Genome Relations among Octaploid Species of *Sesamum* L. (Pedaliaceae)

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Summary Genome relations among 3 octaploid species of *Sesamum* namely *S. radiatum*, *S. occidentale* and *S. schinzianum* were analyzed. These 3 African wild species exhibited normal 32 bivalents at diakinesis and metaphase-I and showed high degree of pollen fertility and seed set. In *S. radiatum*×*S. occidentale* F₁ hybrids, mean chromosome pairing of 0.81_I+31.6_{II} per cell was found. About 86% of the pollen mother cells revealed 32 bivalents formation. This suggests that genomes of *S. radiatum* and *S. occidentale* are similar and homologous. Both these parental species are morphologically similar. F₁ and F₂ progenies are highly fertile. Based on these evidences it is proposed that *S. occidentale* be treated as a variety of *S. radiatum*. Mean chromosome pairing of 0.154_I+31.79_{II}+0.11_{IV} per cell was found in *S. radiatum*×*S. schinzianum* hybrid. About 92% of the PMCs revealed 32 bivalents. This pairing behaviour indicates that genomes of *S. radiatum* and *S. schinzianum* are similar and homologous. These 2 species have diverged and differentiated by 2 translocations as evident from 2 quadrivalent formation in the hybrid. It is proposed that *S. radiatum* and *S. schinzianum* have descended from a common ancestral octaploid species. Isolating barriers preventing gene flow between species in nature is discussed.

Key words Genome homology, Hybridization, *Sesamum radiatum*, *Sesamum occidentale*, *Sesamum schinzianum*.

Genome homologies and differentiation in genus *Sesamum* is poorly understood. This genus of Pedaliaceae with 30 species exhibits 2 basic chromosome numbers, $x=8$ and 13 (Stapf 1906, Bruce 1953, Merxmüller 1968, Ihlenfeldt and Scidensticker 1968, 1979, Kobayashi 1993). Oil seed crop sesame *S. indicum* Linn., *S. mulayanum* Nair., *S. alatum* Thonn., *S. capense* Burm., *S. triphyllum* Welw. ex. Aschers. and *S. angustifolium* (Oliv) Engl. are diploid on $x=13$ base number. In contrast, *S. laciniatum* Klein., *S. angolense* Welw., *S. latifolium* Gillett. and *S. prostratum* Retz. are tetraploid based on basic chromosome number of $x=8$. On this base number *S. schinzianum* Aschers., *S. radiatum* Schumacher and *S. occidentale* Heer and Regel. are octaploids. Chromosome counts are unknown in 17 species of *Sesamum*.

Cytogenetics of genus *Sesamum* has been reviewed by Joshi (1961), Kobayashi (1993) and Hiremath and Patil (1999). Not much work has been done on the cytogenetics of genus *Sesamum*. Genome relations and differentiation in *Sesamum* species is not well understood. Present work was undertaken to establish genome relationships in octaploid species *S. radiatum*, *S. occidentale* and *S. schinzianum* through morphological and cytological analysis of hybrids and assessing their fertility.

Material and method

Seed material of *S. radiatum*, *S. occidentale* and *S. schinzianum* were obtained from Dr. Daniel Abbiw, Botany Department, University of Ghana, Legon, Ghana, West Africa., Tamil Nadu Agri-

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culture University, Coimbatore, India., and Dr. Kyojiro Masuda Department of Biology, Toyama University, Toyama, Japan respectively. Plants were raised in the experimental garden of the department. The identification of these species were confirmed by Royal Botanic Garden, Kew (U.K.). One set of vouched specimen has been filed at the above herbarium and another set is available in the herbarium, Department of Botany, Karnatak University, Dharwad, India. Flower buds destined to open next day morning were emasculated in the previous evening and bagged. Pollen from desired male parent was collected and dusted on the stigmatic surface of the female parent between 7 a.m. to 8 a.m. Pollinated flowers were bagged and labelled. Capsule formation and its growth indicate the success of the cross and the same was inspected after 5 to 6 d after pollination. All the crossing data like number of flowers pollinated, number of fruit sets *etc.* were carefully recorded.

For cytological analysis, flower buds were fixed in Carnoy's fluid and PMCs were stained with 1% acetocarmine. The slides were made permanent using Celariar's (1956) butyl alcohol schedule and mounted in euparal. Chromosome analysis was done from permanent slides. Photomicrographs were taken under Carl Zeiss standard GFL research photomicroscope using Kodak 2415 Technical Pan film. Pollen fertility was estimated on the basis of stainability of pollen in Muntzing's solution.

Results

Crossability

Large number of crosses were made among octaploid species of *Sesamum* in various combinations (Table 1). Only *S. radiatum*×*S. occidentale*, *S. radiatum*×*S. schinzianum* and their reciprocal crosses were successful and these hybrids have been analyzed.

Fruit set was not low in both way crosses. In *S. radiatum*×*S. occidentale* cross, from 18 hybrid capsules were yielded 704 seeds. Out of these only 474 seeds germinated and 45 of them reached to maturity. In the reciprocal cross, about 812 hybrid seeds were extracted from 24 capsules and 451 seeds germinated and about 41 seedlings reached to maturity. Hybrids were confirmed by morphological genetic markers. F₁ hybrids were selfed to get F₂ progeny.

In *S. radiatum*×*S. schinzianum* cross, from 20 hybrid capsules were yielded 760 seeds. Out of them 390 seeds germinated and 9 seedlings reached to maturity. In the reciprocal cross about 828 hybrid seeds were extracted from 23 hybrid capsules. Out of which only 31 seeds germinated and none of them reached to maturity. Majority of the F₁ hybrid seeds were shriveled but were nonviable.

Characterization of hybrids

In *S. radiatum*×*S. occidentale* cross, the female parent, *S. radiatum* is erect with basal branching, has opposite phyllotaxy, leaf margin being serrate, extrafloral nectaries well developed and browned in colour. The corolla is light violet. The capsule is broad oblong and the seed is gray. The

Table 1. Inter species crossability data among octaploid species of *Sesamum*

Sl. No.	Combinations	No. of flowers pollinated	No. of hybrid capsules obtained	No. of seeds obtained	crossability %	No. of hybrid seeds germinated	No. of seedlings reached to maturity
1	<i>S. radiatum</i> × <i>S. occidentale</i>	44	18	704	41	474	45
2	<i>S. occidentale</i> × <i>S. radiatum</i>	59	24	812	41	451	41
3	<i>S. radiatum</i> × <i>S. schinzianum</i>	52	20	760	38	390	9
4	<i>S. schinzianum</i> × <i>S. radiatum</i>	58	23	828	40	31	—
5	<i>S. schinzianum</i> × <i>S. occidentale</i>	75	20	100	27	—	—
6	<i>S. occidentale</i> × <i>S. schinzianum</i>	60	21	98	35	—	—

male parent, *S. occidentale* has erect habit with top branching. Leaf position mixed and leaf margin is entire, nectaries well developed and browned in colour. Corolla is white, seed is black. Hybrid resembled female parent with respect to basal branching and violet corolla. The F_1 hybrids are intermediate with regard to most of the quantitative characters. The hybrid resembled their male parent with respect to mixed phyllotaxy, entire leaf margin and black seed coat. Comparative morphologi-

Table 2. Morphological characters of *S. radiatum*, *S. occidentale* and their F_1 hybrid

Characters	<i>S. radiatum</i>	F_1 hybrid	<i>S. occidentale</i>
Branching habit	Erect, basal branching	Erect, basal branching	Erect, top branching
Leaf position	Opposite	Mixed	Mixed
Leaf margin	Serrate	Entire	Entire
Corolla colour	Violet	Violet	White
Capsule	Broad oblong	Narrowly oblong	Narrowly oblong
Seed coat colour	Gray	Black	Black

Figs. 1–9. 1) Flowering twig of female parent *S. radiatum*. 2) Flowering twig of F_1 hybrid. 3) Flowering twig of male parent *S. occidentale*. 4) MI showing 32 bivalents in *S. radiatum*. 5) MI showing 32 bivalents in *S. occidentale*. 6) MI showing $30_{II} + I_{IV}$ in *S. occidentale*. 7–9) Meiosis in F_1 hybrid. 7) MI showing 32 bivalents. 8) MI showing $10_I + 27_{II}$. 9) MI showing $6_I + 29_{II}$.

cal characters of parents and their hybrids are given in Table 2 and Figs. 1–3.

In the cross *S. radiatum* × *S. schinzianum*, both the parental taxa are distinct species. Female parent, *S. radiatum* has elliptical leaves with serrate margin, white corolla with violet shading and brown dots on the inner surface. Capsule is broadly oblong and seed is gray with rough seed coat. In male parent *S. schinzianum*, lower leaves are ovate with serrate margin and corolla is light violet with purple dots and streaks on the inner surface of the corolla. The capsule is narrowly oblong and

Table 3. Morphological characters of *S. radiatum*, *S. schinzianum* and their F_1 hybrid

Characters	<i>S. radiatum</i>	F_1 hybrid	<i>S. schinzianum</i>
Lower leaf shape	Elliptical	Ovate	Ovate
Corolla colour	White with violet shade	Violet with purple dots on inner surface	Light violet with purple dots
Capsules	Broadly oblong	Narrowly oblong	Narrowly oblong
Seed coat colour	Gray	Black	Black

Figs. 10–18. 10) Flowering twig of female parent *S. radiatum*. 11) Flowering twig of F_1 hybrid. 12) Flowering twig of male parent *S. schinzianum*. 13) MI showing 32 bivalents in *S. radiatum*. 14) MI showing 32 bivalents in *S. schinzianum*. 15–18) Meiosis in F_1 hybrid. 15) MI showing 32 bivalents. 16) MI showing $2_1 + 27_{II} + 2_{IV}$. 17) MI showing $2_1 + 29_{II} + 1_{IV}$. 18) MI showing $4_1 + 30_{II}$.

the seed is black in colour. The hybrids resembled male parent with regard to ovate lower leaf, serrate margin, violet coloured corolla, narrowly oblong capsules and black coloured seed. Hybrid is intermediate for most of the quantitative characters. Comparative morphological characters of parents and their hybrids are given in Table 3 and Figs. 10–12.

Meiotic behaviour

In both the crosses, the female parent, *S. radiatum* exhibits regular meiosis with 32 bivalent formation at MI. In male parent, *S. occidentale*, the meiosis is slightly abnormal. However regular 32 bivalents were observed in 92% of PMCs analyzed (Fig. 5). About 8% of cells showed the formation of one quadrivalent (Fig. 6). Further meiotic divisions were normal. In another male parent, *S. schinzianum* meiotic behaviour appears to be normal with 32 bivalents at MI (Fig. 14).

The F_1 hybrids of *S. radiatum* $\times$ *S. occidentale* showed slightly irregular meiosis. About 86% of PMCs showed the formation of 32 bivalents (Fig. 7). In F_1 hybrid mean chromosome pairing of $0.8_{\text{I}} + 31.6_{\text{II}}$ per cell was found. Four to 10 univalents have been observed in about 14% of cells (Figs. 8, 9). Multivalent formation is conspicuously absent. Micronuclei were found at telophase-I. Further meiotic divisions were normal. Different chromosome configurations and their frequencies are given in Table 4.

In the hybrid of *S. radiatum* $\times$ *S. schinzianum*, mean chromosome pairing of $0.154_{\text{I}} + 31.79_{\text{II}} + 0.11_{\text{IV}}$ per cell was found. Nearly 92% of PMCs showed regular 32 bivalent formation (Fig. 15). One to 2 quadrivalents were noticed in 8% of cells (Figs. 16, 17). Number of univalents ranged from 2 to 4 (Fig. 18). Trivalents were conspicuously absent. Frequencies of different chromosome configurations are given in Table 4. Anaphase-I was normal. Small percentage of PMCs showed micronuclei at telophase-II and tetrad. Other wise meiotic behaviour was regular.

Fertility

In the cross *S. radiatum* $\times$ *S. occidentale*, the female parent, *S. radiatum* shows around 87% pollen fertility and 84% seed setting. In male parent pollen fertility was 83% with 70% seed setting. In the F_1 hybrid 46% of pollen was assessed good and seed set was 30%. Large number of hybrid seeds germinated but only about 9% of seeds germinated and reached to maturity.

Table 4. Chromosome pairing in *S. radiatum*, *S. occidentale*, *S. schinzianum* and their F_1 hybrids

Species/Hybrid	Chromosome association				No. of cells analyzed	% of cells
	I	II	III	IV		
<i>S. radiatum</i>	—	32	—	—	113	100
$\bar{X}$ chromosome pairing/cell	—	32	—	—	113	100
<i>S. occidentale</i>	—	32	—	—	61	92
	—	30	—	1	5	8
$\bar{X}$ chromosome pairing/cell	—	32	—	0.1	66	100
<i>S. schinzianum</i>	—	32	—	—	48	100
$\bar{X}$ chromosome pairing/cell	—	32	—	—	48	100
<i>S. radiatum</i> $\times$ <i>S. occidentale</i> F_1 hybrid	—	32	—	—	72	86
	4	30	—	—	6	7
	6	29	—	—	4	5
	10	27	—	—	2	2
$\bar{X}$ chromosome pairing/cell	0.8	32	—	—	84	100
<i>S. radiatum</i> $\times$ <i>S. schinzianum</i> F_1 hybrid	—	32	—	—	60	92
	2	27	—	2	2	3
	2	29	—	1	3	5
$\bar{X}$ chromosome pairing/cell	0.2	32	—	0.1	65	100

In *S. radiatum* × *S. schinzianum* hybrid, the female parent, *S. radiatum* exhibits 99% pollen fertility with 97% seed set. In *S. schinzianum* pollen fertility was 96% and seed set was 92%. In the F_1 hybrid 41% of pollen was assessed good with 10% seed setting. Hybrid seeds were shriveled and only about 8% of the germinated seeds reached to maturity. The F_2 seeds were nonviable.

Discussion

Among *Sesamum* species, currently 3 taxa, *S. radiatum*, *S. occidentale* and *S. schinzianum* are known to be octaploid ($2n=8x=64$) based on $x=8$ base number (Kobayashi 1993). In an attempt to understand genome relations among these octaploid species, large number of crosses were made in various combinations. Only 2 combinations, *S. radiatum* × *S. occidentale* and *S. radiatum* × *S. schinzianum* were successful.

In *S. radiatum* × *S. occidentale* cross, both the parental species revealed regular 32 bivalent formation during meiosis indicating their allooctaploid nature. Both the parents are highly fertile. In the F_1 hybrid mean chromosome pairing of $0.81_I + 31.6_{II}$ per cell was found and 86% of PMCs showed regular 32 bivalent formation at metaphase-I. This suggests that genomes of *S. radiatum* and *S. occidentale* are similar and homologous. Conspicuously no multivalents were observed. F_1 and F_2 progenies were fertile. Both the parental species are distributed in South Africa. *S. radiatum* and *S. occidentale* are morphologically similar. *S. occidentale* differs from *S. radiatum* in possessing entire leaf margin and whiter corolla. There is a passing reference regarding the hybrid, *S. radiatum* × *S. occidentale* in Joshi's (1961) monograph on *Sesamum*. No details are available on this hybrid. Information on flowering, sympatric distribution and other ecological data from their natural habitat are not available on these species. Morphological similarities between *S. radiatum* and *S. occidentale*, ease of crossability, 32 bivalent formation in their hybrid and high fertility in F_1 and F_2 progenies indicate that *S. occidentale* is not a distinct species and it should be treated as a variety of *S. radiatum* as suggested by Stapf (1906).

In the hybrid, *S. radiatum* × *S. schinzianum* both the parental taxa are distinct species with South African distribution. *S. radiatum* can be easily distinguished by its unpleasant odour. Further, both taxa are morphologically distinct in many characters. Meiotic behaviour is normal in both the species and 32 bivalents are formed at metaphase-I. This pairing behaviour indicates their allooctaploid nature. In the hybrid, *S. radiatum* × *S. schinzianum* mean chromosome pairing of $0.15_I + 31.79_{II} + 0.11_{IV}$ per cell was found. Nearly 92.31% of the PMCs showed 32 bivalents. This pairing behaviour shows that genomes of *S. radiatum* and *S. schinzianum* are similar and homologous. Genomes of these 2 species have diverged and differentiated through 2 translocation as evident from 2 quadrivalent formation in the hybrid. It appears that these 2 species have descended from a common octaploid species. About 160 F_2 seeds were recovered from 9 selfed F_1 plants of *S. radiatum* × *S. schinzianum* cross. F_1 were partially fertile but only 8 F_2 seeds germinated and none of them reached to maturity, indicating F_2 seed sterility. *S. radiatum* and *S. schinzianum* occur together in South Africa but their sympatric occurrence and introgression is not known. The isolating barrier between these 2 species may be ecological and hybrid seed sterility operating through the failure of hybrid derivatives become established in the second generation (Stebbins 1950). The reason for hybrid seed sterility in this case is not defined but endosperm degeneration is considered to be the usual cause for such sterility in plants (Salimath *et al.* 1995).

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