Theor Appl Genet (1986) 72:164–169

Utilization of wild relatives in the genetic improvement of *Arachis hypogaea* L.

7. Autotetraploid production and prospects in interspecific breeding *

A. K. Singh

Groundnut Improvement Program, ICRISAT, Patancheru P.O. 502 324, A.P., India

Received October 20, 1985; Accepted November 25, 1985
Communicated by G. S. Khush

Summary. Autotetraploids were established from 8 diploid wild species of section *Arachis*. In all the autotetraploids the chromosomes paired largely as bivalents even though they possess the ability to pair as multivalents. Pollen and pod fertility in the C_1 generation were not directly associated with chromosome pairing. The C_2 generation autotetraploids showed a gradual increase in bivalent associations and pollen and pod fertility. The identification of two genomes, A and B, in the diploid species and in the tetraploid, *A. hypogaea*, of the section *Arachis*, a fairly good crossability, and the type of chromosome associations observed in hybrids between *A. hypogaea* and the autotetraploids of wild *Arachis* species indicated good prospects of utilizing autotetraploids as genetic bridges in transferring desired traits from these taxa into groundnut.

Key words: *Arachis* – Autotetraploid – Chromosome pairing – Genomic relationships – Recombinations

Introduction

Diploid wild species of the section *Arachis*, genus *Arachis*, are not commercially important but are valuable as a gene pool which afford resistance against several pests and pathogens of groundnut (Abdou et al. 1974; Subrahmanyam et al. 1985; Amin 1985).

Experimental autotetraploids of the species in this genus could provide an insight into the role played by polyploidy in the evolution of the genus. They could also be utilized as genetic bridges for overcoming ploidy differences for the transfer of desirable features from these and other diploid species into cultivated species, as observed in other crops such as cotton (Knight 1953, 1954) and potato (Livermore and Johnson 1940). Raman and Kesavan (1963) produced an autotetraploid in *A. duranensis*, section *Arachis*, and described the morphological and cytological features.

The present paper reports the production of autotetraploids of *A. batizocoi*, *A. duranensis*, *A.* species GKP 10038, *A.* species HLK 410, *A. cardenasii*, *A. chacoense*, *A. correntina* and *A. villosa*. Colchiploids have been studied for their cytogenetical stability and fertility. Crossability and chromosome pairing in hybrids between *A. hypogaea* and these autotetraploids are discussed in order to understand the prospects of utilizing them for the incorporation of desirable features into the cultivated groundnut.

Materials and methods

The identities and sources of the eight diploid wild species used have been previously listed (Singh and Moss 1982).

Production of autotetraploids

Seedlings from each species were grown in a greenhouse at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Centre, Patancheru, near Hyderabad, India. To induce polyploidy the apical meristem was continuously kept moist by means of an absorbant cotton wool ball soaked in colchicine solutions of different concentrations, for 6 h a day for 2 to 3 days. A film of solution was maintained around the cotton wool ball by dropping the colchicine solution over it at frequent intervals. After each daily treatment, the apical tip was washed thoroughly with water. Preliminary observations on the mitotic index indicated 0600 h to 1200 h to be the best time for effective treatment (Singh and Moss 1982). To ensure uniform ploidy levels, only treated meristems were allowed to grow.

Hybridization, and cytological and pollen fertility analyses were done as described earlier (Singh and Moss 1984).

* Submitted as Journal Article No. 516 by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

Chromosome associations were statistically analysed using one way analysis of variance (Cochran and Cox 1957).

Observations

Autotetraploids

The 6 h daily treatments with 0.25% colchicine for 3 days produced the maximum number (50%) of tetraploid plants while 0.35% colchicine produced 30% tetraploid plants.

Morphology. The affected plants showed a stunted growth and produced thicker and variously deformed leaves. The stunted growth in the autotetraploids lasted for a period of 15–45 days and thereafter became nearly normal. At maturity all the autotetraploid plants, irrespective of the species, were more vigorous than their diploid counterparts. The tetraploids had thicker stems, large dark green leathery leaflets and larger flowers than normal. Autotetraploids of all the annual species, *A. batizocoi, A. duranensis* and *A.* species GKP 10038 were seed fertile and could be advanced to the C_2 generation whereas amongst the perennial species, only the autotetraploid of *A. villosa* could be advanced to the C_2 generation. However, an extended period of growth and flowering in perennial species autotetraploids allowed us to utilize them in our breeding programme despite their seed sterility. Pods from the autotetraploids were noticeably larger than those from their diploid counterparts.

Cytology. At least one autotetraploid plant from each of the diploid species was cytologically analysed. These autotetraploids, except those of *A. cardenasii* and *A.* species HLK 410, showed a mean frequency of around 4 quadrivalents per pollen mother cell (PMC) (Table 1, Fig. 1). Autotetraploids of *A. batizocoi* and *A. villosa* had the highest mean quadrivalent frequencies (4.8 and 4.2, respectively). At anaphase I (AI) and the subsequent stages, about 50% of the PMCs showed an equal distribution of the chromosomes while the remainder revealed irregularities such as unequal segregation, laggards and stretching of the associations appearing as bridges (Table 2, Figs. 2 and 3). Most of these irregularities were the result of a nonsynchronous movement of chromosomes at anaphase. Pollen fertility was moderate in all the tetraploids but significant differences were recorded even between autotetraploid plants of the same species (Table 2).

C_2 generation. Observations were possible only in tetraploids of the annual species and of *A. villosa* that had produced pods and progenies. The autotetraploid progenies bred true for their ploidy level with the exception of certain progenies of *A. batizocoi.*

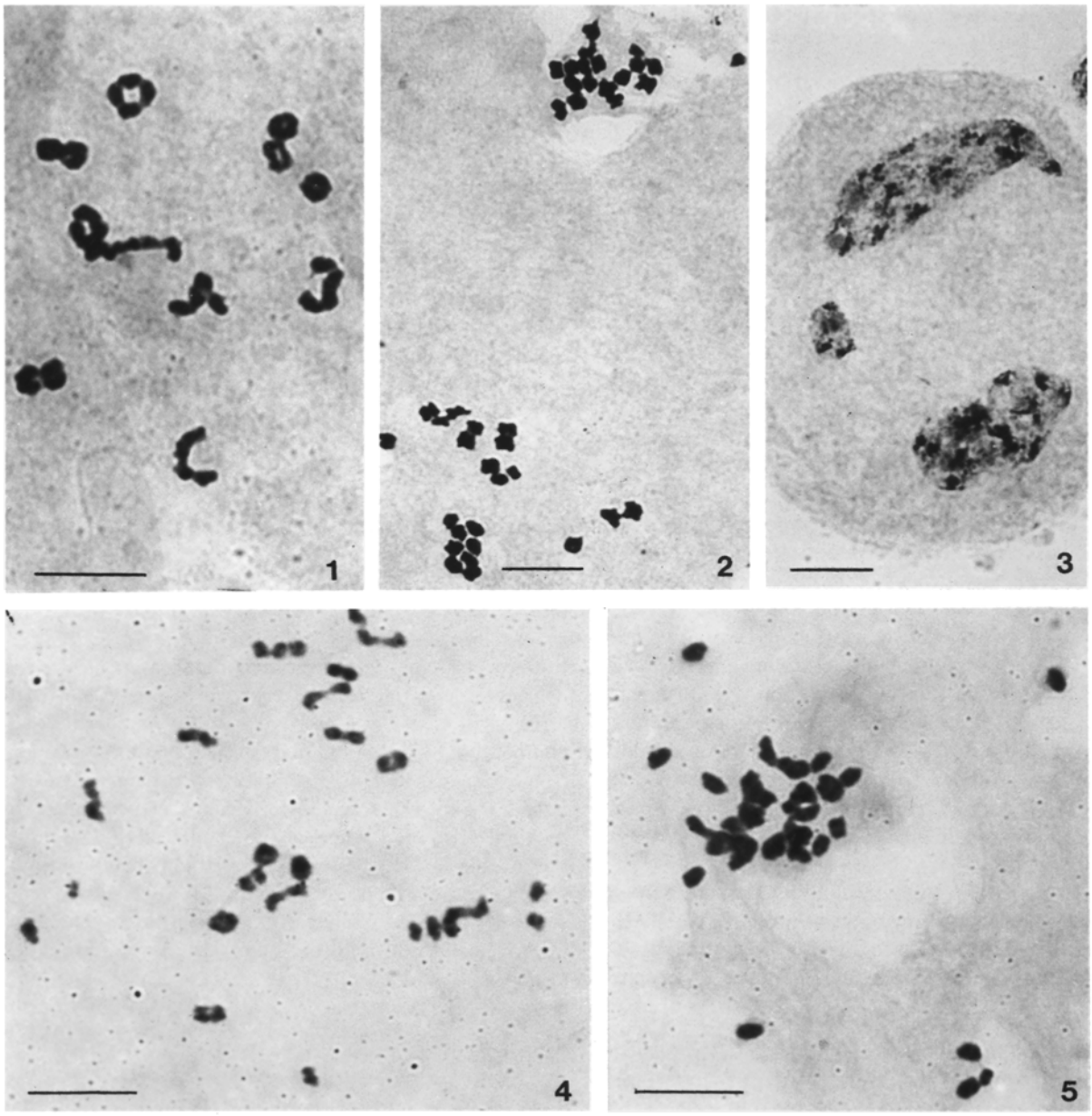
Morphologically C_2 tetraploid plants were as vigorous as C_1 progenies. They showed higher levels of pollen and pod fertility than the tetraploid C_1 plants, but lower than their diploid counterparts. However, considerable variation was recorded in pollen and pod fertility of sister plants. Pod and seed sizes were comparable to those obtained from C_1 plants.

Cytologically, autotetraploid C_2 progenies of *A. batizocoi* and *A. villosa* showed a significant increase in bivalent formation whereas those of *A. duranensis* did not (Table 1). This resulted in an improved normal segregation of chromosomes at AI and subsequent stages of meiosis. Pollen and pod fertility were also improved but not in all the plants of an autotetraploid (Table 2).

Table 1. Chromosome association at Metaphase I in autotetraploids of diploid species of section *Arachis*

Name of species	Mean chromosome association				No. of cells analysed	No. of chiasma formed	No. of chiasma terminalized
	I	II	III	IV			
C1 generation							
A. batizocoi[a]	4.0±0.6	7.3±0.9	0.5 ±0.2	4.8±0.5	24	35.2±0.7	35.0±0.7
A. duranensis[a]	0.9±0.4	12.2±0.8	0.0 ±0.0	3.6±0.5	20	33.9±0.6	33.1±0.7
A. sp. GKP 10038[a]	0.9±0.3	12.0±0.9	0.4 ±0.1	3.5±0.4	20	36.8±1.6	35.8±1.6
A. sp. HLK 410[b]	2.5±0.7	12.5±1.1	0.6 ±0.4	2.8±0.6	8	–	–
A. cardenasii[b]	2.1±0.4	13.0±0.5	0.8 ±0.2	2.4±0.3	25	36.2±0.4	35.7±0.4
A. chacoense[b]	3.1±0.5	10.1±0.5	0.24±0.1	4.0±0.3	29	35.4±0.81	35.3±0.8
A. correntina[b]	1.8±0.3	12.0±0.7	0.4 ±0.1	3.4±0.4	25	35.3±0.3	35.2±0.4
A. villosa[b]	4.5±0.39	8.1±0.94	0.9 ±0.25	4.2±0.40	19	34.0±0.46	32.4±0.48
C2 generation							
A. batizocoi	1.7±0.5	12.7±0.8	0.6 ±0.2	2.8±0.4	18	–	–
A. duranensis	0.5±0.2	13.7±0.6	0.6 ±0.2	2.7±0.3	13	–	–
A. villosa	1.0±0.27	15.9±0.36	0.1 ±0.07	1.7±0.18	34	–	–

[a] = Annuals; [b] = Perennials



Figs. 1–5. **1–3** Pollen mother cells of autotetraploids showing **1**) at metaphase I 8II + 6IV in *A. correntina,* **2**) chromosome distribution of 18–22 in *A. villosa* and **3**) chromosome complement fractionation in *A. chacoense* at anaphase I. **4–5** PMCs of *A. hypogaea* × autotetraploids at metaphase I showing **4**) 12II + 3III + 7I in *A. hypogaea* × *A. batizocoi* (B genome), and **5**) 10II + 2IV + 1III + 9I in *A. hypogaea* × *A. species* HLK 410 (A genome) (Scale bar = 10 μm)

Hybrids between A. hypogaea and autotetraploids

Crossability. Of the autotetraploids established in 8 species, 6 were crossed as male parent with cultivars belonging to the three botanical varieties and two subspecies of *A. hypogaea;* no reciprocal crosses were attempted. Cultivars belonging to the subspecies *fastigiata* appeared to produce more pods per 100 pollinations except in case of *A. correntina* (Table 3). However, crossability varied significantly between the autotetraploids of a species on one hand and different cultivars of the same subspecies or botanical variety of *A. hypogaea,* on the other.

Morphology. The hybrids were vegetatively vigorous. They had intermediate or large leaflets as in *A. hypogaea.* Genetically dominant features of the wild species, such as perenniality of perennial species, pigmentation

Table 2. Chromosomal distribution at Anaphase I, and pollen and pod fertility in autotetraploids of diploid species of section *Arachis*

Species	AI distribution (%)				% Pollen stainability (range)	Pods produced (range)
	Equal	Unequal	Laggard	Bridges		
C1 generation						
A. batizocoi	50	3	26	21	40 (17–67)	(9–13)
A. duranensis	–	–	–	–	50 (24–77)	16
A. sp. GKP 10038	54	42	4	0	62	(3–5)
A. sp. HLK 410	–	–	–	–	67	8[a]
A. cardenasii	56	44	0	0	44 (23–65)	0
A. chacoense	47	41	12	0	35 (14–56)	1[a]
A. correntina	36	52	12	0	41	0
A. villosa	58	6	36	0	44	4
C2 generation						
A. batizocoi	63	25	13	0	64 (58–69)	2
A. duranensis	52	35	13	0	73 (65–75)	(2–18)
A. villosa	64	16	20	–	70 (67–75)	(13–16)

[a] Did not germinate or 2x; – = not analysed

Table 3. Crossability between cultivars of two subspecies of *A. hypogaea* and autotetraploids of 6 wild species of section *Arachis*

A. hypogaea ♀ / Wild species ♂	*A. hypogaea* subsp. *hypogaea* var. ('Virginia')			*A. hypogaea* subsp. *fastigiata* var. ('Spanish')		
	Pollinations	Pods	Pods/100 pollinations	Pollinations	Pods	Pods/100 pollinations
A. villosa	136	20	15	138	46	32
A. correntina	–	–	–	207	29	14
A. sp. HLK 410	132	14	11	–	–	–
A. sp. GKP 10038	191	27	14	73	21	29
A. cardenasii	70	5	7	66	15	23[a]
A. batizocoi	–	–	–	314	44	28

[a] Valencia

Table 4. Chromosome associations at Metaphase I, and pollen and pod fertility in F1 hybrids between *A. hypogaea* and autotetraploids of diploid species of section *Arachis*

A. hypogaea × Autotetraploid	No. cells analysed	Chromosome associations				% Pollen stainability (range)	Pods produced (range)
		I	II	III	IV		
A. batizocoi	23	5.8±0.52	14.1±0.56	0.9±0.23	0.9±0.19	43–64	(5–26)
A. sp. HLK 410	21	10.1±0.78	11.2±0.52	1.9±0.28	0.5±0.18	–	(2–5)
A. villosa	9	7.2±0.68	11.8±0.94	2.2±0.4	0.3±0.17	26	(2–4)
A. sp. GKP 10038	12	7.8±0.91	12.8±0.68	1.1±0.29	0.8±0.22	–	(2–5)

of *A.* species HLK 410, villose or pubescence nature of *A. villosa* and *A. duranensis,* hairy leaflet margin of *A. batizocoi,* yellow flower of *A. batizocoi, A.* species HLK 410 and *A.* species GKP 10038 and resistance to rust (*Puccinia arachidis*) of *A. batizocoi* were expressed in tetraploid hybrids of the respective wild species. Hybrids involving the autotetraploids of both annual and perennial species produced a few pods that were bigger than those from diploid parents but smaller than those from *A. hypogaea.*

Cytology. Cytological analysis was done in four hybrid combinations combining *A. hypogaea* with autotetraploids of (1) *A. batizocoi,* the only representative of the B genome in section *Arachis,* (2) *A. villosa* and *A.* species HLK 410, two perennial species with an *AA* ge-

nome constitution, and (3) *A.* species GKP 10038, an annual species with the *AA* genome constitution. The hybrid between *A. hypogaea* and *A. batizocoi* (4x) showed a significantly higher bivalent association (14.1) than those from crosses with other three (Table 4, Fig. 4). Chromosome association in two hybrids involving perennial species *A.* species HLK 410 and *A. villosa,* both with the same genomic constitution (*AA*), was nearly the same (Table 4, Fig. 5). In the hybrid, *A. hypogaea* × *A.* species GKP 10038 autotetraploid, bivalent association was a little higher, approaching that found in the hybrid between the *A. hypogaea* and *A. batizocoi* autotetraploid. The hybrids between *A. hypogaea* and *A. batizocoi* autotetraploid also showed the highest pollen stainability and produced the highest number of pods (5–26).

Discussion

These induced experimental autotetraploids in different *Arachis* species are not commercially important. Nevertheless, they can be very useful as genetic bridges in overcoming ploidy barriers and the transfer of some of their traits into the cultivated groundnut. The autotetraploids also provide an extra gene dose of the desired trait in crosses with the cultivated species.

Colchiploids showed no change in ploidy level in the C_2 generation, suggesting their true breeding nature for ploidy level. However, from certain autotetraploid plants of *A. batizocoi,* both diploid and tetraploid progenies were obtained in the C_2 generation. This may be attributed to the 2n and 4n sectoral nature of some colchiploids, and/or to the complement fractionation recorded in *A. chacoense* (Fig. 4).

A greater vigour, prolonged growth duration and flowering are common features of colchiploids that facilitate their use as tetraploid pollen parents for an extended period.

The pattern of chromosome association in these colchiploids reveals that the mean quadrivalent frequency is far less than the expected value of 2/3, based on an average of two chiasmata per bivalent in the diploids of these species (Singh and Moss 1982). The reduction in multivalent association may be due to (a) the small size of chromosomes with a smaller number of chiasmata (b) a preferential pairing between specific chromosomes in the case of a species that is relatively heterozygous, (c) a low frequency of chiasma formation in a raw autotetraploid (Table 1) due to the genetic imbalance (leading to the breakdown of multivalent associations into bivalents at metaphase I, or (d) disturbances in zygomere activity. However, the formation of 6–8 quadrivalents in a few PMCs of these autotetraploids (Fig. 1) suggests that the possibility of a genetic regulatory mechanism is lower for bivalent formation.

A. species HLK 410 and *A. cardenasii* autotetraploids have the lowest quadrivalent frequencies (2.8 and 2.4, respectively). It is believed that taxa of relatively recent origin and structurally heterozygous or with specifically localized chiasmata may not show many quadrivalents in their tetraploids (Sybenga 1975). *A.* species HLK 410 and *A. cardenasii* may be such species. Bivalent associations were highest in these two species but not significantly different from that in others except *A. batizocoi* and *A. villosa* (Table 1). The autotetraploids of *A. batizocoi* and *A. villosa* showed the highest quadrivalent frequency which may be either due to their greater homozygosity and primitive nature, or the more random distribution of chiasmata.

Pollen fertilities were relatively high in autotetraploids of annual species irrespective of chromosome associations (Table 2). However, significant variations in pollen fertility were recorded within autotetraploid plants of the same species. These results suggest that the segregation of multivalents and univalents need not always influence pollen fertility. The disturbed genetic and physiological equilibrium in a raw autotetraploid may also lower the pollen viability and the seed set. Viable pods and seeds were obtained in all the annual species and *A. villosa* but not in the other perennial species despite similar chromosomal associations and pollen fertility (Tables 1 and 2). These results suggest that annual species are probably sexually better equipped for exploiting the gamete potential for fertilization than are the perennials (Stebbins 1971).

The most interesting observation in the C_2 generation was the increase in the frequency of bivalents compared to that observed in the C_1 plants. Diplontic (bivalent) behaviour has the advantage of meiotic regularity and stabilization. Therefore, in subsequent generations, selection generally favours gametes which are the products of regular bivalents and the normal segregation of chromosomes. This results in a shift from the multivalent to bivalent associations, i.e. to a diplontic behaviour which suppresses multivalent formation. However, the degree of the shift may vary and eventually result in differences in bivalent associations, and, therefore, in differences of pollen and pod fertility between the plants of the C_2 generation of the same species and different species. Progenies that are the products of fertilization between gametes resulting from normal segregation will have a more regular bivalent association and segregation of chromosomes, and therefore, greater pollen and pod fertility. This may also explain the differential increase in the bivalent association in *A. batizocoi, A. duranensis* and *A. villosa.* Such differences may also be attributed to the differential nature of a multivalent suppressor mechanism or to the evolutionary flexibility of species. A shift to the diplontic behaviour is an adaptive evolutionary process. It may also involve the development of barriers between originally homologous chromosomes by improvements in the recognition system through the reduction of pairing initiation to a single site per chromosome, and differentiation of zygomere DNA base sequences and their regulation system (Sybenga 1969, 1973; Watanabe 1983).

Breeding prospects

There is now strong evidence that there are two homoeologous genomes, *AA* or *BB* among investigated the diploid wild species that together constitute *A. hypogaea,* all in section *Arachis* (Singh and Moss 1982, 1984). Autotetraploids of these diploid wild species can be useful bridges, as in cotton (Knight 1953, 1954), for the transfer of desirable characters. Besides bridging the ploidy gap, this option would provide an extra dose of desired traits in crosses with cultivated species (*AAAB* or *ABBB* hybrids). Intragenomic homologous (*A-A*) or (*B-B*) pairing may form up to 10 bivalents and trivalents (*A-A-A* or *B-B-B*). Homoeologous associations between the *A* and *B* genome would result in additional bivalents (*A-B*) or multivalents (*A-A-B* or *A-B-B/A-A-A-B* or *A-B-B-B*). The latter type of pairing can force alterations in the other genome of the cultivated species, which can be useful. Furthermore, autotetraploids of annual species can be used in bridge crosses with certain wild species of other sections such as *Rhizomatosae* and *Erectoides. Arachis* species of these sections are not crossable with the cultivated species but do however cross with diploid annual species of the section *Arachis* (Gregory and Gregory 1979). Efforts in this direction are currently in progress at the ICRISAT Center.

Hybridization between *A. hypogaea* and the autotetraploids of diploid species from section *Arachis* did not show any change in crossability over that of the diploid crosses (Singh and Moss 1984). Crossability between the autotetraploids of different species was also not significantly different. However, the autotetraploids produced more pods per 100 pollinations when cultivars of *A. hypogaea* subspecies *fastigiata* were used as the female parent instead of *A. hypogaea* subspecies *hypogaea* (Table 3). The variation observed in the crossability of different cultivars belonging to the same subspecies suggests that crossability differences are more dependent on the cultivar rather than on the autotetraploid of a species.

Cytological analyses of these hybrids reinforce our inferences on the genomic constitution of the wild diploid species and *A. hypogaea.* These observations confirmed theoretically expected prospects for the utilization of these autotetraploids in interspecific breeding. The formation of predominantly bivalents, besides univalents and multivalents in hybrids between *A. hypogaea* (*AABB*) and autotetraploids of either *AA* genome or *BB* genomes species suggest that 10 bivalents are formed mostly as a result of intragenomic homologous (*A-A* or *B-B*) pairing between the chromosomes of these wild species and *A. hypogaea.* Additional bivalents and multivalents are formed as a result of homoeologous pairing between the *A* and *B* genome (*A-B; A-A-B, A-B-B* or *A-A-A-B, A-B-B-B*) (Table 4). Among these hybrids, the one between *A. hypogaea* and *A. batizocoi* (*B* genome) showed the highest mean bivalent association (Table 4, Fig. 4). This was indeed expected based on chromosome associations seen in the triploid hybrids of these two species (Singh and Moss 1984).

We have incorporated rust resistance from *A. batizocoi* via its autotetraploids into *A. hypogaea* (ICRISAT 1983). Efforts are on with the autotetraploid of the *AA* genome species that will be of greater significance. In these hybrid combinations homoeologous pairing in the form of multivalents and more than 10 bivalents may help in the genetic alteration of the *B* genome chromosome(s) of *A. hypogaea,* that probably carry dominant susceptibility to late leafspot. Autotetraploids of *A* genome species resistant to both the leafspots and rust, such as *A. cardenasii* and *A.* species HLK 410, have been produced, crossed and backcrossed with *A. hypogaea.* It is hoped to produce *A. hypogaea*-like tetraploid derivatives by these methods.

This technique might be effective in other segmental allotetraploid species also where homoeology in addition to homology of progenitor genome/species may facilitate desired recombinations.

Acknowledgements. The author is indebted to Mr. R.W. Gibbons, Leader, Groundnut Improvement Program ICRISAT for his critical reading of the manuscript and for his suggestions.

References

Abdou YAM, Gregory WC, Cooper WE (1974) Sources and nature of resistance to *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk and Curt.) Deighton in *Arachis* species. Peanut Sci 1:6–11

Amin PW (1985) Resistance of wild species of groundnut to insect and mite pests. In: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). Proc Int Workshop on Cytogenetics in *Arachis.* Patancheru AP, India, pp 57–61

Cochran WC, Cox GM (1957) Experimental designs. Wiley and Sons, New York, pp 95–102

Gregory MP, Gregory WC (1979) Exotic germplasm of *Arachis* L. interspecific hybrids. J Hered 70:185–193

ICRISAT (1983) (International Crops Research Institute for the Semi-Arid Tropics) Annu Rep 1982. Patancheru AP, India

Knight RL (1953) The genetics of blackarm resistance. 9. The gene *B6m* from *Gossypium arboreum.* J Genet 51:270–275

Knight RL (1954) Cotton breeding in the Sudan-part I and II Egyptian cotton. Emp J Exp Agric 22:68–92

Livermore JR, Johnson EE (1940) The effect of chromosome doubling on the crossability of *Solanum chacoense, S. jamsii* and *S. bulbocastarum* with *S. tuberosum.* Am Potato J 17:170

Raman VS, Kesavan PC (1963) Induced autotetraploidy in *Arachis duranensis.* Indian J Genet 23:320–324

Singh AK, Moss JP (1982) Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. 2. Chromosome complements of species of section *Arachis.* Theor Appl Genet 61:305–314

Singh AK, Moss JP (1984) Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. 5. Genome analysis in section *Arachis* and its implications in gene transfer. Theor Appl Genet 68:355–364

Subrahmanyam P, Ghanekar AM, Knolt BL, Reddy DVR, McDonald D (1985) Resistance to groundnut diseases in wild *Arachis* species. In: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). Proc Int Workshop on Cytogenetics in *Arachis.* Patancheru AP, India, pp 49–55

Stebbins GL (1971) Chromosomal evolution in higher plants. Edward Arnold, London

Sybenga J (1969) Allopolyploidization of autopolyploids 1. Possibilities and limitations. Euphytica 18:355–371

Sybenga J (1973) Allopolyploidization of autopolyploids. 2. Manipulation of chromosome pairing system. Euphytica 22:433–444

Sybenga J (1975) Meiotic configurations. In: Frankel R, Gall GAE, Grossmann M, Linskens HF, Zeeuw D de, Riley R (eds) Monographs on theoretical and applied genetics, vol. 1. Springer, Berlin Heidelberg New York, pp 169–178

Watanabe K (1983) Studies on control of diploid like meiosis in polyploid taxa of *Chrysanthemum.* 4. Colchiploids and the process of cytogenetical diploidization. Theor Appl Genet 66:9–14