Theor Appl Genet (2006) 112: 1342–1348
DOI 10.1007/s00122-006-0236-z

ORIGINAL PAPER

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Molecular analysis of *Arachis* interspecific hybrids

Received: 8 December 2005 / Accepted: 29 January 2006 / Published online: 18 February 2006

Abstract Incorporation of genetic resistance against several biotic stresses that plague cultivated peanut, *Arachis hypogaea* ($2n = 4x = 40$), is an ideal option to develop disease resistant and ecologically safe peanut varieties. The primary gene pool of peanut contains many diploid wild species ($2n = 2x = 20$) of *Arachis*, which have high levels of disease and insect resistances. However, transfer of resistant genes from these species into *A. hypogaea* is difficult due to ploidy level differences and genomic incompatibilities. This study was conducted to monitor alien germplasm transmission, using Random Amplified Polymorphic DNA (RAPD) markers, from two diploid wild species, *A. cardenasii* and *A. batizocoi*, into *A. hypogaea*. Triploid interspecific hybrids were produced by crossing two *A. hypogaea* cultivars (NC 6 and Argentine) with the two species and by colchicine-treating vegetative meristems, fertility was restored at the hexaploid (C_o) level in the four hybrids. Hexaploids were allowed to self-pollinate for four generations, each referred to as a cycle (C_1, C_2, C_3, and C_4). At each cycle, a backcross was made with the respective *A. hypogaea* cultivar as the maternal parent and only lineages tracing back to a single hexaploid hybrid were used for RAPD analysis. Analysis of mapped, species-specific RAPD markers in BC_1F_1 to BC_1F_3 hybrids indicated that alien germplasm retention decreased every generation of inbreeding, especially in Argentine and in *A. batizocoi* crosses. A similar trend was also observed for every cycle in BC_1F_2 and BC_1F_3 families, possibly, due to the loss of alien chromosomes following selfing of hexaploids. RAPD marker analysis of 40–chromosome interspecific hybrid derivatives from the four crosses supported previous reports that reciprocal recombination and/or translocations are the predominant mechanisms for exchange of chromosomal segments. No evidence was found for preferential transfer of alien chromosomal regions to specific linkage groups. The implications for developing disease resistant peanut breeding lines are discussed in light of these findings.

Communicated by M. Kearsey

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Introduction

The cultivated peanut (*Arachis hypogaea* L.) is a major oilseed crop of the tropics and subtropics and its cultivation extends also to warm areas of the temperate regions. The seeds are rich in protein and oil and make a substantial contribution to human nutrition. Members of the genus *Arachis* are widely distributed south of the Amazon river where speciation has generally followed waterways (Valls et al. 1985). Together, Krapovickas and Gregory (1994) and Valls and Simpson (2005) described 80 species and divided the genus into nine sections based on morphological similarities and geographical distribution. They also concluded that species in section *Arachis* are cross compatible with the cultivated species, *A. hypogaea* and the section contains 31 species. Most section *Arachis* species have an A-genome, a few with B-genome, and one species, *A. glandulifera* has a D-genome (Holbrook and Stalker 2003). The cultivated tetraploid ($2n = 4x = 40$) species, *A. hypogaea* and a wild tetraploid species, *A. monticola*, have AB genomes.

Many of the high yielding peanut cultivars available to growers are susceptible to several major pests and pathogens and chemical control has become a necessity to achieve high yields. Genotypes with satisfactory resistance are rare in the *A. hypogaea* gene pool. In contrast, high levels of resistance have been reported in wild species of section *Arachis* for many pathogens and

insect pests (Stalker and Moss 1987; Holbrook and Stalker 2003). However, transfer of resistant genes from these wild species into improved peanut cultivars has been very difficult because of genomic and ploidy barriers which restrict hybridization or cause hybrid failure/sterility (Tallury et al. 2005a).

In spite of this challenge, several breeding strategies have been proposed to utilize the diploid *Arachis* species for peanut improvement (Simpson 1991). Direct hybridization of cross compatible diploid species with *A. hypogaea* results in the production of sterile triploid hybrids, which, upon colchicine doubling of chromosomes result in fertile hexaploids (triploid–hexaploid route). Also, autotetraploids and amphidiploids can be produced prior to crossing with *A. hypogaea* to directly produce F_1 tetraploid interspecific hybrids. But the most frequent method used in peanut interspecific hybrid breeding is the triploid–hexaploid cytological route because of the ease of direct hybridization, but the chromosome number also must be lowered to $2n = 4x = 40$ from $2n = 6x = 60$. Hexaploid × diploid species crosses would be the fastest method to reduce the ploidy level, but Halward and Stalker (1987) were unable to recover progenies using this approach. Additionally, hexaploids hybrids can be selfed repeatedly to impose recombination between useful wild species chromosome segments and the chromosomes of *A. hypogaea*, and the continuous selfing may eventually lead to spontaneous chromosome loss to the tetraploid level. Finally, backcrossing hexaploid plants with *A. hypogaea* produces pentaploids, which can be selfed to obtain 40-chromosome derivatives, again, by spontaneous loss of chromosomes. The climax is that obtaining fertile 40-chromosome interspecific hybrid progenies does not insure useful introgression. The quickest methods to obtain tetraploid plants have not been highly productive for recovering germplasm with agronomic value (Stalker 1992). Thus, the transfer of desirable genes from the wild species into *A. hypogaea* remains unpredictable and difficult.

At present, very few 40-chromosome *Arachis* interspecific hybrid derivatives have resulted in populations with potential for agronomic improvement (Simpson and Starr 2001; Stalker and Beute 1993; Tallury et al. 2005b). The best example includes the progenies from the interspecific hybrid, *A. hypogaea* × *A. cardenasii* Krapov. and W.C. Gregory, produced by Smartt and Gregory (1967). Forty-chromosome lines were observed after five to eight selfing generations of the original hexaploid (Stalker et al. 1979). Stable introgression lines from this cross have since been identified with very high levels of resistance to nematodes (Stalker et al. 1995, 2002a), *Cercospora arachidicola* Hori (Stalker 1984; Stalker and Beute 1993; Stalker et al. 2002b), *Cercosporidium personatum* (Berk. et Curt.) Deighton (Stalker and Beute 1993; Stalker et al. 2002b), *Puccinia arachidis* (Reddy et al. 1996), *Sclerotium rolfsii* Sacc. (Reddy et al. 1996), leafhopper and corn earworm (Stalker and Campbell 1983; Stalker and Lynch 2002), and high yield (Guok et al. 1986; Reddy et al. 1996; Tallury et al. 2005b). All these traits, except for high yield, were directly derived from *A. cardenasii*. Further, RFLP and RAPD analyses of 46 interspecific hybrid lines revealed introgression of *A. cardenasii* chromosome segments into 10 of 11 linkage groups and into both genomes of the cultivated peanut (Garcia et al. 1995). They concluded that recombination between *A. hypogaea* and *A. cardenasii* genomes occurred at the hexaploid level via translocations and reciprocal recombination.

Overall, information regarding efficiency of gene transfer between wild and cultivated peanut is lacking. The objective of this investigation was to evaluate the triploid–hexaploid cytological route using RAPD markers to trace the eventual transfer of alien germplasm from diploid wild species to *A. hypogaea*. Families from four interspecific crosses at different selfed- and backcross generations were analyzed with 24 RAPD markers of known map locations to determine the amount and stability of wild species germplasm retention in interspecific hybrid derivatives.

Material and methods

Plant material and establishment of interspecific hybrid progenies

Two *A. hypogaea* cultivars (subsp. *hypogaea* var. *hypogaea* cv. NC 6 and subsp. *fastigiata* var. *vulgaris* cv. Argentine) were used as female parents in crosses with two section *Arachis* diploid speciges, *A. cardenasii* (GKP 10017, PI 262141) and *A. batizocoi* Krapov. and Rigoni (K 9484, PI 298639) during the summer of 1990 to produce F_1 triploid interspecific hybrids. The diploid species were chosen as the donor parents because they have different genomes, *A. cardenasii* having the A genome, and at the time the crosses were made, *A. batizocoi* is the only known B genome species. Also, these two species have resistance to late leafspot (*Cercospora personatum*), early leafspot (*C. arachidicola*), root knot nematode (*Meloidogyne arenaria*), rust (*P. arachidis*), and several insects (Abdou et al. 1974; Reddy et al. 1996; Stalker and Campbell 1983; Subrahmanyam et al. 1985; Stalker and Moss 1987; Nelson et al. 1990).

When the triploid hybrids were fully established with several branches, vegetative branches, which were at least 10 cm long with developing apical leaflets, were cut and the cut end was placed overnight into an aqueous solution of 0.2% colchicine to induce doubling of chromosome number and restore fertility at the hexaploid level. Seeds from fertile sectors (C_0 generation) were planted to establish the C_1 generation hexaploids. Hexaploids were self-pollinated for four generations and used as pollen parents (C_1, C_2, C_3, and C_4 generations) in crosses with the respective *A. hypogaea* parent. Backcross families were derived from each hexaploid generation and shown as a lateral branch (Fig. 1). All families tracing back to a single hexaploid hybrid were referred to as a cycle. To make valid comparisons among cycles and among gen-

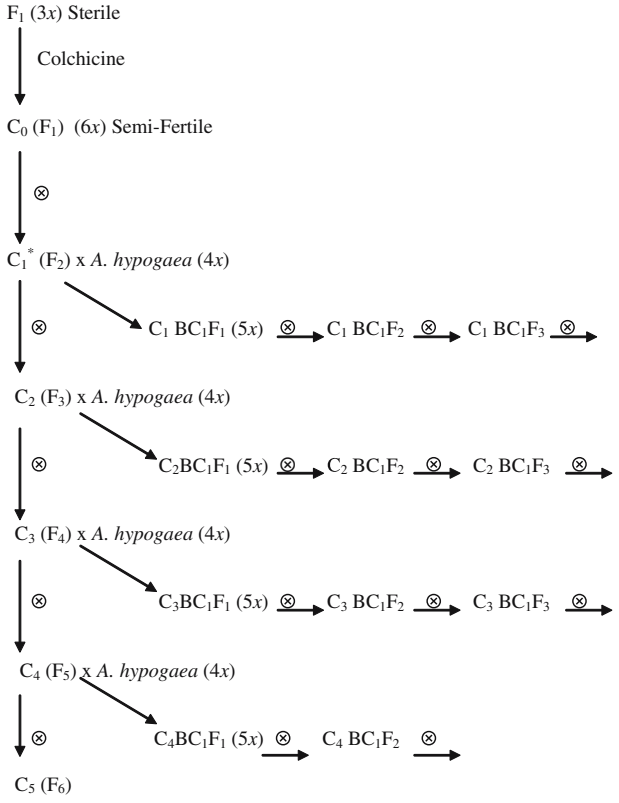

Fig. 1 Breeding scheme used to develop interspecific hybrid populations

erations within cycles, only lineages tracing back to a single hexaploid hybrid were used in each lateral branch. Many families were thus discarded because most pentaploid hybrids did not produce seeds (Table 1).

RAPD analysis

DNA isolation, reaction mixtures, PCR amplification conditions, and DNA size fractionation were as described by Garcia et al. (1995). Because the molecular linkage map of peanut was generated from the backcross population [*A. stenosperma* × (*A. stenosperma* × *A. cardenasii*)] (Garcia et al. 2005), mapped markers were screened against NC 6, Argentine, *A. cardenasii*, and *A. batizocoi* to identify polymorphisms before they were used to detect alien germplasm transmission and possible retention. This screening provided a number of markers that are specific to detect wild species alleles. Because most of these markers were polymorphic in both *A. cardenasii* crosses (Argentine × *A. cardenasii* and NC 6 × *A. cardenasii*), 17 RAPD markers were selected according to their map position to provide the best possible genome coverage (Garcia et al. 2005) An additional eight *A. cardenasii*-specific unmapped markers were also used to monitor alien germplasm transfer. Despite the high level of phenotypic variation between *A. batizocoi* and

Table 1 Number of interspecific hybrids from four crosses using two diploid species obtained in different generations from triploid–hexaploid pathway

Generation	Pollinations	Plants which produced seed	Plants obtained	Plants analyzed[a]
F_1	520		120	4
$C_1(F_2)$			31	4
$C_1BC_1F_1$	3,354		161	5
$C_1BC_1F_2$		40	139	19
$C_1BC_1F_3$		51	223	28
$C_2(F_3)$		30	235	7
$C_2BC_1F_1$	2,317		20	7
$C_2BC_1F_2$		11	39	19
$C_2BC_1F_3$		16	98	45
$C_3(F_4)$		22	48	6
$C_3BC_1F_1$	1,736		75	14
$C_3BC_1F_2$		17	57	5
$C_4(F_5)$		29	169	4
$C_4BC_1F_1$	3,570		64	14

[a]Plants analyzed for introgression

A. hypogaea, only seven of the mapped RAPD markers were polymorphic between these species (Garcia 1995). Thus, most of the scored markers (27) in *A. batizocoi* derived populations were of unknown map position.

When the banding pattern of an interspecific hybrid was similar to either *A. cardenasii* or *A. batizocoi*, the result was scored as a positive signal for the evidence of retention of a wild species locus. Retention at each locus was then calculated as the number of plants showing the band at that locus over the total number of plants in the generation being studied (expressed as a percentage). Comparisons were then made among cycles and among generations within cycles. Among selfed generations within each cycle (lateral branch, Fig. 1), a total retention percentage was calculated as the number of plants showing the corresponding species-specific band in the last generation of the cycle over the total number of plants in the cycle. This accounts for plants with potential wild species germplasm incorporation but did not produce progeny, and serves as a correction factor for reduced fertility in some hybrids. Within each cross, families from different cycles but in the same generation were compared for retention at each locus utilizing a chi-square contingency table (retained versus not retained in different cycles). Hexaploids plants (C_1, C_2, C_3, and C_4 generations) also were scored for germplasm retention as indicated by the presence of species-specific RAPD bands.

Cytological analysis

Mitotic preparations of plants utilized root tips from germinated seedlings, rooted lateral cuttings, or from immature leaf tissues (Dhesi and Stalker 1994). Chromosome numbers were obtained from all plants at every generation and after every backcrossing.

Results

Chromosome analysis in BC_1F_1 and BC_1F_2 progenies of the four crosses

Based on our past observations, progenies from BC_1F_1 pentaploid ($2n = 5x = 50$) interspecific hybrids were expected to reach 40 (or near 40) chromosomes after two generations of selfing. On the whole, the chromosome number of BC_1F_1 and BC_1F_2 plants from the four crosses ranged from $2n = 40$ to $2n = 55$ (Table 2). Sister progenies varied in chromosome number and had mostly reduced chromosome numbers than their progenitor plants. Thirty percent of the BC_1F_3 progenies had 40 chromosomes as compared to 10.8% of the BC_1F_2 progenies (data not shown; Garcia 1995). Higher numbers of progenies from the Argentine crosses reached 40 (or near 40) chromosomes after two generations of selfing than progenies from NC 6 crosses. Progenies from *A. batizocoi* crosses also reached 40 (or near 40) chromosomes more often and faster than progenies from *A. cardenasii* crosses (Table 2).

RAPD marker analysis

A total of 59 RAPD markers, including 25 (17 mapped plus eight unmapped *A. cardenasii*-specific markers) and 34 (7 mapped plus 27 unmapped *A. batizocoi*-specific markers), were used to evaluate progenies of four interspecific crosses at different selfing and backcross generations. The C_1 and C_2 hexaploids retained over 90% of the analyzed markers in both the crosses. However, NC 6 × *A. cardenasii* hexaploid hybrids started to lose markers in the C_3 and C_4 generations (Table 3).

The four crosses in all cycles showed a substantial decrease of retention with every generation of selfing after the initial backcross (Table 3). The decrease was more pronounced in *A. batizocoi* crosses. However, the Argentine × *A. cardenasii* C_2 BC_1F_2 progeny were derived from a single pentaploid plant (BC_1F_1) that showed positive signal in five of the 25 markers and expressed a low initial retention of 47.9% (Table 3).

Table 3 Mean marker retention of *Arachis* species germplasm in interspecific hybrids

Cross	Progeny			
	(6*x*) % Retention	BC_1F_1 (5*x*)	BC_1F_2	BC_1F_3
C_1				
NC 6 × *A. cardenasii*	96.20	94.70	57.00	60.50
Arg. × *A. cardenasii*	92.00	83.51	70.20	42.50
NC 6 × *A. batizocoi*	95.70	89.60	37.90	13.80
Arg. × *A. batizocoi*	93.75	85.70	58.00	17.00
C_2				
NC 6 × *A. cardenasii*	92.60	80.95	52.80	23.94
Arg. × *A. cardenasii*	92.00	47.90	31.70	11.40
Arg. × *A. batizocoi*	93.75	93.10	46.55	11.40
C_3				
NC 6 × *A. cardenasii*	88.40	76.20	61.90	30.95
Arg. × *A. cardenasii*	92.00	79.20		
C_4				
NC 6 × *A. cardenasii*	79.30	83.80	34.90	

Marker retention among cycles

A. cardenasii crosses

For all markers in NC 6 × *A. cardenasii*, except AA12/850, no significant differences for germplasm retention were observed among cycles at the BC_1F_1 generation (pentaploids), which showed a high percentage of retention (mean = 83.9%) (Table 3). In the BC_1F_2, nine of 20 markers analyzed showed significant ($P < 0.05$) differences among cycles and, in the BC_1F_3, 18 out of 20 showed highly significant ($P < 0.001$) differences among cycles for germplasm retention. In all cases, retention was higher in the first cycle (C_1) and decreased with succeeding cycles, with the most pronounced decrease among the BC_1F_3 generation plants (two generations of selfing after backcrossing) across different cycles.

Similarly, in Argentine × *A. cardenasii* crosses, except for marker P9/920, there were no significant differences in germplasm retention among cycles in the BC_1F_1 generation. However, the mean percentage of retention was lower (70.2%) than for the NC 6 cross with *A. cardenasii*, which was 83.9% (Table 3). Thirteen of 22 markers showed significant ($P < 0.05$) differences among cycles in the BC_1F_2 generation, and all markers except

Table 2 Distribution of chromosome numbers in progenies from BC_1F_1 ($2n = 50$) and BC_1F_2 plants

Cross	Progeny from	Chromosome numbers															
		40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
NC 6 × *A. card.*	BC_1F_1				1	3	2	3	1	1	1			2	1		1
	BC_1F_2	3	2	4	1	3		2		1							6
Argentine × *A. card.*	BC_1F_1	3		1		1		2	1	1		1	1				1
	BC_1F_2	11	1	3	2	2	4	4				1					
NC 6 × *A. bati.*	BC_1F_1							1		1							
	BC_1F_2	2	2	1				1									
Argentine × *A. bati.*	BC_1F_1	1					1	1		1				3			
	BC_1F_2	5	4	3	1		1		1							1	
Totals	BC_1F_1	4		1	1	4	3	7	2	4	1	1	1	2	4		2
	BC_1F_2	21	9	11	4	5	5	7	1	1		1				1	6

C5/1200 showed highly significant ($P < 0.001$) differences among cycles in the BC_1F_3 generation. Although only two cycles could be compared in the BC_1F_2 and BC_1F_3 generations, this cross showed the same tendency of decaying retention within and among cycles as the NC 6 × *A. cardenasii* cross; however, more pronounced differences among cycles seemed to start at earlier generations (BC_1F_2) and the overall initial level of retention was lower in Argentine × *A. cardenasii* progenies.

A BC_1F_3 40-chromosome derivative from a NC 6 × *A. cardenasii* cross (line 745-1) showed *A. cardenasii* DNA in five different linkage groups and three unmapped markers. The presence of *A. cardenasii*-specific markers in linkage groups 3 and 11 could have resulted from a chromosome substitution because both markers at each linkage group were scored positive; but the presence of markers C5/1200, A11/640, and P9/650 in linkage groups 1, 5, and 7, respectively, indicated that translocations and/or recombination were responsible for the occurrence of these markers. Likewise, introgression detected by markers, C5/1200 in linkage group 1 and I17/850 in linkage group 7, in the related 40-chromosome BC_1F_3 lines 771-1/6 and 772-1/6 also suggested that recombination or translocations resulted in the transfer of *A. cardenasii* chromosomal segments. Because these 40-chromosome sister lines and their 40-chromosome progenitor plant showed incorporation at the same linkage group, a single recombination event must have occurred during an early hybrid generation (probably between BC_1F_1 and BC_1F_2). C_3, C_4, and C_5 plants (from F_4, F_5, and F_6 hexaploids, respectively) were negatively scored for markers at four different linkage groups, suggesting that F_4 hexaploids started to drop chromosomes.

A. batizocoi crosses

None of the markers showed significant differences among cycles in the BC_1F_1 generation of Argentine × *A. batizocoi*. Twelve of the 30 markers analyzed showed significant ($P < 0.05$) differences among cycles in the BC_1F_2 generation, and all the markers showed highly significant ($P < 0.001$) differences among cycles in the BC_1F_3 generation. Like the *A. cardenasii* crosses, and despite a high retention percentage in the BC_1F_1 generation (81.4%), retention decayed with every cycle for almost 50% of the markers in the BC_1F_2 and for all markers in the BC_1F_3. The final retention of *A. batizocoi* crosses was lower than for *A. cardenasii* crosses (Table 3). Because only one cycle could be completed in the NC 6 × *A. batizocoi* cross (due to very low fertility of the pentaploids), comparisons among cycles could not be performed.

Although very few markers could be assigned to specific linkage groups in BC_1F_3 lines, 40-chromosome plants were scored for several markers. This suggests that introgression is not restricted to a specific chromosome (e.g., lines 798-1 and 754-6 showed introgression at four different loci).

Discussion

Direct hybridization, chromosome doubling to obtain hexaploid hybrids and derivation of stable-tetraploid germplasm lines from the hexaploids, has been considered as a practical means to utilizing potential germplasm resources in genus *Arachis* (Stalker 1992). However, transfer of desirable genes from wild species to *A. hypogaea* has been a slow and laborious process. This is largely due to cross-incompatibilities and sterility barriers, which limit gene transfer between species. Nonflowering or nonpegging plants are commonly obtained at several ploidy levels and creation of 40-chromosome populations is indeed a frustrating challenge.

The most tenable pathway from 60 to 40-chromosome plants is to backcross hexaploids as male parents with the cultivated parent to produce 50-chromosome (pentaploid) intermediates. Some pentaploid hybrids are semi-fertile and upon selfing produce 40 or near 40-chromosome progenies. However, two major genetic bottlenecks of this pathway include (a) recovery of fertile hexaploid plants from colchicine-treated triploid F_1 hybrids and (b) obtaining BC_1F_2 progenies from the pentaploid hybrids (Table 1). Use of molecular markers should enhance the ability to follow specific chromosome segments and, therefore, to identify progenies with stable incorporation of the desired chromosomal regions. The rationale for the crossing scheme utilized in this study was to compare the efficiency of retention or the rate of loss of alien germplasm at different stages of inbreeding after chromosome doubling and backcrossing. From our earlier observation of hexaploid hybrid progenies, it was realized that selfing with selection of hexaploids for several generations before backcrossing would provide more opportunity for intergenomic chromosome pairing and recombination between the chromosomes of *A. hypogaea* and their counterparts in wild species.

In the present study, the retention of wild species germplasm within each cycle of backcrossing decreased with every generation of selfing for the four crosses. The somatic chromosome number of these lines ranged from 40 to 55 with a marked tendency for chromosome reduction after each generation of selfing. This indicates that the decrease in wild species germplasm retention is due mainly to chromosome loss. Derivatives from *A. batizocoi* crosses showed a steeper rate of germplasm decay, but also a higher number of 40 or near 40-chromosome progenies after two generations of selfing than derivatives from *A. cardenasii* cross. This could have been because *A. batizocoi* has a lower chromosome affinity with *A. hypogaea*, as indicated by several studies which have shown that it forms very distinctive cytological and genetic groups from other section *Arachis* diploid and tetraploid species (Stalker and Dalmacio 1981; Halward et al. 1991; Kochert et al. 1991; Bianchi-Hall et al. 1993; Stalker et al. 1994; Tallury et al. 2001; Milla et al. 2005); however, more progenies and additional cycles need to be analyzed before definite

conclusions can be made. The lower retention and faster reduction in chromosome number of Argentine versus NC 6 crosses also may have been due to different affinity of *A. cardenasii* and *A. batizocoi* with *A. hypogaea* types. *Arachis hypogaea* subsp. *hypogaea* (e.g., cv. NC 6) is considered more primitive (Stalker and Simpson 1995) and therefore may show more affinity with the wild species genome than subsp. *fastigiata* (e.g. Argentine).

Comparisons among families at the same generation from different cycles (C_1 to C_3 BC_1F_1s, C_1 to C_3 BC_1F_2s, or C_1 to C_3 BC_1F_3s) showed a significant reduction of wild species germplasm retention for every cycle that the hexaploid is self-pollinated before backcrossing was initiated. No differences were detected among BC_1F_1 families where the level of retention was high; however, almost 50% of the markers showed a significant decay in retention when BC_1F_2 families were compared, and 100% of the markers showed a highly significant ($P < 0.01$) decrease in retention among BC_1F_3 families. This was unexpected because it was opined that self-pollinating hexaploids for several generations would increase the frequency of intergenomic pairing and recombination. Selection for fertile hexaploids, which presumably is a manifestation of regular bivalent pairing, could reduce intergenomic exchange and could have been responsible for the reduced retention observed after each generation of selfing.

On the other hand, chromosome loss during the selfing of initial hexaploid hybrids could have been partially responsible for the progressive reduction in germplasm retention observed in cycles 2, 3, and 4. Although not analyzed cytologically, F_4, F_5 and F_6 progenies from the initial hexaploid hybrid were scored negative for a series of markers in four linkage groups. Spielman et al. (1979) and Company et al. (1982), after analyzing hexaploid plants from several interspecific crosses, suggested that the probable mechanism leading to chromosome loss was heterogenetic pairing, giving rise to gametes with unbalanced sets of chromosomes leading to aneuploid progenies. They also assumed that the loss of chromosomes was a random event involving both *A. hypogaea* and wild species genomes. If the reduction in alien germplasm retention observed in this study was due to preferential wild species chromosome loss, then the reduction should have been restricted to markers associated with the lost chromosomes. However, the reductions involved the majority of the analyzed markers. Unfortunately, no estimates of *A. hypogaea* marker retention were made because only diploid species-specific RAPD markers were studied.

The analysis of a group of 40-chromosome sister lines in the present study which traced back to a common pentaploid hybrid indicated that in some cases stability of germplasm retention occurred just one generation after backcrossing because a majority of observed markers were homozygous. In other progenies, there were several markers still segregating two generations after backcrossing, which indicated that recombination of wild species chromosomes occurred at a later time.

Because the number of 40-chromosome derivatives obtained in each cycle was similar and the amount of alien germplasm retention in tetraploid derivatives was not significantly different, backcrossing hexaploids to the cultivated parent should start as soon as fertility is restored. To mitigate the genetic bottlenecks found at different cytological stages, crossing efforts should be concentrated in obtaining a large number of pentaploid hybrids. Because pentaploids are usually vegetatively robust plants, which produce few seeds, they ideally need to be planted outdoors in a location with a long growing season to acquire progenies. Unfortunately, this has been a major limitation of our research program in North Carolina, where the first frost, usually in early to mid October, does not provide a long enough growing season for interspecific hybrids to produce viable seeds in large numbers. Further, evaluating these progenies for a high percentage of germplasm retention from the wild species should increase the frequency of lines probably with desired germplasm. When species-specific markers are linked to desired traits to be introgressed, the overall efficiency of genetic advancement will be increased by allowing selection of progenies exhibiting the linked markers. This will also reduce linkage drag in interspecific breeding by discarding progenies with a large amount of wild species DNA other than the specific desired trait.

Acknowledgments The research reported herein was a cooperative effort of the North Carolina Agric. Res. Serv., a USDA competitive grant (#92-37300-7516) from the National Research Initiative Plant Genome Program, and the Peanut CRSP, USAID grant no. DAN-4048-G-SS-2065-00 and the recommendations represent neither an official position nor policy of the NCARS, USDA, or USAID. We also wish to thank Dr. J.S. Dhesi and J.A. Burns for their help with the chromosome counts and S.C. Copeland for her technical help during the crossing programs, seed planting, leaf collection, and DNA extractions.

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