosomes with arm ratio (r) < 2 ranges in interval 0.99 – 0.51 (0.67 for Gsq-4 genotype, and 0.83 for the other ones), so that according to Stebbins' classification (1971), the *G. squarrosa* karyotypes are quoted as 2A. Gsq-6 karyotype (classified as 1A) has no chromosome with r > 2, but we should mention that the arm ratio in pair III is very close to 2.0 (1.96). Anyway, both categories (1A and 2A) reflect an increased level of karyotype symmetry and include primitive karyotypes. Although the differences are small, the comparative inter-karyotypic analysis could indicate that Gsq-2 and Gsq-4 karyotypes are slightly more asymmetrical than the other five because they have the greatest values of R and AsI% index, and the smallest TF% level. Additionally, Gsq-4 genotype has the highest proportion of chromosomes with an arm ratio greater than 2, which fact also confers it an increased asymmetry.

In order to construct the *G. squarrosa* idiogram, we used the average values of cytogenetic parameters determined for the seven karyotypes (*Table 2*).

The mean chromosome length calculated for the idiogram is $\bar{X}$ ± SE = 2.6 ± 0.1, and the average sizes of the longest (I) and shortest (VI) chromosome pairs are 2.98 ± 0.13 µm and 2.17 ± 0.11 µm, respectively. The relative chromosome length ranges between 14.20 ± 0.59% – 19.58 ± 0.78%. The final results stated the presence of only one chromosome pair with sub-median placed centromere (sm), namely the satellite-bearing chromosome pair (III). In the idiogram, the average data led to the establishment of the following karyotype formula for the haploid complement: n = x = 6 = 5m + 1sm-SAT (*Figure 4*).

Discussion

Taking into account the similarity of some morphological traits (shape of disc corollas, for example), the same chromosome number, and some molecular data (chloroplast DNA restriction site), the genus *Grindelia* is closely related to the genera *Isocoma* Nutt., *Olivaea* Schultz-Bip. Ex-Benth., *Rayjacksonia* Hartman & Lane, *Stephanodoria* Greene, and *Xanthocephalum* Willd. (LANE and HARTMAN, 1996; NESOM, 2000). However, adjustments of generic boundaries are to be made, and *Grindelia* is one of the genera from the Asteraceae family subjected to possible taxonomic revisions because some current proposals on systematic affinities and phylogenetic relationships remain controversial (BARTOLI and TORTOSA, 2003). A phylogenetic analysis of the *Grindelia* species based on molecular investigations (internal transcribed spacer nuclear sequence, micro satellite markers, fluorescence *in situ* hybridization with rDNA) is yet unsolved; only some clades have been recovered so far (BAEZA and SCHRADER, 2005; MOORE *et al.,* 2009). Therefore, any new scientific data, including those provided by classical cytogenetics, can contribute to the clearing of incompletely known aspects of the inter-specific relationships, especially as the available information on karyotype traits in the members of the *Grindelia* genus is relatively scarce.

Until now, the cytotaxonomic studies in the *Grindelia* species from South and Central America (Argentina, Chile, Bolivia, Southern Brazil, Paraguay and Uruguay) have indicated only the basic number (x = 6) and the somatic chromosome number (2n = 2x = 12) (BARTOLI and TORTOSA, 1998; TORTOSA *et al.,* 2000; BAEZA *et al.,* 2004;

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Baeza and Schrader, 2005). Few are the details concerning karyotype features (Bartoli and Tortosa, 2004; Baeza and Schrader, 2005). Both diploids (2n = 2x = 12) and tetraploids (2n = 4x = 24) have been reported only for *G. chiloensis* in South America (Bartoli *et al.,* 1990, 1993; Bartoli and Tortosa, 1998), while for North America, two levels of chromosome numbers were recognized in the *Grindelia* genus, namely 2n = 12 (diploid) and 2n = 24 (tetraploid) (*G. camporum* Greene) (Dunford, 1970, 1983). In *G. camporum* supernumerary chromosomes (0–3) have been evidenced while in *G. humilis* and *G. stricta* var. *platyphylla* aneuploids are sometimes present (Stiefkens *et al.,* 2011).

The present study shows that the investigated genotypes of *G. squarrosa* have exclusively 12 somatic chromosomes and our results confirm the basic chromosome number x = 6 and the diploid number 2n = 2x = 12, reported for other species of the genus (Lane and Hartman, 1996; Tortosa *et al.,* 2000; Baeza *et al.,* 2004; Bartoli and Tortosa, 2004; Baeza and Schrader, 2005).

If the data available in literature for different *Grindelia* species have shown constancy of the diploid number, some variation is displayed concerning the centromere position and chromosome morphotypes. For some *Grindelia* diploid species, the reported karyotype formula was KF = 2n = 12 = 10m + 2sm, with no specification concerning satellite position (Stiefkens *et al.,* 2011). A more diversified karyotype formula, compared to that of *G. squarrosa*, was established for two South American species – *G. pygmaea* and *G. coronensis* (Tortosa *et al.,* 2000; Bartoli and Tortosa, 2004) in which three pairs of metacentric chromosomes with median placed centromere, and three telocentric chromosome pairs are recorded: KF = 2n = 12 = 6m + 4st + 2st-SAT. In this case, the satellite is located on the smallest chromosome pair of the complement. Other two South American species (*G. anethifolia* and *G. prunelloides*), newly analyzed with molecular-cytological methods, have exclusively metacentric chromosomes: 2n = 12 = 10m + 2m-SAT, and symmetric karyotypes (AsI% = 55.46% and R = 1.27, 55.95% and R = 1.3, respectively) (Baeza and Schrader, 2005). In these species, the nucleolar organizing region (NOR) was detected by fluorescence *in situ* hybridization (FISH) with 18/25S rDNA in the satellite-bearing chromosome 2, but some specific signals were also identified by FISH with 5S rDNA in the short arms of the chromosomes 3 or 4, which are not significantly different in their length.

The results we obtained reveal that the seven karyotypes of *G. squarrosa* show low variation concerning chromosome morphometric features and that they display a high degree of symmetry. Consequently, *G. squarrosa* karyotypes can be considered as less evolved and less subjected to significant genetic restructurings during their evolution. The symmetrical karyotypes with small chromosomes (< 4 µm, according to Lima-de-Faria, 1980) and predominantly of the metacentric and submetacentric type are considered as being more primitive, little evolved, because they have not undergone significant structural changes and rearrangements during their evolution (Stebbins, 1971; Acosta *et al.,* 2005). Increasing asymmetry can occur either through the shifting of centromere position from median/submedian to terminal/subterminal, or through the accumulation of differences in the relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous (Paszko, 2006).

Stebbins (1971) stated that the tendency towards karyotype asymmetrization by the increase of the number of telocentric chromosomes to the detriment of the metacentric and submetacentric ones marks a progressive step in karyotype evolution, with repercussions on the evolution of the species.

The high symmetry and homogeneity of the karyotypes made difficult the detection of intra-specific differences, as reported for other species as well (Martinello and Schifino-Wittmann, 2003). The absence of basic chromosome numbers other than x = 6 in the *Grindelia* species suggests that the diversification at inter-specific level has occurred through the structural alteration of chromosomes rather than through numerical change. These conclusive data on the diploid chromosome number and on the resemblance of karyotype features in *G. squarrosa* with the other species of the genus additionally prove that the evolution has not been accompanied by large karyotypic changes, although small chromosomal rearrangements have certainly occurred (e.g., the number and position of 5S rDNA in the karyotype – Baeza and Schrader, 2005).

The results of this study contribute to the knowledge of chromosome constitution in *G. squarrosa*, an invasive plant in Romania, ensuring thus the enlargement of karyological databases for the *Grindelia* genus. Obviously, such classical cytogenetic investigations must be completed and correlated, in the future, with thoroughgoing advanced approaches such as CMA_3/DAPI banding, fluorescence *in situ* hybridization (FISH) or multicolour fluorescent *in situ* hybridization (McFISH), for identifying new molecular markers that could enable the unequivocal chromosome pairing, or the precise identification of the presence of nucleolar organizing regions as well as the formulation of an accurate view on the karyotype evolutionary trends in the Asteraceae family.

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