 Cytologia **73**(3): 305–311, 2008

Karyotypic Variability in Experimental Diploid and Triploid Hybrids of *Aloe vera*×*A. saponaria*

José Imery-Buiza[1,*], María B. Raymúndez[2] and Andrea Menéndez-Yuffa[2]

[1] Laboratorio de Genética Vegetal, Herbario IRBR, Departamento de Biología, Universidad de Oriente, P.O. Box 245, Cumaná 6101, Venezuela
[2] Centro de Botánica Tropical, Instituto de Biología Expimental, Facultad de Ciencias, Universidad Central de Venezuela, P.O. Box 47114, Caracas 1041A, Venezuela

Received July 28, 2008; Accepted September 28, 2008

Summary Cytogenetic traits were examined in *Aloe saponaria* (2*S*), *A. vera* (2*V* and 4*V*), and the experimental diploid (*VS*) and triploid (*VVS*) hybrids. Karyotype $2n=2x=14$ (8L+6S) was confirmed in 2*S* and 2*V*, whereas $2n=4x=28$ (16L+12S) was ascribed to 4*V*. *VS* showed the expected karyotype $2n=2x=14$ (8L+6S), with slight differences between the long (L) homologues. *VVS* also showed the expected karyotype $2n=3x=21$ (12L+9S); however, several chromosome deviations reflecting the absence or addition of small (S) chromosomes, terminal deletions of variable size which led to the formation of atypical chromosomes, or the loss of a L chromosome were also observed. The implication of these karyological variations is discussed.

Key words *Aloe*, Aneuploid, Chromosome, Deletion, Hybrids, Polyploidy.

Aloe vera (L.) Burm.f. (=*A. barbadensis* Mill.) is a succulent plant highly exploited agroindustrially due to its multiple medicinal and cosmetic properties. It was introduced into the Americas in the 16th century during the Spanish occupation and today it is cultivated extensively on the Caribbean islands and other arid or semiarid regions (Carter 1994). Incidence of the bacterial leaf rot caused by *Erwinia chrysanthemi* in plantations from Aruba, Mexico, Venezuela and India (De Laat *et al.* 1994, Mandal and Maiti 2005), has led to define control strategies (Lugo 1999), and the search for new varieties. In order to increase genetic variability and the possibility of achieving promising genotypes we suggest here reap the benefits vegetative already reached with tetraploides *A. vera* (Imery and Cequea 2001) and combine them with genetic variability generated by hybridization with *A. saponaria* Haw. (=*A. maculata* Medik.), an exotic species with therapeutic properties (Reynolds 2004), grown in South America chiefly as an ornamental plant, less affected by the pathogenic bacteria and highly cross-compatible with *A. vera* (Imery and Cequea 2008). Thus, the aim of the present work was to characterize the cytogenetic variation of the parental species, and hybrids obtained from the direct crossing between *A. saponaria* as pollen parent and the diploid and tetraploid *A. vera* as the female/seed parents.

Materials and methods

Parental species and their interspecific hybrids were grown under greenhouse conditions. For experiments we used three parental genotypes: diploid *A. saponaria*, named 2*S*, as male, obtained from Mesa Garden (NM, USA), two *A. vera* types as females, one a diploid named *2V* and was collected from a naturalized population on the peninsula of Araya (10°36′34″N, 64°07′18″W), and the second was a tetraploid induced by Imery and Cequea (2001) and named 4*V*. The diploid (*VS*) and

* Corresponding author, e-mail: jimeryb@cantv.net

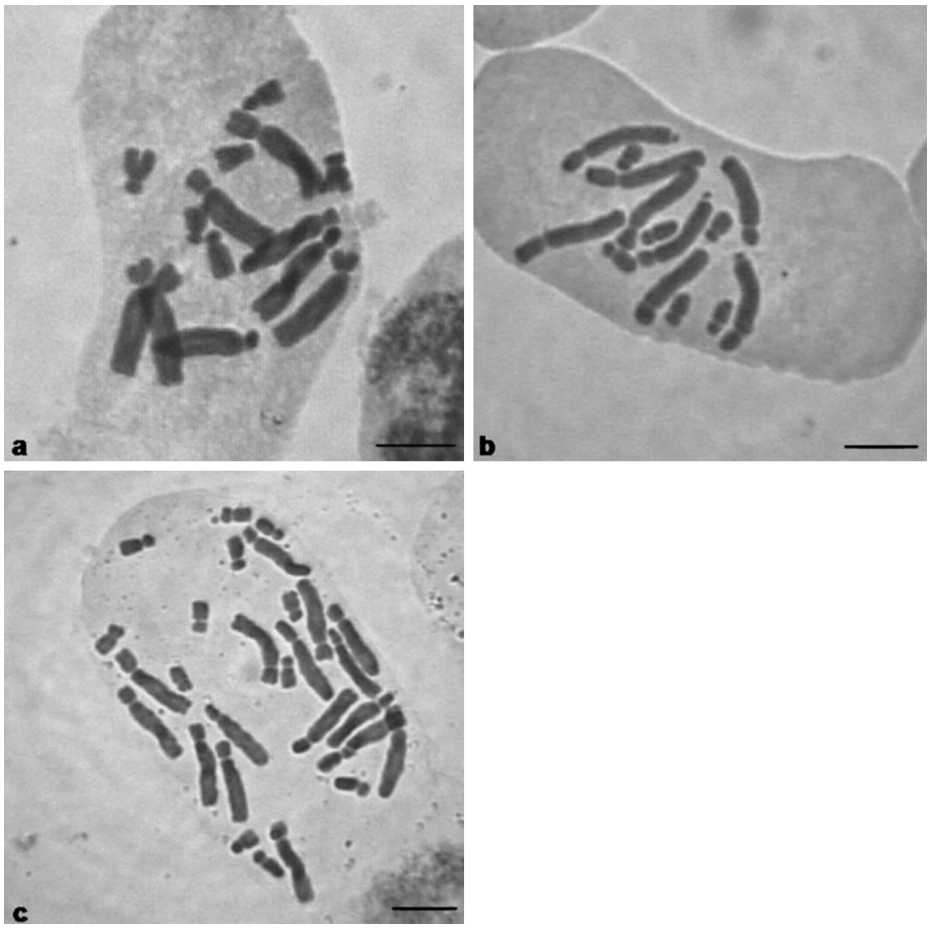

Fig. 1. Mitotic chromosomes from parental *Aloe saponaria*, 2*S* (a), *A. vera* diploid, 2*V* (b), and tetraploid 4*V* (c). Scale Bars=10 μm.

triploid (*VVS*) hybrids were obtained by direct pollination of the stigmas of the 2*V* and 4*V* plants with pollen from 2*S* flowers.

Mitotic chromosomes were examined using meristematic tissues from the roots: a) cutting root tips of 2–2.5 cm (7:30–8:15 am); b) pretreatment with colchicine 0.05% for 3 h at room temperature (RT) and in total darkness, followed by a wash in distilled water (H_2O_d) for 5 min; c) fixing in Carnoy's fixative (3 : 1 abs. ethanol/glacial acetic acid) for 24 h at 4°C; d) rehydration in H_2O_d for 20 min, and hydrolysis with 1 N HCl for 10 min at RT, followed by washing in H_2O_d for 10 min; e) individual dissection of the root tips, suspended in a drop of H_2O_d on a coverglass, for the selection of the meristematic zone; f) staining of the dissociated meristem with two drops of 2% propionic-orcein for 3–4 min; g) placement of cover-slip, eliminating the excess stain from the edges with absorbing paper, and a gentle squash over the stained root tips.

The karyotype characterizations were carried out using five metaphasic cells for each plant. Photomicrographs were taken at 1500 X on a Nikon Optiphot microscope and the images were later analyzed by computer with the SigmaScan Pro 5 program. The features examined were the number of chromosomes, short arm (sa), large arm (la), and chromosome length (L=sa+la), total length of the karyotype (LK=#L), and arm ratio (r=la/sa) of each chromosome. These mitotic chromosomes were then classified according to size (Brandham 1971) and position of the centromere (Levan *et al.* 1964).

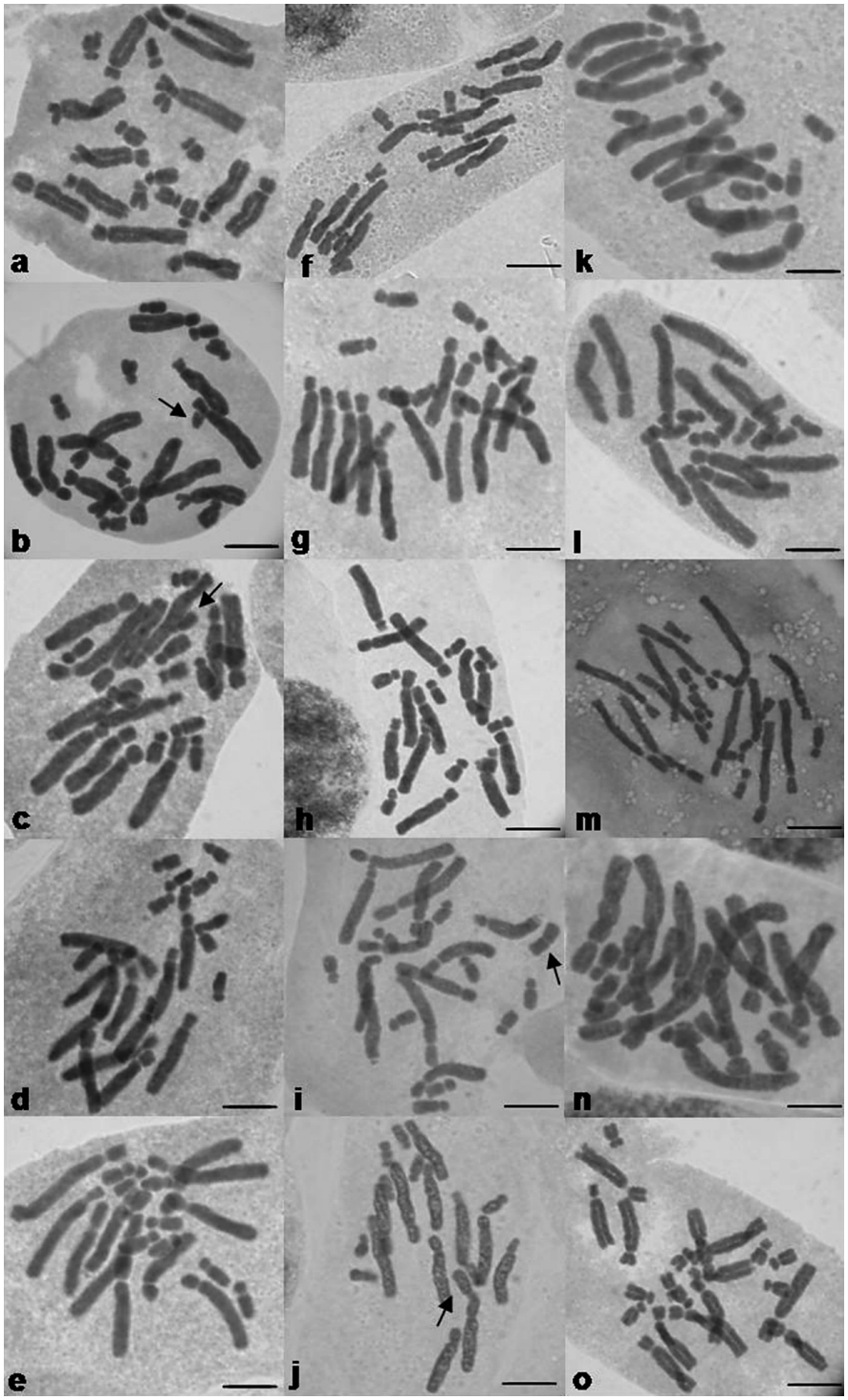

Fig. 2. Mitotic chromosomes from triploid hybrids (*VVS*), stained with propionic orcein. (a) VVS_{38}, (b) VVS_{41}, (c) VVS_{46}, (d) VVS_{52}, (e) VVS_{66}, (f) VVS_{69}, (g) VVS_{73}, (h) VVS_{77}, (i) VVS_{86}, (j) VVS_{113}, (k) VVS_{121}, (l) VVS_{140}, (m) VVS_{172}, (n) VVS_{179}, and (o) VVS_{185}. Arrows indicate atypical chromosomes (in b, c, i and j). Scale Bars = 10 μm.

Table 1. Number, size and classification of mitotic chromosomes, and karyotype length in the parental species *Aloe saponaria* (♂, 2*S*), *A. vera* (♀, 2*V* and 4*V*) and their experimental diploid (*VS*) and triploid (*VVS*) hybrids

Plant	Number and size	Karyotype formula	Variation	Karyotype Length (μm)
2*S*	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	165.6±2.5
2*V*	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	133.0±2.4
4*V*	2*n*=4*x*=28=16L+12S	4L*sm*+12L*st*+12S*sm*	—	262.4±3.9
VS_{03}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	148.9±1.9
VS_{07}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	150.3±8.3
VS_{13}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	146.3±2.9
VS_{35}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	148.1±5.5
VS_{37}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	158.6±4.6
VS_{54}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	148.0±1.2
VS_{67}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	158.9±9.5
VS_{74}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	145.5±6.8
VS_{91}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	148.9±6.1
VS_{92}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	152.4±3.8
VS_{95}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	147.9±1.9
VS_{100}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	150.8±2.5
VS_{112}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	145.8±1.5
VS_{118}	2*n*=2*x*=14=8L+6S	2L*sm*+6L*st*+6S*sm*	—	145.3±3.8
VS_{132}	2*n*=2*x*=14=8L+6S	1L*sm*+7L*st*+6S*sm*	—	152.8±2.9
VVS_{38}	2*n*=3*x*=21=12L+9S	3L*sm*+9L*st*+9S*sm*	—	215.6±3.1
VVS_{41}	2*n*=3*x*=21=11L+10S	2L*sm*+9L*st*+10S*sm*	$-1L_4+1S_{(at)}$	203.6±7.0
VVS_{46}	2*n*=3*x*+2=23=12L+11S	2L*sm*+9L*st*+12S*sm*	$+1S_1+1S_{(at)}$	224.7±9.9
VVS_{52}	2*n*=3*x*+1=22=12L+10S	1L*sm*+11L*st*+10S*sm*	$+1S_2$	217.3±3.5
VVS_{66}	2*n*=3*x*-1=20=11L+9S	3L*sm*+8L*st*+9S*sm*	$-1L_4$	199.9±9.7
VVS_{69}	2*n*=3*x*+1=22=12L+10S	1L*sm*+11L*st*+10S*sm*	$+1S_2$	219.5±7.7
VVS_{73}	2*n*=3*x*+1=22=12L+10S	2L*sm*+10L*st*+10S*sm*	$+1S_1$	221.1±9.6
VVS_{77}	2*n*=3*x*-1=20=12L+8S	1L*sm*+11L*st*+8S*sm*	$-1S_1$	209.9±9.7
VVS_{86}	2*n*=3*x*+1=22=12L+1M+9S	2L*sm*+10L*st*+1M*m*+9S*sm*	$+1M_{(at)}$	221.0±4.2
VVS_{113}	2*n*=3*x*-1=20=11L+1M+8S	2L*sm*+9L*st*+1M*sm*+8S*sm*	$-1L_4$-$1S_3+1M_{(at)}$	202.3±9.8
VVS_{121}	2*n*=3*x*-1=20=11L+9S	2L*sm*+9L*st*+9S*sm*	$-1L_4$	198.8±9.9
VVS_{140}	2*n*=3*x*-1=20=11L+9S	1L*sm*+10L*st*+9S*sm*	$-1L_4$	199.7±6.5
VVS_{172}	2*n*=3*x*+1=22=12L+10S	2L*sm*+10L*st*+10S*sm*	$+1S_1$	218.5±5.1
VVS_{179}	2*n*=3*x*-1=20=12L+8S	2L*sm*+10L*st*+8S*sm*	$-1S_3$	205.1±5.7
VVS_{185}	2*n*=3*x*=21=12L+9S	1L*sm*+11L*st*+9S*sm*	—	212.4±2.2

Abbreviations: L, M, and S=large, intermediate, and small chromosomes, respectively, in accordance with Brandham (1971). *m*, *sm*, and *st*=chromosome with median, submedian, and subterminal centromere, respectively, according to Levan *et al.* (1964). at = atypical chromosome (not expected from parental karyotypes). Plus or minus signs indicate the presence or lack of a chromosome, respectively. Karyotype lengths represent means±S.D. (n=5) in metaphasic cells.

Results and discussion

In the meristematic of root tips, we consistently found $2n=2x=14$ chromosomes in the 2*S* and 2*V* parental species, and $2n=4x=28$ in tetraploid *A. vera* (Fig. 1). In the *VS* hybrids, the karyotypes showed the expected formula of $2n=2x=14$ chromosomes, whereas in the *VVS* hybrids the karyotypes varied between $2n=3x-1=20$ to $2n=3x+2=23$ chromosomes (Fig. 2, Table 1). Most of the species of the family Aloaceae show a bimodal karyotype, with $2n=2x=14$ chromosomes, with the exception of a few polyploid species with 21, 28, 35 or 42 somatic chromosomes. The basic number $x=7$ comprises four large acrocentric chromosomes (L_1–L_4) of 12–18 μm and three small submetacentric ones (S_1–S_3) of 4–6.5 μm (Brandham 1971). Within the karyotypes, the *VS* hybrids showed slight differences of length among homologues, and even variations of morphology in the pair L_1 of the specimens identified as VS_{35}, VS_{54}, VS_{74}, VS_{91}, VS_{95}, and VS_{132} (Table 1). This karyo-

logical variability may be attributed to the greater length of the chromosomes of *A. saponaria* and the heteromorphism detected in its pair L_1, which is inherited in a Mendelian manner among the progeny.

In our work, although the parental diploid species showed the same number of mitotic chromosomes $2n=2x=14$ (8L+6S), the LK of 2*S*/LK of 2*V* (1.25) points to the existence of karyological differences between *A. vera* and *A. saponaria*, probably due to the longer length of the chromosomes of the latter. On the other hand, the relation $4V/2V=1.97$ confirms the direct duplication of the ploidy level from $2n=2x=14$ chromosomes in *A. vera* (2*V*) to its descendant experimental tetraploid 4*V* ($2n=4x=28$).

Brandham and Doherty (1998) had examined the morphometric variations among chromosomes from various species of the family Aloaceae and reached the conclusion that the increased amounts of DNA occurred in a proportional way in large and small chromosomes, thus maintain their relative lengths and the bimodality of the karyotype of most *Aloe* species. Imery and Caldera (2002), who detected significant differences between *A. saponaria* and *A. vera* when comparing the lengths of each pair of homologues.

In *VVS* hybrids, besides the variations of the parental chromosome lengths, we noticed terminal deletions of variable size in the longer arms of L chromosomes, as signaled by the presence of atypical chromosomes in the specimens VVS_{41}, VVS_{46}, VVS_{86}, and VVS_{113}. Other abnormalities involved the lack of a L_4 chromosome in VVS_{66}, VVS_{121}, and VVS_{140}, the absence of an S_1 or S_3 chromosome in VVS_{77} and VVS_{179}, and the addition of one S_1 or S_2 in VVS_{52}, VVS_{69}, VVS_{73}, and VVS_{172} (Fig. 1, Table 1). Such differences in structure and numbers of chromosomes between triploid hybrids incide directly on the total length of their karyotypes. The VVS_{38} and VVS_{185} hybrids with the expected karyotype $2n=3x=21$ (12L+9S), gave a mean karyotype length of 214 μm but the other triploid hybrids also showed values ranging between 198.8 μm (VVS_{121}) to 224.7 μm (VVS_{46}).

Diploid plants in the genera *Aloe*, *Gasteria* and *Haworthia*, showed no signs of chromosomic deletion; however, in polyploid species the frequencies of such abnormalities were of 5.8% in *Aloe* and 2.5% in *Haworthia*, indicating that those genomic changes were deleterious in diploid species and that individuals with the highest levels of ploidy withstand better the genic unbalance caused by the loss of a chromosomic fragment (Brandham 1976). These arguments are supported by our findings, bearing in mind that deletions and chromosome losses were seen only in the *VVS* triploid hybrids. It seems likely that the karyological abnormalities inherited from parental 2*V* are deleterious to *VS* embryos, surviving only those with a balanced complement of parental genomes. This last genetic condition might be the chief reason for the greater amount of fruits and seeds formed by parental 4*V* following the crossing with pollen from 2*S* plants, and may explain the superior rate of germination and survival of the *VVS* progeny during its initial stages of development.

Aneuploidy in interspecific hybrids has been explained in terms of the loss of chromosomes as a consequence of incomplete DNA synthesis during interphase, physiological abnormalities of the achromatic spindle or the asynchrony of cell cycles in parental species, all leading to the elimination of chromosomal material from a given parental genome during the embryonic development of the progeny (Humphreys 1978, Mujeeb *et al.* 1978, Jackson 1985). Such variations have been found in polyploid hybrids in *Hordeum* (Linde-Laursen and Von Bothmer 1998), *Brassica* (Song *et al.* 1995), *Carica papaya*×*C. cauliflora* (Magdalita *et al.* 1997), *Helianthus annuus*×*H. tuberosus* (Natali *et al.* 1998), *Cucumis hystrix*×*C. sativus* (Chen *et al.* 2004) and in intergeneric hybrids of *Brassica napus*×*Orychophagmus violaceus* (Cheng *et al.* 2002). If this were the case concerning *A. saponaria* and *A. vera*, it could explain at least in part the origin of the aneuploidy $2n=3x-1=20$ (11L+9S) and (12L+8S). Nonetheless, it fails to elucidate the reasons for the aneuploidy $2n=3x+1=22$ (12L+10S), or the presence of atypical chromosomes in karyotypes $2n=3x=21$ (11L+9S+$1S_{(at)}$), $2n=3x+2=23$ (12L+10S+$1S_{(at)}$), $2n=3x+1=22$ (12L+$1M_{(at)}$+9S), and $2n=3x-1=20$ (11L+$1M_{(at)}$+8S).

Previous findings describing the presence of an intermediate (M) chromosome in the haploid set $n=x=7$ (3L+1M+3S) of *A. vera* (Marshak 1934) and in the triploid karyotype $2n=3x=21$ (11L+1M+9S) of the hybrids of *A. rauhii*×*A. dawei* (Brandham 1975), and *Gasteria nigricans*× *G. crassifolia* (Brandham 1977), point towards deviations from the expected bimodality $n=x=7$ (4L+3S) and $2n=3x=21$ (12L+9S), respectively. The morphometric similarity between those M chromosomes and some of the atypical ones seen in *VVS* hybrids suggest that the loss of chromosomal fragments occurs at a prezygotic level and not at the embryonic phase.

Dicentric bridges between large meiotic homologues, acentric fragments and additional microspores, indicate pairing abnormalities, spontaneous ruptures, sticking of sister chromatids, and a possible paracentric inversion in *A. vera* (Imery and Cequea 2002). These observations suggest that in our parental 4*V*, unequal ruptures in the dicentric bridges between L4 homologues, together with the formation of univalents and multivalents at prophase I and the subsequent unbalanced migration of small (S) chromosomes at anaphase I (Ramsey and Schemske 2002), ought to be the meiotic events responsible for the chromosome deletions, losses and additions transmitted to the *VVS* hybrids. Further research on karyological aspects of megasporogenesis in 2*V* and 4*V* plants may establish the existence of meiotic abnormalities equivalent to those seen during microsporogenesis and this may help to clarify the origin of variability in interspecific progenies.

Artificial hybridization between *A. vera* and *A. saponaria* freed the genetic variability retained by the asexual propagation of both species, and favored the formation of numerous genotypic combinations as a result of the intra- and interchromosomal recombinations from each progenitor. The meiotic abnormalities from *A. vera* introduced a further source of variation, as reflected by the karyological analyses of its progeny. These sources of variation, added to the effects of polyploidy, give rise to the range of morphotypes potentially useful in breeding programs.

Acknowledgments

Financial support was provided by funds from the Universidad de Oriente, Consejo de investigación CI-5-010101-1223/05.

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