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Origin, evolution and genome differentiation in *Guizotia abyssinica* and its wild species

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Abstract. *Guizotia abyssinica*, *G. schimperi* and *G. scabra* are diploid species (2n = 2x = 30) characterised by 15 bivalents during prophase-I/metaphase-I of meiosis. The former species is cultivated whereas the latter two are wild. Interspecific hybrids between these three species were generated and the F_1 hybrids were analysed to assess cytogenetic relationships and crop evolution within the genus *Guizotia*. Meiotic chromosome configurations at diakenesis/metaphase-I in the pollen mother cells of hybrids averaged $0.25_{I} + 14.60_{II} + 0.15_{IV}$ for *G. abyssinica* × *G. schimperi*, $0.05_{I} + 13.6_{II} + 0.14_{III} + 0.58_{IV}$ for *G. abyssinica* × *G. scabra*, and $0.8_{I} + 12.7_{II} + 0.08_{III} + 0.88_{IV}$ for *G. schimperi* × *G. scabra*. Based upon the results of our investigations we conclude that the genomes of *G. abyssinica* and *G. schimperi* are similar and homologous, whereas the *G. scabra* genome is only partially homologous to that of *G. abyssinica*/*G. schimperi*. Furthermore, the crop species *G. abyssinica* might have originated from *G. schimperi* through selection and cultivation; chromosome translocations appear to have played a significant role in the divergence and differentiation of these three species.

Key words: *Guizotia abyssinica* – Genome – Interspecific hybridization – Evolution – Karyotype

Introduction

The exclusively diploid (2n = 2x = 30) African genus *Guizotia* Cass. (tribe Heliantheae, family Asteraceae) comprises three annual and four perennial species (Hiremath et al. 1992). The genus is of economic value since one of the species, *G. abyssinica* (L. f.) Cass. (Common name: niger), is an edible oil seed crop cultivated in Ethiopia, Sudan, Uganda, Tanzania, Malawai and in India. Niger seeds are rich in oil content, and they carry about 25–60% edible oil (Weiss 1983; Seegler 1983). *G. schimperi* SCH. BIP. in WALP, *G. villosa* SCH. BIP. in WALP (both annual), *G. scabra* (Vis.) Chiov., *G. reptans* Hutch., *G. zavattarii* Lanza in Chiov et al. and *G. arborescens* I. Friis (all four perennial) are wild species endemic to East Africa, especially Ethiopia (Baagoe 1974; Hiremath et al. 1992).

Baagoe (1974) revised the taxonomy of the genus *Guizotia* and recognised six species within it. Based upon the results of her investigations Baagoe (1974) further proposed some evolutionary trends and phylogenetic relationships within the genus, suggesting the derivation of the niger crop from *G. scabra* most probably from *G. scabra* ssp. *schimperi*. We undertook a detailed cytogenetic analysis of this genus with two major objectives: (1) to clarify the origin and evolution of *G. abyssinica* and (2) to analyse the cytogenetical mechanisms underlying speciation and the differentiation patterns within and between the species of *Guizotia*. The present communication deals with origin and evolution of *G. abyssinica*, and the genome relationships between three of the species of *Guizotia* assessed on the basis of meiotic chromosome pairing and the fertility of the species and their F_1 hybrids.

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Table 1. Interspecific hybridization in *Guizotia*

Sl. no.	Cross	No. of florets pollinated	No. of F_1 hybrid seeds obtained	No. of F_1 hybrid seeds germinated	No. of seedlings reached to maturity	Percentage of cross-ability
1	*G. abyssinica* × *G. schimperi*	112	58	46	30	51.8
2	*G. schimperi* × *G. abyssinica*	96	38	17	11	39.6
3	*G. abyssinica* × *G. scabra*	120	19	8	5	15.8
4	*G. scabra* × *G. abyssinica*	192	30	–	–	15.6
5	*G. schimperi* × *G. scabra*	96	14	3	2	14.6
6	*G. scabra* × *G. schimperi*	192	11	–	–	5.7
7	*G. abyssinica* × *G. villosa*	116	4	3	–	3.4
8	*G. schimperi* × *G. villosa*	124	4	–	–	3.2
9	*G. villosa* × *G. scabra*	208	1	–	–	0.5
10	*G. abyssinica* × *G. reptans*	28	–	–	–	–
11	*G. schimperi* × *G. reptans*	124	–	–	–	–
12	*G. scabra* × *G. reptans*	180	–	–	–	–
13	*G. abyssinica* × *G. zavattarii*	168	–	–	–	–
14	*G. scabra* × *G. zavattarii*	84	–	–	–	–

Materials and methods

Plants of *Guizotia* species were raised from seeds collected from various germplasm banks and individual botanists (Murthy 1987). Herbarium specimens were confirmed by Dr. Charles Jeffrey, Royal Botanic Gardens, Kew, UK.

Guizotia species are highly cross pollinated, and self incompatible. Their disk florets are hermaphrodite whereas the ray florets are female. For hybridization all the disk florets were removed and the female ray florets were pollinated with the pollen of the desired male parent. Crosses were performed in various combinations involving six *Guizotia* species (see Table 1). However, F_1 hybrids identified on the basis of their morphological genetic marker characters, were recovered in only three combinations, namely, *G. abyssinica* × *G. schimperi*, *G. abyssinica* × *G. scabra* and *G. shimperi* × *G. scabra*. These were subsequently analysed for their meiotic behaviour and fertility. For meiotic study young capitula (or head inflorescence) were fixed in Carnoy's fluid (ethanol : acetic acid : chloroform = 6 : 3 : 1) and the pollen mother cells were stained in 1% acetocarmine (Hiremath and Murthy 1986). Pollen fertility was estimated on the basis of the stainability of pollen in a 1 : 1 acetocarmine glycerol mixture.

Results

Crossability

The number of interspecific hybridizations attempted, the number of F_1 hybrid seeds harvested, the number of F_1 seedlings reaching maturity, and the per cent crossability between the cultivated and wild species of *Guizotia* are presented in Table 1. Crosses were successful in only three combinations: (1) *G. abyssinica* × *G. schimperi* (both directions), (2) *G. abyssinica* × *G. scabra*, (3) *G. shimperi* × *G. scabra*.

Systematics

As is evident from their descriptions, *G. abyssinica*, *G. schimperi* and *G. scabra* are distinct species. Although Baagoe (1974) grouped *G. schimperi* and *G. scabra* as subspecies of *G. scabra*, viz., *G. scabra* (Vis.) Chiov. ssp. *schimperi* SCH. BIP. in WALP Baag. and *G. scabra* (Vis.) Chiov. ssp. *scabra* respectively, we have recently considered them to be independent species (Hiremath et al. 1992).

The F_1 hybrids raised in the present study were intermediate between both the parents in most quantitative characters. Whereas, a majority of the qualitative characters of the male parent were of the dominant type (Tables 2, 3 and 4).

Table 2. Morphological characters of *G. abyssinica*, *G. schimperi* and their F_1 hybrid

Character	*G. abyssinica*	F_1 hybrid	*G. schimperi*
Length of leaves	Long	Medium	Short
Width of leaves	Wide	Narrow	Narrow
Diameter of head	Large	Medium	Small
Length of outer involucral leaves	Long	Medium	Medium
Width of outer involucral leaves	Wide	Narrow	Medium
No. of disk florets	> 50	< 50	< 50
Tip of paleae	Non-pigmented	Purple pigmented	Purple pigmented
Glands on paleae	Sessile	Stalked	Stalked
Multicellular hairs on paleae	Absent	Present	Present
Length of achene	Long	Medium	Short
Seed weight	Heavy	Medium	Light

Table 3. Morphological characters of *G. abyssinica*, *G. scabra* and their F_1 hybrid

Character	*G. abyssinica*	F_1 hybrid	*G. scabra*
Habit	Annual	Perennial	Perennial
Height	Medium	Medium	Tall
Diameter of head	Large	Medium	Medium
Length of outer involucral leaves	Short not exceeding disk centre	Long exceeding disk centre	Long exceeding disk centre
Breadth of outer involucral leaves	Wide	Medium	Narrow
Number of outer involucral leaves	5	5	8
Number of ray florets	8	8	12
Paleae	5 nerved	3–5 nerved	3 nerved
Length of achene	Long	Medium	Short
Seed weight	Heavy	Medium	Light

Table 4. Morphological characters of *G. schimperi*, *G. scabra* and their F_1 hybrid

Character	*G. schimperi*	F_1 hybrid	*G. scabra*
Habit	Annual	Perennial	Perennial
Height	Medium	Tall	Tall
Oil drop deposits on leaves	Present	Absent	Absent
Number of outer involucral leaves	5	6–8	8
Length of outer involucral leaves	Not exceeding the disk centre	Not exceeding the disk centre	Long exceeding the disk centre
Width of outer involucral leaves	Wide	Medium	Narrow
Number of ray florets	8	8–12	12
Number of disk florets	< 50	> 50	> 50
Paleae	Purple pigmented	No pigmentation	No pigmentation

Cytogenetics

All the *Guizotia* species included in the present investigation are typical diploids with 30 chromosomes in their somatic cells and 15 bivalents in the pollen mother cells at diakinesis/metaphase-I of meiosis (Hiremath and Murthy 1992). Except for the sporadic occurrence of 2–4 univalents at diakinesis/metaphase-I and/or one or two lagging univalents at anaphase-I the meiotic divisions in *G. abyssinica*, *G. schimperi* and *G. scabra* were assessed to be normal, revealing respectively 15 bivalents in 97%, 94% and 92% of the pollen mother cells examined (Table 5, Fig. 1A–D).

Table 5. Meiotic chromosome configurations in three *Guizotia* species and their F_1 hybrids

Species/ hybrid	No. of cells analysed	Chromosome configuration			
		I	II	III	IV
		Mean (range)	Mean (range)	Mean (range)	Mean (range)
G. abyssinica	85	0.1 (0–2)	14.95 (14–15)	–	–
G. schimperi	110	0.08 (0–2)	14.95 (14–15)	–	–
G. scabra	98	0.25 (0–4)	14.87 (13–15)	–	–
G. abyssinica × *G. schimperi*	185	0.25 (0–4)	14.60 (12–15)	–	0.15 (0–1)
G. abyssinica × *G. scabra*	135	0.05 (0–1)	13.6 (11–15)	0.14 (0–2)	0.58 (0–2)
G. schimperi × *G. scabra*	102	0.08 (0–1)	12.7 (11–15)	0.08 (0–1)	0.88 (0–2)

Hybrid meiosis was slightly disturbed in the *G. abyssinica* × *G. schimperi* F_1. A mean chromosome pairing of $0.25_I + 14.60_{II} + 0.14_{IV}$ was observed. Nearly 81% of the pollen mother cells contained 15 bivalents (Fig. 1E), while about 14% of the pollen mother cells showed the presence of a single quadrivalent (Fig. 1F). Zero to four univalents were encountered in 5% of the cells (Fig. 1G). These univalents subsequently formed laggards in 12% of the cells at anaphase-I and anaphase-II. Consequently, micronuclei were observed in over 11% of the tetrads examined.

Meiosis in the F_1 hybrids of both *G. abyssinicia* × *G. scabra* and *G. schimperi* × *G. scabra* was abnormal and they respectively showed a mean chromosome pairing of $0.05_I + 13.6_{II} + 0.14_{III} + 0.58_{IV}$ and $0.08_I + 12.7_{II} + 0.08_{III} + 0.88_{IV}$ per cell. The different chromosome configuration observed are illustrated in Fig. 1I–P. Hybrids in both these combinations exhibited a similar meiotic behaviour though they differed slightly in the per cent and frequency of the different chromosome configurations. *G. abssinica* × *G. scabra* F_1s contained 15 bivalents in 43.4% of the cells, a single trivalent and a single univalent and/or two trivalents in 10% of the cells, and up to two quadrivalents in 46% of the cells. Nearly 30% of the cells exhibited lagging univalents at anaphase-I and anaphase-II stages and almost the same per cent of tetrads contained one-to-several micronuclei. In F_1 hybrids of *G. schimperi* × *G. scabra* 102 pollen mother cells were available for analysis; 54% of these showed 15 regular bivalents, with a single univalent and a trivalent in 8.6% of the cells and up to two quadrivalents in 38% of the cells. At the subsequent anaphase-I one to several laggards were seen in about

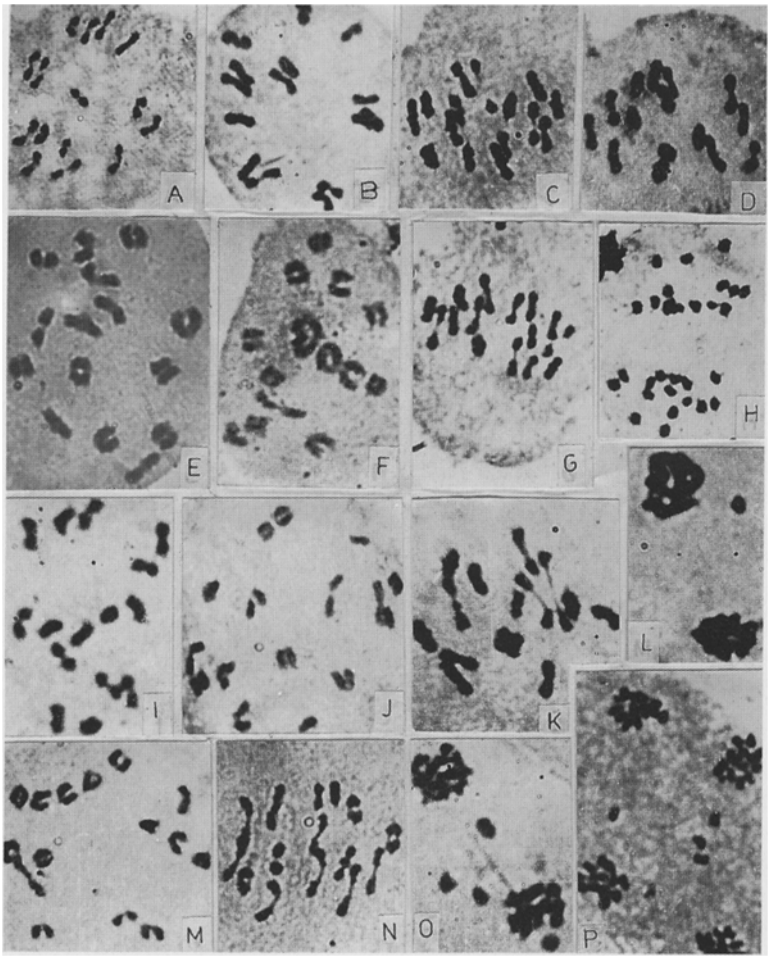

Fig. 1A–P. Meiosis in three *Guizotia* species and their F_1 hybrids. **A** *G. abyssinica*, MI, 15_{II}; **B** *G. schimperi*, MI, 15_{II}; **C** *G. scabra*, MI, $2_I + 14_{II}$; **D** *G. scabra*, MI, 15_{II}. **E–H** *G. abyssinica* × *G. schimperi* F_1 hybrid: **E** MI, 15_{II}; **F** MI, $13_{II} + 1_{IV}$; **G** MI, $2_I + 14_{II}$; **H** Anaphase-I, normal. **I–L** *G. abyssinica* × *G. scabra* F_1 hybrid: **I** MI, 15_{II}; **J** MI, $1_I + 13_{II} + 1_{III}$; **K** $11_{II} + 2_{IV}$; **L** Anaphase-I with a laggard. **M–P** *G. schimperi* × *G. scabra* F_1 hybrid: **M** MI, $13_{II} + 1_{IV}$;**N** MI, $11_{II} + 2_{IV}$; **O** Anaphase-I with laggards; **P** Telophase-II with laggards

24% of the 58 cells observed. Stages of meiosis-II were also found to be abnormal as evidenced by laggards at anaphase-II and micronuclei in tetrad cells.

Pollen fertility in *G. abyssinica* was nearly 80% whereas seed set was 70%. *G. schimperi* and *G. scabra* respectively showed 68% and 65% pollen fertility while the seed set recorded in both of them was around 60%. Pollen fertility and seed set for *G. abyssinica* × *G. schimperi* F_1 hybrids was 41% and 32% respectively. In the *G. abyssinica* × *G. scabra* F_1 hybrid, only 11% of the pollen was assessed as being fertile with an 8% seed set, whereas in *G. schimperi* × *G. scabra* F_1s, about 15% of the pollen was fertile and the seed set was nearly 10%.

Discussion

Several techniques and methodologies have been developed to investigate the plant genome – defined as the basic chromosome number with attendant genes (Jackson 1985) – and to determine its role in the genetic and evolutionary differentiation of plant taxa (Grant 1987; Greilhuber and Ehrendorfer 1988; Jauhar and Crane 1989). The classical method developed by Kihara (1930), that of observing the degree of chromosome pairing in the meiosis of interspecific F_1 hybrids, is regarded as the best and most extensively practised method to elucidate species/genome relationships (Kimber et al. 1981; Singh and Hymowitz 1985; Singh 1986; Wang 1988; Hiremath and Salimath 1992). In addition to yielding significant information regarding species relations the cytogenetic analysis of interspecific hybrids in a genus containing wild, weedy, and cultivated species may also be helpful in devising procedures for the transfer of desirable characters from wild to cultivated forms (Leggett 1983; Bothmer and Jacobsen 1986).

The genus *Guizotia* consists of seven species including an oil seed crop, *G. abyssinica*. Studies on the cytogenetics and species relationships in this important genus are of recent origin (Hiremath and Murthy 1992; Hiremath et al. 1992). Surprisingly, the origin and evolution of *G. abyssinica* remains unknown.

In the present study a detailed meiotic analysis of *G. abyssinica*, *G. schimperi* and *G. scabra* revealed 15

regular bivalents at diakinesis/metaphase-I. Although Hiremath and Murthy (1992) recorded quadrivalent formation in some populations of *G. scabra* and *G. abyssinica*, none of the species accessions examined in the present paper showed multivalent formation. A couple of accessions of *G. scabra* are also known to carry B-chromosomes (Hiremath and Murthy 1986) but those used in the present study were devoid of such chromosomes. In the *G. abyssinica* × *G. schimperi* F_1s nearly 81% of the pollen mother cells at diakinesis/metaphase-I contained 15 regular bivalents. Furthermore, the F_1s exhibited high pollen and seed fertility. The preponderance of bivalent formation and high fertility in the hybrid strongly suggests that the genomes of *G. abyssinica* and *G. schimperi* are basically similar and fully homologous. Thus *G. abyssinica* might have originated from *G. schimperi* through selection and further cultivation of a "large achened mutant". In other words humans would have selected for mutations which determined the differences, especially between the seeds/achenes, of *G. abyssinica* and *G. schimperi*. Based upon the results of our investigation, therefore, we propose a common genomic formula 'G' for these two taxa. Such a proposition finds additional support from the fact that the karyotypes of several collections of *G. abyssinica* and *G. schimperi* are alike and uniform, most notably in respect of similar chromosome size, absolute chromosome length, the type and number of SAT chromosomes and satellite size (Hiremath and Murthy 1992). This is true despite the fact that *G. abyssinica* and *G. schimperi* show a large difference in genome size with *G. abyssinica* having nearly 78% more DNA than *G. schimperi* (Hiremath et al. 1992). As evidenced by about 14% of pollen mother cells with a single quadrivalent in the F_1 hybrid plants, the genomes of *G. abyssinica* and *G. schimperi* have become distinguished, during the course of evolution, by a single reciprocal translocation.

G. abyssinica is cultivated in the African and Indian subcontinents whereas *G. schimperi* is a weed in Ethiopia despite the cultivation of *G. abyssinica* (Riley and Belayneh 1989). *G. abyssinica* and *G. schimperi* share several common morphological features (Baagoe 1974). Based upon evidence from morphology, phytogeography and cytogenetics, Hiremath and Murthy (1988) concluded that *G. abyssinica* was domesticated in Northern Ethiopia as early as 3000 B.C. and that the crop was introduced into India, probably through trade routes, prior to the Christian era. Baagoe (1974) recognised *G. abyssinica* and its companion weed *G. schimperi* as distinct species. She treated *G. schimperi* as a subspecies of *G. scabra*, i.e., *G. scabra* (Vis.) Chiov. ssp. *schimperi* (SCH. BIP. in WALP) Baag. Considering the differences in habit, distribution, karyotype, breeding behaviour, and genome size of ssp. *scabra* and ssp. *schimperi* we treated them as independent species (Hiremath et al. 1992). It is also amply clear from the present study that *G. abyssinica* and *G. schimperi* cross easily; their F_1 meiosis is nearly complete, the F_1s are highly fertile, and these two taxa share a common 'G' genome. Therefore, both *G. abyssinica* and *G. schimperi* are conspecific members of the same species, and form, or belong to, a common gene pool. Hence, *G. schimperi* may be an important gene source in the improvement of *G. abyssinica*.

In both *G. abyssinica* × *G. scabra* and *G. schimperi* × *G. scabra* the F_1 hybrid plant meiosis was irregular. Theoretically, if the genomes of *G. abyssinica*/*G. schimperi* and *G. scabra* are similar and homologous then in their F_1 hybrids one should observe 15 regular bivalents (GG), as seen in *G. abyssinica* × *G. schimperi* F_1s. On the other hand, if their genomes are completely nonhomologous the F_1 plants are expected to show 30 univalents (GH). From Table 5 it is quite clear that both *G. abyssinica* × *G. scabra* and *G. schimperi* × *G. scabra* the hybrid combinations showed a similar meiotic behaviour. None of the hybrid plants contained either 15 regular bivalents or 30 univalents in a majority of the pollen mother cells. The frequency of pollen mother cells with 15 regular bivalents observed in *G. abyssinica* × *G. scabra* and *G. schimperi* × *G. scabra* F_1s was respectively about 43% and 54%. From this chromosome pairing data we conclude that *G. abyssinica* and *G. schimperi* are almost equivalent in their relationship to *G. scabra*. In this sense the common genome (G) of *G. abyssinica* and *G. schimperi* is partially homologous to that of *G. scabra*. Thus *G. scabra* also belongs to the 'G' genomic category within the genus *Guizotias*. During the course of evolution, however, the *G. scabra* genome has undergone extensive differentiation, primarily through translocations, resulting in an asymmetric karyotype that is different from the symmetric karyotype of both *G. abyssinica* and *G. schimperi* (Hiremath and Murthy 1992). This is further substantiated by our observation of a high frequency of multivalents in *G. abyssinica* × *G. scabra* and *G. schimperi* × *G. scabra* F_1 plants and also a significant genome size difference between *G. scabra* (2C DNA = 5.26 pg). *G. abyssinica* (7.57 pg) and *G. schimperi* (4.25 pg) (Hiremath et al. 1992). Finally, we assign a genomic symbol 'G_e' for the differentiated *G. scabra* genome. The poor fertility of *G. abyssinica* × *G. scabra* F_1s indicates that *G. scabra* may not be of potential value for the genetic improvement of *G. abyssinica*.

The genomic designation of the remaining species of *Guizotia*, viz., *G. reptans*, *G. villosa*, *G. zavattarii*, and their potential utility in a crop improvement programme could not be ascertained as they did not yield viable F_1s with *G. abyssinica* or in any other combination.

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