Cytogenetical Studies in the Diploid Interspecific Hybrids of Section Erectoides in the Genus Arachis

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The genus *Arachis* which includes the cultivated groundnut *A. hypogaea* has been divided into seven botanical sections based on morphological similarities, cross compatibility and fertility of hybrids (Gregory *et al.* 1973, 1980). The wild species of *Arachis* are restricted to South America in Brazil, Bolivia, Argentina, Uruguay and Paraguay. The genus contains diploids and tetraploids. Section Erectoides with which we are dealing in this paper includes a few named species and a large number of unnamed taxa all of which are perennial diploids. Interspecific hybridization in *Arachis* is possible to certain extent. The first reported interspecific hybrid in this genus was between *A. hypogaea* L. and *A. villosa correntina* (Benth.) Burk. (Krapovickas and Regoni 1951). Later several people reported interspecific hybrids in *Arachis* (Raman 1958, Smartt and Gregory 1967, Varisai Muhammad 1973, Smartt *et al.* 1978, Gregory and Gregory 1979, Stalker *et al.* 1979, Stalker and Wynne 1979). Meiotic behaviour in the diploid interspecific hybrids is regular showing mostly bivalents (Raman and Kesavan 1962, Smartt *et al.* 1978, Stalker and Wynne 1979, Ressler and Gregory 1979). Husted (1936) reported the formation of multivalents in the cultivated groundnut. Raman (1973) reported the formation of one tetravalent in about 4 percent of the cells. Based on this *A. hypogaea* has been suggested to be a segmental allopolyploid.

In the present study the diploid interspecific hybrids of section Erectoides reveal the formation of meiotic abnormalities like univalents and tetravalents at metaphase-I and laggards and chromatin bridges at anaphase. The formation of ring of four chromosomes and chromatin bridges due to structural changes may play a significant role in diversification of taxa in the section Erectoides.

Materials and methods

The three species of section Erectoides viz., *A. regonii*, *A. sp. 9990*, and *A. sp. 10002* used in the present crosses were obtained from ICRISAT, Hyderabad, India. For hybridization plants were grown in earthen pots. Suitable flower buds of female parents were emasculated by using fine forceps between 2.00 p.m. and 6.00 p.m. After emasculation small threads were tied to identify the hybrid seeds when they were harvested. In the next day morning between 6.00 a.m. and 8.00 a.m. cross-pollinations were conducted. After sufficient crosses have been completed subsequent flower buds were plucked off from the plants to avoid the development of selfed pods. Sixty to hundred days after the last crossing the seeds were collected.

The F₁ seeds were germinated in the laboratory in petridishes. After germination the seedlings were transferred to the field. The hybrids were identified based on their leaflet morphology, flower colour and pollen stainability which were studied in the plants grown in the field.

For meiotic studies appropriate flower buds were fixed between 8.00 a.m. and 10.00 a.m. in 1:3:6 glacial acetic acid, chloroform and ethyl alcohol respectively. After two days they
were transferred to acetic alcohol into which few drops of ferric chloride were added. Anthers were smeared using 2 percent acetocarmine stain.

Results

Morphologically the parents *A. sp. 9990* and *A. sp. 10002* have erect growth habit, lanceolate leaflets and orange flowers while *A. regonii* has spreading type of growth habit, obovate

Figs. 1–9. 1, F₁ hybrid *A. regonii × A. sp. 9990*. Late anaphase-II showing chromatin bridge. 2–5: F₁ hybrid *A. sp. 9990 × A. regonii*. 2, late metaphase-I showing 8 bivalents and 4 univalents (due to early separation). 3, metaphase-I showing early separation of 3 bivalents. 4, anaphase-II showing chromatin bridge and laggards. 5, anaphase-II showing dicentric bridge. 6, F₁ hybrid *A. regonii × A. sp. 10002*. Early anaphase-I showing late disjunction of 3 bivalents. 7–9: F₁ hybrid *A. sp. 9990 × A. sp. 10002*. 7, diakinesis showing 8 bivalents and a ring of four chromosomes (note the ring of four chromosomes associated with the nucleolus). 8, metaphase-I showing 8 bivalents and one tetravalent. 9, metaphase-II showing two chromosomes away from the groups.
levaves and yellow flowers. Hybrids were produced between \( A. \) sp. 9990\( \times A. \) regonii, \( A. \) sp. 10002\( \times A. \) regonii, their reciprocals and between \( A. \) sp. 9990\( \times A. \) sp. 10002. The first two hybrids and their reciprocals show spreading type of growth habit and yellow flowers resembling \( A. \) regonii. But the leaflets are intermediate in shape, while the other hybrid \( A. \) sp. 9990\( \times A. \) sp. 10002 has erect growth habit, lanceolate leaflets and orange flowers as both the parents involved have similar characters.

Cytological analysis in the parents reveals the presence of 10 bivalents at diakinesis and metaphase-I. The subsequent stages are also normal. Meiotic behaviour is considerably irregular in the hybrids. The chief meiotic abnormalities include the formation of univalents and tetravalents at metaphase-I, early separation of bivalents, late disjunction of bivalents, laggards and bridges at anaphase and elimination of chromosomes.

The hybrid \( A. \) regonii\( \times A. \) sp. 9990 and its reciprocal shows ten bivalents in approximately 89 percent of the PMCs while in the remaining 11 percent of the cells 8 bivalents and 4 univalents are noticed. As many as 3 bivalents disjoin precociously. Anaphase-I shows one to two laggards in 17.5 percent of the cells. One chromatin bridge is noticed in 8 percent of the PMCs at anaphase-II (Fig. 1). Its reciprocal cross viz., \( A. \) sp. 9990\( \times A. \) regonii also shows similar meiotic abnormalities of univalents (Fig. 2), early separation of three bivalents (Fig. 3) and anaphase bridge (Figs. 4 and 5). Pollen stainability in these hybrids is approximately 35 percent.

The hybrid \( A. \) regonii\( \times A. \) sp. 10002 and its reciprocal reveals the occurrence of ten bivalents in 86 percent of the PMCs and 14 percent of the cells show 8 bivalents and 4 univalents. At anaphase late disjunction of three bivalents is seen (Fig. 6). One or two laggards are seen in about 4 percent of the PMCs. But no bridges were recorded either at anaphase-I or anaphase-II. Pollen stainability is recorded in approximately 33.5 percent in the hybrid \( A. \) regonii\( \times A. \) sp. 10002 and 37.5 percent in its reciprocal cross.

Meiotic observations in the hybrid \( A. \) sp. 9990\( \times A. \) sp. 10002 reveal the formation of ten bivalents in 82 percent of the pollen mother cells and in the remaining 18 percent of the cells 8 bivalents and a ring of 4 chromosomes are noticed at diakinesis (Fig. 7) and metaphase-I (Fig. 8). The ring of four chromosomes is found to be associated with the nucleolus. Anaphase-I shows 1 to 2 laggards in about 10 percent of the cells. In 12 percent of the cells two chromosomes are found to be separated from the other chromosomes at metaphase-II (Fig. 9). The pollen stainability in this hybrid is 43 percent.

Discussion

The spreading type of growth habit and yellow flower colour noticed in the hybrids \( A. \) regonii\( \times A. \) sp. 9990 and \( A. \) regonii\( \times A. \) sp. 10002 and reciprocals suggest their dominant nature over erect growth habit and orange flowers respectively in section Erectoides. Even the shape of the flower of the parent \( A. \) regonii is inherited as a dominant character. However the leaflet shape in these hybrids is intermediate between the lanceolate leaflets of the erect forms and obovate leaflets of the spreading type. Hence dominant gene controlling the shape of the leaflets appears to be absent.

The successful formation of interspecific hybrids irrespective of their differences in morphology and growth habit indicates that the different species in section Erectoides are closely related. Thus they do not seem to have sufficiently diverged to form distinct taxonomic species.

Further, the occurrence of ten bivalents in majority of the pollen mother cells indicates the presence of a more or less similar genome in all three species examined here. Earlier Raman and Kesavan (1962), Smartt et al. (1978), Stalker and Wynne (1979), Resslar and Gregory (1979) and Singh and Moss (1984) who analysed the cytology of diploid interspecific
hybrids in section Arachis emphasized the presence of a common genome in all the members. Stalker (1981) who analysed the intersectional hybrids between section Arachis and section Erectoides is of the opinion that the genomes of these two sections were similar. However, interspecific hybrids of section Arachis show less abnormalities when compared to the interspecific hybrids of section Erectoides.

The formation of a ring of four chromosomes in the hybrid \( A. \) sp. 9990\( \times A. \) sp. 10002 is an indication of the possible segmental exchange (translocations) in the genome. Although the two parents viz., \( A. \) sp. 9990 and \( A. \) sp. 10002 are more or less similar in exomorphic characters they seem to have diverged from each other by translocation. Another interesting aspect is that the ring of four chromosomes is always seen associated with the nucleolus in the hybrid. Perhaps one of the chromosomes involved in translocation is the region of a nucleolar organiser with another chromosome. Singh and Moss (1982) who analysed the karyomorphology in the species of section Arachis found some differences in the nucleolar organiser chromosomes of \( A. \) stenosperma and \( A. \) cardenasii. Obviously the nucleolar organiser chromosomes of this genus appear to be susceptible to breakages.

Apart from the above the formation of chromatin bridge in about 8.5 percent of the cells at anaphase-II in the hybrid \( A. \) sp. 9990\( \times A. \) regonii and its reciprocal suggests the possible presence of a paracentric inversion. Earlier Ressalar and Gregory (1979) who analysed the hybrid of \( A. \) hypogaea cv. NC 4\( \times A. \) cardenasii observed the formation of anaphase bridge in 4.7 percent of the PMCs and this was attributed to the presence of one paracentric inversion.

As mentioned earlier the genus \( A. \) arachis comprises 22 named and described species and 50 to 80 or more as yet undescribed taxa which have been divided into seven botanical sections (Gregory et al. 1973, 1980). This genus comprises both diploids and tetraploids with a basic chromosome number \( x=10 \). Diversity and speciation in this genus appear to have followed different lines of evolution in each section. The tetraploids of sections Arachis and Rhizomatosae have been suggested to be allopolyploids orginated by hybridization between two species followed by the doubling of the chromosome complements. The annual tetraploid \( A. \) hypogaea is suggested to have two genomes 'A' and 'B' both of which are derived from the same section where the cultivated groundnut is included (Smartt et al. 1978 and Singh and Moss 1982). On the other hand the perennial tetraploids of section Rhizomatosae is presumed to have originated from species which are similar to diploid Rhizomatosae members and Erectoides by hybridization followed by doubling of chromosomes (Gregory and Gregory 1979). The female sterility and their propagation by rhizomes also support the intersectional origin of tetraploid Rhizomatosae. In section Extranervosae, Raman and Manimekalai (1973) reported the spontaneous origin of autotetraploid from diploid \( A. \) villosulicarpa Stock, which they maintained in their field at Coimbatore, India.

Cytological analysis carried out by different people in the diploid interspecific hybrides of section Arachis suggests the presence of a common genome in all species except \( A. \) batizocoi. Raman (1976), who analysed the meiotic behaviour of diploid interspecific hybrids, found only ten bivalents and the possible involvement of large structural differences between the genomes involved were omitted. However, Singh and Moss (1984) reported the formation of trivalent and tetravalent in less frequency. The above events suggest that diversity among diploid members of section Arachis appears to have taken place mainly at genic level and to some extent by chromosomal repatterning. The genome of section Erectoides appears to be susceptible to more of structural changes compared to the other sections of genus \( A. \) rarachis studied. The presence of large number of taxa in this section also supports the flexible nature of the genome. Cytogenetical investigation in other four sections is meagre and needs attention from workers.
Summary

Three diploid interspecific hybrids and two reciprocal hybrids were produced between the species of section Erectoides. The cytological analysis in two hybrids and their reciprocal crosses shows 10 bivalents at diakinesis and metaphase-I in most of the PMCs and 2-4 univalents are seen in about 10 to 15 percent of cells. Further the hybrid between A. sp. 9990 × A. sp. 10002 reveals a ring of four chromosomes in approximately 18 percent of the PMCs. In the hybrid A. regonii × A. sp 9990 and its reciprocal, chromatin bridge is seen in 8.4 percent of the cells at anaphase-II, thus providing cytological evidences for the possible role of structural changes in speciation of the section Erectoides.

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