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Cytogenetics of new *Guizotia* Cass. (Compositae) interspecific hybrids pertaining to genomic and phylogenetic affinities

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Abstract. The following crosses were made using four recognized species/subspecies and a new population of *Guizotia*, referred to as Chelelu after the name of the locality in Ethiopia from which it was collected: *G. scabra* subsp. *schimperi* × Chelelu, Chelelu × *G. scabra* subsp. *scabra*, *G. zavattarii* × *G. arborescens* and Chelelu × *G. zavattarii* (all accessions with $2n = 30$). Plant morphology as well as mitotic and meiotic chromosome analysis confirmed the hybrid nature of the obtained progeny. At metaphase I of meiosis, the F_1 hybrid plants ($2n = 30$) showed a mean of about 95%, 31%, 63% and 0.50% of the pollen mother cells with 15 bivalents, and a mean of about 14.95, 13.75, 14.40 and 7.86 bivalents per cell, respectively. The respective mean pollen stainability was about 67%, 19%, 31% and 2%. From the results it was concluded that Chelelu is more closely related to *G. scabra* subsp. *schimperi* than to *G. scabra* subsp. *scabra* but more to the latter than to *G. zavattarii*. *Guizotia zavattarii* and *G. arborescens* are closely related to each other. Based on the cytological observations made, the probable basic chromosome number for the genus is discussed.

Key words: *Guizotia arborescens*, *G. scabra* subsp. *scabra*, *G. scabra* subsp. *schimperi*, *G. zavattarii*, Chelelu, hybrids, morphology, mitosis, meiosis, genomic and phylogenetic affinity.

Guizotia Cass. (Compositae) is a small African genus belonging to the family Compositae. According to its revised taxonomy (Baagøe 1974) the genus consists of the following species and subspecies: *G. abyssinica* (L. f.) Cass., *G. arborescens* I. Friis, *G. reptans* Hutch., *G. scabra* (Vis.) Chiov. subsp. *scabra*, *G. scabra* subsp. *schimperi* (Sch. Bip.) Baagøe, *G. villosa* Sch. Bip., and *G. zavattarii* Lanza (all with $2n = 30$). However, later studies have comprised new populations of *Guizotia* which do not exactly fit into any of the above recognized taxa. The two collections referred to as "Chelelu" and "Ketcha" populations ($2n = 30$), in Dagne (1995), are the case in point. They were discovered, identified as *Guizotia*, and named after the localities from where they were collected in Ethiopia by Dr. Mesfin Tadesse (National Herbarium, Addis Ababa University). The two populations hereafter will be referred to as Chelelu and Ketcha. The former is included in the present study.

Guizotia abyssinica is the only cultivated form. It is believed to have been domesticated in Ethiopia (Baagøe 1974, Hiremath and Murthy 1988), and today is cultivated mainly in Ethiopia and India for its seed oil. On a small scale, it is also grown in some other African and Asian countries (Seegeler 1983, Riley and Belayneh 1989, Getnet and Sharma 1996). The other members of the genus are

either wild or weedy plants (Baagøe 1974). All taxa of the genus, except *G. reptans*, have been recorded from Ethiopia which is probably the center of genetic diversity, if not the center of origin, for the genus (Baagøe 1974).

In spite of their smaller number, the phylogenetic relationships between the different taxa and the origin of the cultivated form are largely speculative (Baagøe 1974; Hiremath and Murthy 1988, 1992; Murthy et al. 1993; Dagne 1994, 1995). However, our understanding about the affinities between the different species has improved, as more and more data have become available. For instance, Dagne (1995) arranged six of the known taxa and the Chelelu and Ketcha populations into three groups on the basis of their relative karyotypic similarity. This was possible following the availability of adequate karyotypic information on these taxa and populations. The three groups are: (1) *G. abyssinica*, *G. scabra* subsp. *schimperi* and Chelelu, (2) *G. arborescens* and *G. zavattarii*, (3) *G. scabra* subsp. *scabra*, *G. villosa* and Ketcha.

Genome analysis by studying meiotic pairing in hybrids has markedly contributed to our increased knowledge of the genome constitution and phylogenetic relationships of many species. To date, there are some data available on the crossability, meiosis and pollen fertility in hybrids between some of the *Guizotia* taxa (Murthy et al. 1993, Dagne 1994). The results mainly indicated the close relationship between *G. abyssinica* and *G. scabra* subsp. *schimperi*, and between *G. scabra* subsp. *scabra* and *G. villosa*, thus corroborating the karyotypic data. However, these findings are neither complete nor enough to make any strong inference about the phylogenetic relationships between all taxa of *Guizotia*.

The objective of the present study was, therefore, to provide additional information on the meiosis and pollen fertility of *Guizotia* hybrids involving four recognized taxa and Chelelu, for which such data have not been available.

Materials and methods

Plant materials. The *Guizotia* taxa included in the study and the origin of the materials from Ethiopia are: *G. arborescens* – Omo-Neda track (33 km), in the vicinity of Jimma town; *G. scabra* subsp. *scabra* – Keffa, Jimma-Bonga road; *G. scabra* subsp. *schimperi* – Addis Ababa; *G. zavattarii* – Mega-Moyale road (2 km); Chelelu – Addis Ababa-Sendafa road (ca. 20 km). Four types of F_1 hybrids obtained from crosses between these materials are the object of the present study.

Crosses. The following four reciprocal crosses were attempted. *G. scabra* subsp. *schimperi* × Chelelu, Chelelu × *G. scabra* subsp. *scabra*, *G. zavattarii* × *G. arborescens* and Chelelu × *G. zavattarii*. Crosses were made by removing disk florets (with forceps) from flower heads that were ready to open within the next two or three days. After the disk florets were removed, the heads were protected by bagging. Flower heads of the male parents were also bagged before they flowered in order to avoid contamination. Hand pollination was made by rubbing freshly dehisced anthers, collected from the male parent, against the stigma of the pistillate ray florets when the latter were opened. Voucher specimens of the parental materials and the hybrids Chelelu × *G. scabra* subsp. *scabra* and *G. zavattarii* × *G. arborescens* are deposited in the National Herbarium, Addis Ababa University.

Plant morphology. An intermediate overall appearance compared to the parents was considered as a criterion of hybridity in the plants obtained from the crosses. The identification of certain paternal morphological characters in these plants was used as an additional evidence of hybridity. Plant growth habit, leaf shape, and number of ray florets were documented.

Somatic chromosome preparations. The methods used for air-dry chromosome preparations, aceto-orcein staining and C-banding, were those described in Dagne and Heneen (1992). The differentiation of specific paternal or both paternal and maternal chromosomes in the plants obtained from the crosses was used to confirm hybridity.

Meiotic chromosome preparations. Young flower heads (capitula) at the right stage of development, as judged from experience, were fixed in ethanol – chloroform – acetic acid (6:3:1) for about 24 h and stored in 70% ethanol at 4 °C until used. The capitula were then transferred from the

storing medium to Snow's carmine (Snow 1963) and allowed to stain for about a week or longer at room temperature. Pollen mother cells (PMCs) were released by mashing the disk florets with a forceps in a drop of 45% acetic acid on a glass slide. After removing the debris of tissues, the PMCs were squashed under a coverslip, and the preparation was made semi-permanent by sealing the edges of the coverslip with paraffin wax.

Pollen fertility. Fresh pollen grains from newly dehisced anthers were released in a drop of cotton blue lactophenol on a glass slide. A coverslip was added, and the pollen grains were allowed to stain from several hours to a day or longer, before they were scored for stainability. Fully stained pollen grains were scored as normal, and unstained or partially stained as aborted.

Results

Crosses. Hybrid plants were obtained from the four crosses indicated below, where the first parent was maternal and the second was paternal.

Two hybrid plants were obtained from *Guizotia scabra* subsp. *schimperi* × Chelelu crosses. The attempts to obtain viable hybrids from the reciprocal crosses were not successful, though hybrid seeds were obtained. Four such seeds did not terminate germination and died after the emergence of the radicle.

Only one plant was produced from Chelelu × *G. scabra* subsp. *scabra* crosses. Seeds from the reciprocal crosses were not able to germinate.

The present crosses between *G. zavattarii* × *G. arborescens* were the first ever attempted between these two species. Crosses in both directions produced seeds. A total of 38 (79.17%) out of 48 pollinated florets set seeds in the *G. zavattarii* × *G. arborescens* combination. In the reciprocal crosses 63 (73.26%) seeds were obtained out of 86 pollinated florets. Six plants were successfully grown from the former combination.

Two plants were produced from Chelelu × *Guizotia zavattarii* crosses. Attempts to germinate seeds obtained from the reciprocal crosses failed.

Morphology. In their overall morphological appearance the obtained hybrids were intermediate between their parents. In certain specific characters the hybrids looked like one or the other parent. The hybrids between *G. scabra* subsp. *schimperi* and Chelelu had an intermediate number of ray florets (mean 10.6 and range 9–13) compared to the pollen parent (mean 12.23, range 9–14) and the mother parent in which almost all heads had 8 florets. The hybrid plants between *G. zavattarii* and *G. arborescens* had a woody and shrubby growth habit like the pollen parent. Also, like the pollen parent, their leaves were petiolated. The petiole possessed narrow extensions of the blade along the lateral sides. The width of these extensions varied from leaf to leaf but generally they were wider than the extensions encountered only in some leaves of *G. arborescens*. The leaf morphology of the hybrid plants between Chelelu and *G. zavattarii* appeared intermediate between that of the two parents.

Somatic chromosomes. Somatic chromosome analysis was useful for confirming hybridity in those cases where it was possible to identify one or more chromosomes of the paternal parent or of both parents in the hybrid, with or without the help of C-banding (Dagne 1995). This was applicable only in two of the four hybrids obtained. Thus, in the hybrid plants between *G. zavattarii* and *G. arborescens* satellited chromosomes of both parental types could be identified by means of their C-bands (Fig. 1a). In some cells a few additional chromosomes of *G. arborescens* were identifiable by their telomeric C-bands of the long arm. Also the large m type chromosome of *G. zavattarii* (Dagne 1995) could easily be identified in the root tip metaphase chromosome preparations of the hybrid plants between Chelelu and *G. zavattarii*. (Fig. 1b).

Meiotic chromosomes. The hybrid plants were analyzed for chromosome pairing at metaphase I, presence of laggards and chromatin bridges at anaphase/telophase I and II, presence of microspores additional to the usual

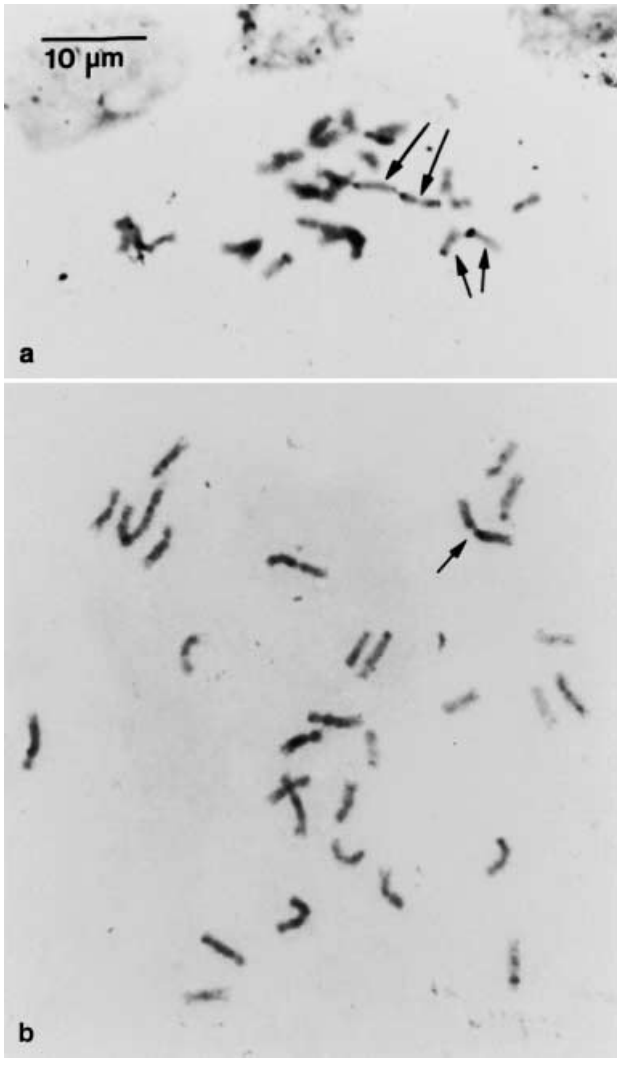

Fig. 1. Somatic metaphase chromosomes of two hybrids. **a** *G. zavattarii* × *G. arborescens* showing C-banded satellited chromosomes with the C-bands at one end (*G. zavattarii*, short arrows) or at both ends (*G. arborescens*, long arrows); **b** Chelelu × *G. zavattarii*, aceto-orcein stained chromosomes showing a large m type chromosome of *G. zavattarii* (arrow)

four at tetrad stage, and pollen stainability (Tables 1 and 2).

***Guizotia scabra* subsp. *schimperi* × Chelelu.** As is evident from Table 1, the mean number of bivalents in the two hybrid plants obtained was very high (14.95) since about 95% of the PMCs formed 15 bivalents (Fig. 2a). The remaining about 5% contained mainly 14 II + 2 I (Fig. 2b) and only a single PMC contained 13 II + 4 I.

Over 95% of the PMCs at anaphase/telophase I and II stages were without laggards or bridges (Table 2). When present, the number of laggards per PMC was usually 1 (Fig. 3a). Chromosome counts made on 40 of the analyzed PMCs at anaphase I showed 15:15 segregation in all. A total of 437 PMCs in the tetrad stage was examined for the presence of minimicrospores and micronuclei that might result from laggards. None of the analyzed PMCs showed these structures. About 65% and 70% of the pollen of plants 1 and 2, respectively, stained normally.

Chelelu × *G. scabra subsp. scabra*. The mean number of bivalents per PMC in the single hybrid plant obtained was 13.75, and about 31% of the PMCs contained 15 bivalents (Table 1). The remaining 69% of the PMCs contained varying numbers of bivalents and univalents ranging from 14 II + 2 I to 10 II + 10 I (Fig. 2c). However, the 14 II + 2 I type of PMCs was the most frequent (about

35% of total PMCs and about 50% of PMCs with univalents).

At anaphase/telophase I, laggards and bridges with fragments were observed in about 35% and 4% of the PMCs, respectively (Table 2). At anaphase/telophase II (Fig. 3b) the corresponding values were about 21% and 8%. At the tetrad stage, about 5% of the 170 PMCs examined contained additional minimicrospores, and about 32% contained micronuclei. Within a tetrad, micronuclei occurred in one or more microspores. The pollen fertility of this hybrid plant was very low. Only about 19% of the 1525 pollen grains scored looked normal. The rest remained unstained or partially stained.

***Guizotia zavattarii* × *G. arborescens*.** Six F_1 hybrid plants were analyzed from this cross. In general, most of the chromosomes formed bivalents as is evident from the mean bivalent value (14.40) for the different plants (Table 1, Fig. 2d). The frequencies of PMCs with 15 bivalents varied among the plants, amounting to 42–83%. If PMCs with 15 II and 14 II + 2 I are combined, the range would narrow to 71–96%, and it would further narrow to 90–99% if PMCs with 13 II + 4 I are included.

Laggards, bridges and fragments (Fig. 3c) were observed in a low frequency. In all the hybrid plants over 90% of the anaphase/telophase I PMCs did not manifest laggards or bridges (Table 2), and about 85–95% of the PMCs at anaphase/telophase II stage were free from laggards or bridges. At the tetrad stage only 0.76% and 1.73% of the observed PMCs contained minimicrospores and micronuclei, respectively. Pollen stainability ranged from about 23% to 45%, with a mean of about 31%.

Chelelu × *G. zavattarii*. Wide variations in bivalent and univalent numbers were observed in the two plants obtained (Table 1; Fig. 2e, f). PMCs with 11 II + 8 I to 4 II + 22 I were the most frequent. The mean number of bivalents per PMC was low (7.86) while that of univalents was high (14.25). PMCs with 15 II or apparently 30 univalents were rare and occurred at the same frequency.

Very high frequencies of PMCs with laggards and bridges were observed at anaphase/telophase I and II (Fig. 3d, e) amounting to 95% and 89%, respectively (Table 2). An unsual observation about these hybrids is the prolonged persistence of the nucleolus in the PMCs (Figs. 2e–f, 3d). Nucleoli were commonly detectable throughout the first meiosis, and in some PMCs they persisted until telophase II. Among a total of 156 PMCs analysed at the tetrad stage, about 39% had four microspores while the remaining 61% contained 5 to 8 microspores of various sizes (Fig. 3f). Micronuclei were also observed in one or more of the microspores in about 18% of the PMCs at the tetrad stage. The frequency of normal pollen was very low. Only about 5% such pollen was found in one and 0.4% in the other plant.

Discussion

Phylogenetic affinity between species can be inferred from the degree with which their chromosomes pair in the hybrids. Obviously, such an approach requires that the species in question are cross compatible and form viable hybrids. As shown by the present study as well as some previous ones (Murthy et al. 1993, Dagne 1994), it is possible to produce hybrids between several taxa of *Guizotia*. Thus, meiotic analysis is applicable for several combinations of *Guizotia* taxa in order to assess their genomic affinities.

The present results have shown different degrees of affinities between the different combinations of *Guizotia* taxa studied. Of these combinations, the highest genomic affinity was observed between *G. scabra* subsp. *schimperi* and Chelelu. This is evident from the almost regular meiosis manifested by the hybrid plants. The present result is comparable to what was observed in the hybrids between *G. scabra* subsp. *schimperi* and *G. abyssinica* (Murthy et al. 1993, Dagne 1994). Even though meiotic data are not available for *G. abyssinica* × Chelelu, it can be inferred from the present result and from the chromosome

Table 1. Frequencies of PMCs with various numbers of bivalents and univalents in the hybrid plants obtained

Cross	Plant	No of PMCs	Number and percentage (between parenthesis) of PMCs with various numbers of bivalents and univalents:															Mean
			15II 0I	14II 2I	13II 4I	12II 6I	11II 8I	10II 10I	9II 12I	8II 14I	7II 16I	6II 18I	5II 20I	4II 22I	3II 24I	2II 26I	0II 30I	II
*G. s.** subsp. *schimperi* × Chelelu	1	145	137 (94.48)	8 (5.52)	–	–	–	–	–	–	–	–	–	–	–	–	–	14.94
	2	84	81 (96.43)	2 (2.38)	1 (1.19)	–	–	–	–	–	–	–	–	–	–	–	–	14.95
		229	218 (95.20)	10 (4.37)	1 (0.44)	–	–	–	–	–	–	–	–	–	–	–	–	14.95
Chelelu × *G.s.* subsp. *scabra*	1	130	40 (30.77)	46 (35.38)	25 (19.23)	11 (8.46)	7 (5.38)	1 (0.77)	–	–	–	–	–	–	–	–	–	13.75
G. zavattarii × *G. arborescens*	1	113	47 (41.59)	43 (38.05)	20 (17.70)	3 (2.65)	–	–	–	–	–	–	–	–	–	–	–	14.19
	2	44	24 (54.55)	12 (27.27)	5 (11.36)	2 (4.55)	–	1 (2.27)	–	–	–	–	–	–	–	–	–	14.25
	3	280	221 (78.93)	42 (15.00)	15 (5.36)	1 (0.36)	1 (0.36)	–	–	–	–	–	–	–	–	–	–	14.72
	4	143	62 (43.36)	39 (27.27)	27 (18.88)	10 (6.99)	3 (2.10)	–	1 (0.70)	1 (0.70)	–	–	–	–	–	–	–	13.97
	5	104	86 (82.69)	14 (13.46)	3 (2.88)	1 (0.96)	–	–	–	–	–	–	–	–	–	–	–	14.78
	6	129	64 (49.61)	36 (27.91)	16 (12.40)	11 (8.53)	2 (1.55)	–	–	–	–	–	–	–	–	–	–	14.16
		813	504 (61.99)	186 (22.88)	86 (10.58)	28 (3.44)	6 (0.74)	1 (0.12)	1 (0.12)	1 (0.12)	–	–	–	–	–	–	–	14.40
Chelelu × *G. zavattarii*	1	231	–	2 (0.87)	6 (2.60)	10 (4.33)	31 (13.42)	31 (13.42)	28 (12.12)	37 (16.02)	36 (15.58)	25 (10.82)	14 (6.06)	6 (2.60)	3 (1.30)	1 (0.43)	1 (0.43)	8.36
	2	150	2 (1.33)	1 (0.67)	3 (2.00)	6 (4.00)	8 (5.33)	12 (8.00)	13 (8.67)	15 (10.00)	17 (11.33)	25 (16.67)	15 (10.00)	25 (16.67)	5 (3.33)	2 (1.33)	1 (0.67)	7.09
		381	2 (0.52)	3 (0.79)	9 (2.36)	16 (4.20)	39 (10.24)	43 (11.29)	41 (10.76)	52 (13.65)	53 (13.91)	50 (13.12)	29 (7.61)	31 (8.14)	8 (2.10)	3 (0.79)	2 (0.52)	7.86

*s = scabra

Table 2. Frequencies of PMCs without (normal) and with laggards and bridges at anaphase/telophase I and II in the hybrid plants obtained

Cross	Plant	Number and percentage (between parenthesis) of PMCs with meiotic aberrations								Pollen stainability (%)
		Anaphase/telophase I				Anaphase/telophase II				
		Normal	Laggard	Bridge	Total	Normal	Laggard	Bridge	Total	
*G. s.** subsp. *schimperi* × Chelelu	1	158 (97.53)	2 (1.23)	2 (1.23)	162					65.21
	2	186 (95.88)		8 (4.12)	194	162 (95.29)	2 (1.18)	6 (3.53)	170	70.11
		344 (96.63)	2 (0.56)	10 (2.81)	356	162 (95.29)	2 (1.18)	6 (3.53)	170	66.53
Chelelu × *G.s.* subsp. *scabra*	1	177 (61.46)	100 (34.72)	11 (3.82)	288	111 (71.15)	33 (21.15)	12 (7.69)	156	19.41
G. zavattarii × *G. arborecens*	1	247 (90.81)	19 (6.99)	6 (2.21)	272	30 (85.71)	3 (8.57)	2 (5.71)	35	33.02
	2	347 (95.59)	13 (3.58)	3 (0.83)	363	54 (94.74)	3 (5.26)		57	45.34
	3	127 (94.07)	7 (5.19)	1 (0.74)	135	154 (85.08)	25 (13.81)	2 (1.10)	181	22.71
	4	190 (90.91)	19 (9.09)		209	46 (92.00)	3 (6.00)	1 (2.00)	50	27.22
	5	106 (94.64)	4 (3.57)	2 (1.79)	112	65 (90.28)	7 (9.72)		72	29.37
	6	24 (92.31)	2 (7.69)		26					37.32
		1041 (93.20)	64 (5.73)	12 (1.07)	1117	349 (88.35)	41 (10.38)	5 (1.27)	395	31.39
Chelelu × *G. zavattarii*[a]	1 & 2	10 (4.90)	190 (93.14)	92 (45.10)	204	18 (11.25)	131 (81.88)	80 (50.00)	160	2.35

[a] Data of plants 1 and 2 are combined. 88 cells at anaphase/telophase I and 69 cells at anaphase/telophase II, containing both laggards and bridges, are counted twice (once under the 'laggard' column and once under the 'bridge' column); *s = scabra

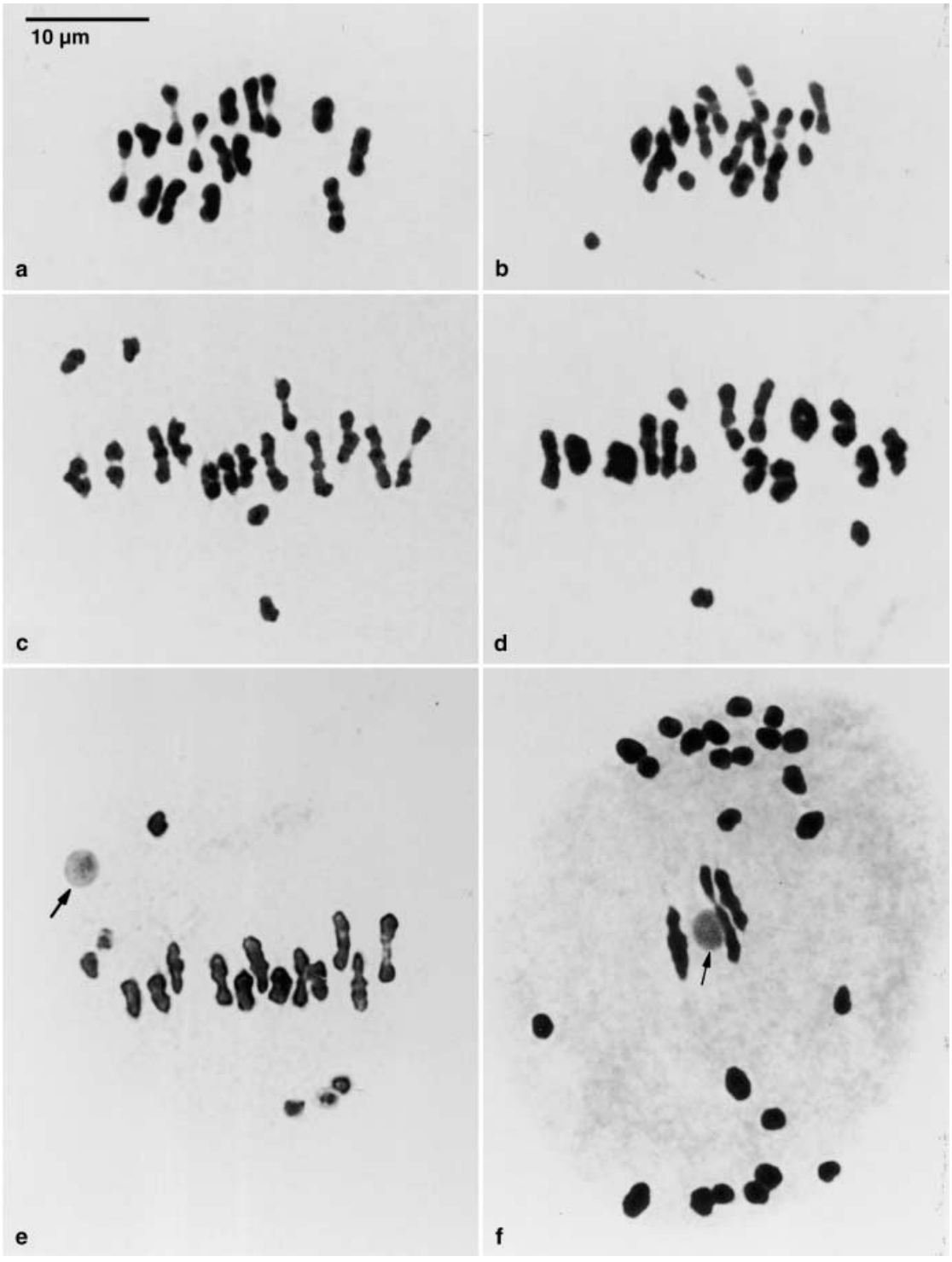

Fig. 2. Meiotic metaphase I PMCs of the hybrid plants. **a, b** *G. scabra* subsp. *schimperi* × Chelelu; **a** 15 II; **b** 14 II + 2 I; **c** Chelelu × *G. scabra* subsp. *scabra*, 13 II + 4 I; **d** *G. zavattarii* × *G. arborescens*, 13 II + 4 I (one pair of the univalents possibly constitute an oriented bivalent); **e, f** Chelelu × *G. zavattarii*; **e** 12 II + 6 I and a nucleolus (arrow); **f** 3 II + 24 I and a nucleolus (arrow)

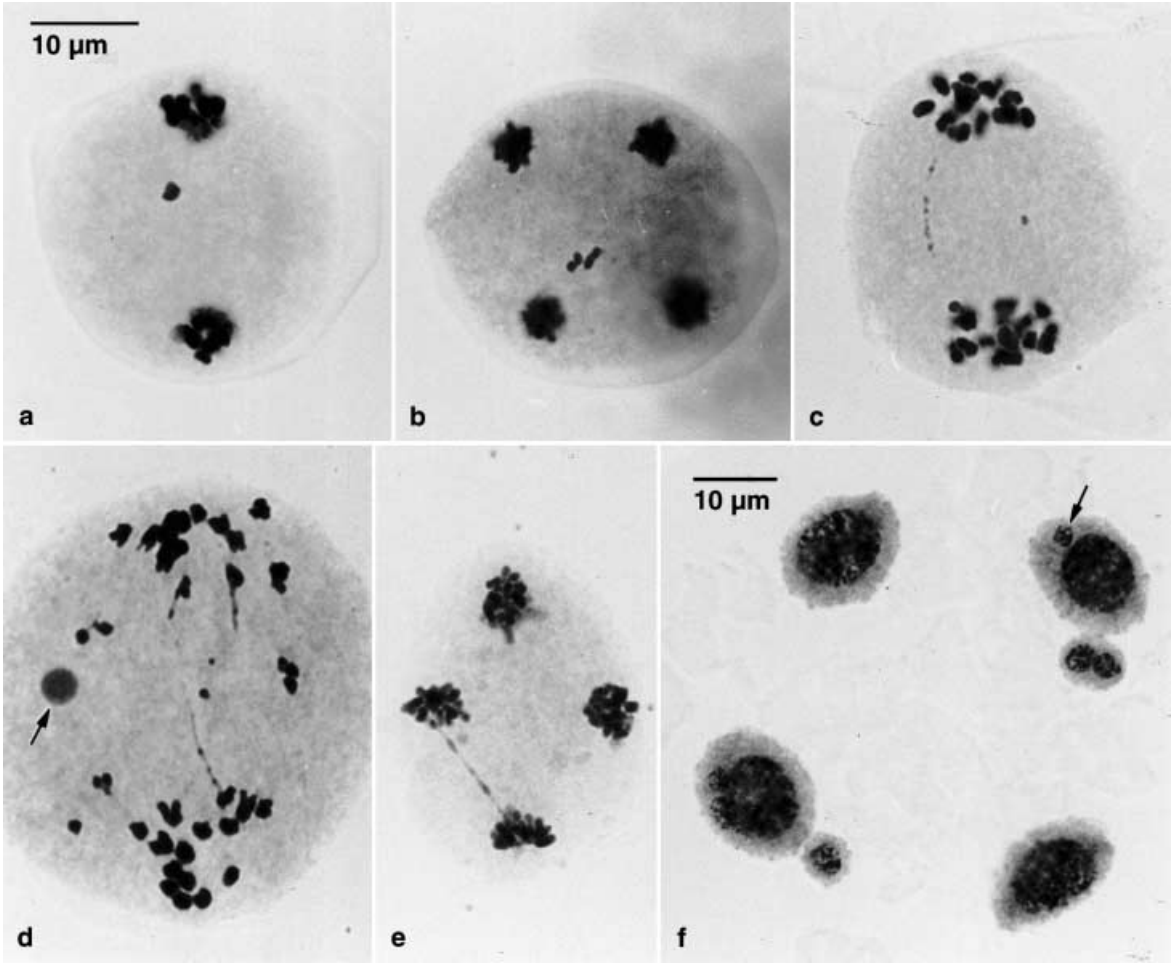

Fig. 3. Anaphase and telophase I and II and tetrad stage PMCs showing aberrations. **a** *G. scabra* subsp. *schimperi* × Chelelu, telophase I with a laggard; **b** Chelelu × *G. scabra* subsp. *scabra*, telophase II with two laggards; **c** *G. zavattarii* × *G. arborescens*, late anaphase I with a bridge and a fragment; **d–f** Chelelu × *G. zavattarii*; **d** anaphase I with laggards, bridges fragments and a persistent nucleolus (arrow); **e** telophase II with a bridge; **f** microspore stage showing four large and two minimicrospores with one of the large microspores containing a micronucleus (arrow); **a–e** the same magnification

morphology data (Dagne and Heneen 1992, Dagne 1995) that *G. scabra* subsp. *schimperi*, *G. abyssinica* and Chelelu are closely related to each other.

The second type of hybrid obtained was between Chelelu and *G. scabra* subsp. *scabra*. Although only one hybrid plant was available for analysis, the result might give some idea about the genomic affinity between the two parents. There was a considerable degree of homology between the chromosomes of the two parents, although it was not as high as in the preceding cross. Even though most of the PMCs at metaphase I contained univalents, the majority had only two univalents. Thus, chromosome pairing and bivalent formation takes place between most of the chromosomes (mean of 13.75 II), and only a few of them fail to do so. This is indicative of a high degree of homology between the genomes of the two *Guizotia* materials in question. However, comparing this with the preceding results shows that Chelelu is more related to *G. scabra* subsp. *schimperi* than to *G. scabra* subsp. s*cabra*. This

finding is in good agreement with the groupings of *Guizotia* taxa and new accessions on the basis of their karyotypic similarity by Dagne (1995).

Regarding the hybrid plants obtained from the *G. zavattarii* × *G. arborescens* cross, the frequencies of PMCs with 15 II varied from plant to plant, although the differences were caused largely by variations in the frequencies of PMCs that contained 2 or 4 univalents. The cause of the differences could be genetical, microenvironmental and/or sample size differences. The result shows a high degree of genomic affinity between the two species as indicated by the high mean values of the bivalents, 13.97 to 14.76. This high frequency of bivalent formation was observed irrespective of the fact that the 20 sm + st chromosomes of *G. zavattarii* are relatively more asymmetrical than the 20 m/sm and sm chromosomes of *G. arborescens* (Dagne 1995; m, sm and st indicate median, submedian and subterminal centromere positions according to Levan et al. 1964). It seems that these differences did not affect chromosome pairing in the hybrids. In general the results indicate that the genomes of the two species have a high degree of homology and that they are closely related. The present finding is thus concordant with the grouping of the two species (Dagne 1995). To be noted here is the high number of hybrid plants obtained and the lower number of bivalents observed in this cross combination compared to that between Chelelu × *G. scabra* subsp. *schimperi*. Thus the ease of obtaining hybrid plants does not need to imply a consequent close relationship and high chromosome pairing tendency. A significant fact to be taken into consideration in this context is seed dormancy. Even in nonhybrid seeds of various *Guizotia* taxa, germination failure was of common occurrence.

In the present study, the highest frequency of meiotic aberrations and the lowest pollen fertility were observed in the hybrids between Chelelu and *G. zavattarii* (Tables 1 and 2). The frequencies of PMCs with high numbers of bivalents were low. The frequent types observed were those with 4 II to 11 II, implying that mainly 10–20 chromosomes form bivalents while the remaining chromosomes rarely form bivalents. This indicates that of the *G. zavattarii* 15 chromosome types, 10 probably show homoeology to, and 5 differ from, Chelelu chromosomes. Chelelu and *G. zavattarii* are thus distantly related to each other, and by inference one is also distantly related to taxa that are closely related to the other. This means that *G. arborescens* which is closely related to *G. zavattarii* is distantly related to *G. abyssinica* and *G. scabra* subsp. s*chimperi* which are closely related to Chelelu.

The karyotypes of *G. zavattarii* and *G. arborescens* contain five pairs of m type chromosomes, which are not differentiable as a distinct group in the karyotypes of the other *Guizotia* materials, including that of Chelelu (Dagne 1995). From their morphological distinctness, it may be assumed that the m type chromosomes of *G. zavattarii* may rarely pair with Chelelu chromosomes in the hybrid. As to the origin of the five pairs of m type chromosomes, it may be assumed that during the course of evolution, these chromosomes were acquired independently by *G. arborescens* or *G. zavattarii*, or were rendered unrecognizable as a distinct group in the other taxa due to chromosome repatterning. If independent acquirement is the case, it would be assumed that the two species evolved through hybridization between $2n = 20$ and $2n = 10$ progenitors, the latter being the source of the five pairs of m type chromosomes. If this would be so, an independent hybridization of the $2n = 20$ progenitor, or another one similar to it, with a different progenitor of $2n = 10$ chromosome constitution would be assumed to originate the rest of the *Guizotia* taxa.

As shown in Dagne and Heneen (1992) and Dagne (1995), the chromosomes in the karyotypes of several *Guizotia* taxa can be arranged into distinct groups of five chromosomes or multiples of this. Thus, the karyotypes of *G. abyssinica* and Chelelu have the formula 10 m + 5 sm; that of *G. arborescens* 5 m + 5 m/sm + 5 sm; whereas that of *G. zavattarii* 5 m + 10 sm/st. This would tempt one to

venture further and suggest that currently known *Guizotia* species are probably ancient hexaploids with $2n = 6x = 30$.

Baagøe (1974) suggested the basic number $x = 5$ for the genus *Guizotia*. However, evidence was not presented. On the basis of the putative basic number $x = 9$, more than 30% of the Compositae species are polyploids (Solbrig 1977). Although the modal number of chromosomes for the family is 9, there are a number of species with $n = 5$ or less (Solbrig 1977). As a result, some workers favour $x = 5$ or 4, or both, and others favour $x = 9$ (Solbrig 1977, Turner 1977, Stuessy 1977). What has been suggested in this paper about the basic number and the evolution of chromosome number in *Guizotia* is in line with Baagøe's (1974) proposition of $x = 5$ for the genus, and the basic number $x = 5$ proposed for the family by some workers. It may be concluded that the available chromosomal data are only suggestive, not conclusive, about the probable basic number and the mechanism by which the chromosome number could have evolved.

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