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The chromosome segment related to apomixis in *Paspalum simplex* is homoeologous to the telomeric region of the long arm of rice chromosome 12

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Abstract

Apomixis is a form of asexual reproduction that in plants leads to the production of seed progeny that are exact copies of the mother individual. A mapping population generated by backcrossing a sexual with an apomictic genotype of *Paspalum simplex*, both at the tetraploid level, was used to find markers co-segregating with apomixis. Genetic analysis showed that apomixis is under the control of a single dominant allele assuming a random chromatid assortment. Five rice markers, mapped in the telomeric region of the long arm of rice chromosome 12, showed tight linkage with apomixis. Genetic and molecular data strongly indicate that the potentiality to express apomixis in *P. simplex* is given by a relatively large chromosome segment that is inherited as a single genetic unit.

Introduction

Apomixis is a form of reproduction that bypasses meiosis and fertilization of egg cells, giving rise to individuals that are exact copies of the mother. Two main forms of apomixis, sporophytic and gametophytic, have so far been recognized in plants. In sporophytic apomixis, commonly referred to as 'adventitious embryony' (Lakshmanan and Amgegaokar 1984), unreduced embryos originate from the somatic tissue of nucellus or the integument of the ovule. In gametophytic apomixis, the female gametophyte arises from a functionally differentiated structure, the embryo sac, which may originate from the megaspore mother cell (diplospory) or from the nucellar cells of the ovule (apospory) (Nogler 1984a). In both cases, the genetic consequence of apomixis is the clonal propagation of the mother plant through seeds (Asker and Jerling 1992). The potential benefits deriving from harnessing apomixis are obvious and vary from the full exploitation of heterosis by reseeding elite hybrids to the clonal propagation of superior genotypes in seed-propagated outcrossing crops (Ramulu et al. 1999). The impact of apomictic crops in agriculture would be comparable to, or even greater than, that of the green revolution, especially in third-world countries (Hanna 1995; Vielle-Calzada et al. 1996a). Barring few exceptions regarding some forage grasses and fruit trees, apomixis is not a common feature among crop species, but studies aimed at the direct transfer of apomixis from wild relatives to crop species have resulted to date in partially fertile, agronomically unsuitable lines with added chromosomes (Vielle-Calzada et al. 1996a). Thus, efforts are being made to gain a better understanding of the molecular mechanisms of apomixis by screening mutants of the female gametophyte development in sexual model species (Ramulu et al. 1998), or isolating differentially expressed genes in apomictic and sexual lines (Vielle-Calzada et al. 1996b; Leblanc et al. 1997), or characterizing loci controlling apomixis in wild apomicts through map-based strategies (Ozias-Akins et al. 1998). The latter strategy consists in the isolation of markers tightly linked to the trait of interest and in tagging the cor-

responding gene by means of 'chromosome walking' or analogous procedures (Martin et al. 1993).

The species *Paspalum simplex* Morong is a warm-season grass adapted to the semi-arid environments of northern Argentina, which displays a number of characteristics that make it useful for tagging the apomixis gene using a map-based approach: chiefly among them the relatively small genome and the existence of sexually compatible apomictic and sexual lines (Caceres et al. 1999). Apospory, followed by parthenogenetic development of the embryo, is the gametophytic type of apomixis in *P. simplex*. The mature embryo sacs in apomictic and sexual lines of *P. simplex* are similar, having both 8 nuclei organized as one egg cell, two synergids, a binucleated central cell and three or sometimes more antipodals (Caponio and Quarín 1987). This type of apomictic gametogenesis is classified as *Hieracium*-type (Nogler 1984a). Apomictic lines of *P. simplex* segregated tetrasomically when used as male parents, reflecting their autotetraploid genetic architecture (Pupilli et al. 1997). Appropriate strategies, such as that of selecting single dose restriction fragments (SDRFs) and the construction of a proper mapping population (Wu et al. 1992), were then adopted in *P. simplex* (Pupilli et al. 1997). Recently, comparative mapping has revealed various extents of conservation of gene orders among Gramineae (Bennetzen and Freeling 1993). Comparative maps within the Gramineae include rice versus maize (Ahn et al. 1993), barley (Saghai-Maroof et al. 1996), oat (van Deynze et al. 1995a), wheat (van Deynze et al. 1995b) and millet (Devos et al. 1998). Moore et al. (1995) showed that all the maps of Gramineae can be combined into a single integrated grass map with rice as its base genome, because this species has the smallest cereal genome that has ever been studied extensively and for which there are the densest maps and most genomic tools available (Moore et al. 1997). Of all the molecular markers exploited to date, RFLPs have proved to be the most reliable and accurate tool for comparative mapping in rice and other species (Mohan et al. 1997). In addition, the use of a publicly available set of markers covering the whole grass genome and used by all scientists would produce interesting information on the relatedness and structure of the different types of apomixis. The aim of the present work was therefore the establishment of possible syntenic relationships between the *Paspalum* chromosome segment carrying the apomictic locus and its homoeologous rice counterpart by using a set of rice molecular markers evenly distributed in the rice map.

Material and methods

Plant material, trait screening and chromosome counts

A mapping population segregating for apomixis was generated by backcrossing an apomictic F_1 developed from a cross between a natural tetraploid apomict ($2n = 4x = 40$) and a colchicine-created sexual autotetraploid ($2n = 4x = 40$) (Caceres et al. 1999). The crosses were performed in Argentina according to procedures previously established (Quarín and Caponio 1995). Seeds were germinated in petri dishes and seedlings transplanted in soil (greenhouse conditions, Italy) in early Spring 1998. A randomly chosen population of 87 BC plants was selected to establish a core mapping population, and progeny tests were used to assess the apo/sex phenotype. The population was kept isolated from any other possible source of *Paspalum* pollen. One spikelet for each BC plant, chosen in such a way that its anthesis was shifted with regard to the other spikelets of the same plant, was emasculated and left to open-pollinate, then 4 cross-derived plants at the seedling stage were analysed with molecular markers for progeny tests. The apo/sex phenotype attribution was confirmed the next year (1999) by repeating the progeny tests on a new seed set from each plant. Chromosome numbers were counted on metaphase plates from root-tips pre-treated with α-bromonaphthalene for 4 h, fixed in Carnoy (glacial acetic acid/ethanol 1:3 v/v) for 24–48 h and hydrolysed in 1 M HCl at 60 °C for 6 min. Root tips were stained according to the Feulgen method and squashes were performed in 1% acetic orcein.

DNA extraction and RFLP procedures

For DNA extraction from seedlings, fresh leaf material (10 ± 2 mg) was ground with liquid nitrogen in a 2 ml Eppendorf tube with a glass pestle and the powder obtained was resuspended in 300 μl 2× CTAB buffer (100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2% w/v CTAB) pre-warmed at 65 °C and containing 2% v/v 2-mercaptoethanol.The mixtures were incubated at 65 °C for 30 min under gentle agitation. A 500 μl aliquot of chloroform/isoamyl alcohol (24:1) was added to the tubes which were agitated in a rotatory mixer for 15 min at 45 rpm at room temperature (RT). After centrifugation at 5000 × g for 7 min at RT, the aqueous phase was collected and the nucleic acids were precipitated by adding an equal volume of chilled isopropyl alcohol. The tubes were kept on ice

for 5 min and then the DNA was pelletted at 6000 × *g* for 10 min at 4 °C, vacuum-dried, resuspended in 40 μl TE buffer (1 mM Tris-HCl, 0.1 mM EDTA) and digested all at once. For DNA extraction from fully developed plants, 0.5 g of fresh leaf material was collected from each plant and the above procedure was scaled up accordingly. Genomic DNA (5–7 μg) was restriction-digested overnight with 20 units of *Hind*III, *Eco*RI, *Bam*HI, *Eco*RV and *Bgl*II (New England Biolabs, NEB) to detect informative polymorphisms between parental lines and with the appropriate enzyme for segregation analysis of all the BC individuals. Digested fragments were electrophoresed in 1% agarose gels and blotted onto Hybond-N^+ membranes (Amersham), according to the standard capillary procedure (Southern 1975). Blots were hybridized with random-primed ^{32}P-labelled probes, washed according to the membrane instruction manual, replacing SSPE with SSC (150 mM NaCl, 15 mM sodium citrate) and omitting the last high-stringency washes. Probed membranes were stripped in 50% v/v formamide, 6× SSC and 0.1% w/v SDS buffer at 74 °C for 30 min and reprobed up to 20 times. The rice probes used belonged to the New Landmarker Set (Nagamura et al. 1997). Inserts were amplified from recombinant plasmids by PCR with M13 (reverse and forward) universal primers. Amplified inserts were checked for molecular weight by electrophoresis and column-purified with the JetQuick-PCR purification spin kit (Genomed). *Paspalum* homologous probes were selected from a *Pst*I partial genomic library (Pupilli et al. 1997) and isolated from plasmids according to standard procedures (Maniatis et al. 1982).

Data analysis

For mapping purposes, only alleles segregating from the apomictic parent were taken into account. One of two different allelic configurations is expected for these fragments. If we call A an hypothetical segregating allele and 0 the corresponding null-allele, we shall have A000 (SDRF) when the segregation ratio of A is 1:1 or 13:15 (presence/absence), depending on whether random chromosome or chromatid assortment is assumed respectively, or AA00 (double-dose restriction fragment, DDRF) when A segregates 5:1 or 11:3 (presence/absence) for random chromosome or chromatid assortment (Allard 1960). On the basis of the composition of the F_1 the natural apomictic parent probably had 2 doses of the apomixis controlling allele whereas the pollinating parent of the mapping population had only one. Therefore apomixis was considered like a SDRF in the mapping population and its presence was assigned to the heterozygous dominant and its absence to the homozygous recessive genotype. Linkage and map order were calculated with MAPMAKER/EXP3 (Lander et al. 1987; Lincoln et al. 1992). This program is able to calculate recombination frequencies among SDRFs and, with minor accuracy, among DDRFs. In any case, the marker order on the map is not affected. Data were analysed by the 'F_2 backcross' option and linkage was detected with a lod score threshold of 3. Recombination frequencies were converted to map distance (cM) using the Haldane mapping function. The recombination frequencies for markers putatively linked in repulsion phase were calculated according to the formula $r = [3(a+d)/n] - 1$ (Hackett et al. 1998) where r is the recombination frequency, $a + d$ is the sum of non-parental progeny and n is the total number of progeny analysed.

Results

Genetic control

The segregation of SDRFs, or the lack of it as revealed by the homologous probes in the cross-pollination progeny of each BC individual, enabled us to confidently attribute either the apomictic or the sexual phenotype to the whole mapping population. The rice probes were in general less effective than the *Paspalum* probes in detecting signals with the small amount of DNA extracted from seedlings, probably because of partial homology. If we assume that each SDRF is present in half of the gametes of each heterozygous BC plant, then the relative abundance of gametes in the pollen pool of the population will be 1/4 for A0 and 3/4 for 00 if A is a SDRF. Similarly, each SDRF present in the mother plant will be inherited by half of its egg cells; therefore the probability of the whole cross-pollination progeny of each BC plant inheriting a SDRF while remaining sexual is $(5/8)^{n \cdot i}$ where n is the number of offspring and i the number of SDRFs considered. With 4 plants and 4 SDRFs this probability is abundantly less than 1%. Following this criterion, the apomictic phenotype was attributed to 32 plants, whereas 55 were considered sexual in the first-year screening. Two of these individuals, although considered sexual in 1998, showed all the apomixis-linked markers. Therefore the progeny tests were repeated on a new seed set for the

whole mapping population the following year and the same two individuals turned apomictic whereas all the other phenotypes were confirmed. Although errors in seed collection cannot be excluded, we speculated that these two plants, though carrying the apomixis locus, retained a high capacity of sexual development. It can be concluded that 34 individuals showed the potentiality to express the apomixis phenotype whereas 53 individuals remained sexual during the two years of evaluation. Chi-square analysis of segregation showed that the ratio of 34 apomicts to 53 sexuals did not fit the 1:1 ratio, consistent with a single dominant allele with either disomic or tetrasomic inheritance and random chromosome assortment ($\chi^2 = 4.15$ significant at $p < 0.05$). Conversely, if we assume tetrasomic inheritance and random chromatid assortment, we can hypothesize that apomixis in *P. simplex* is controlled by a single allele, whether linked (expected ratio 12:15, $\chi^2 = 1.16$, not significant) or not (expected ratio 13:15, $\chi^2 = 1.66$, not significant) to a lethal allele. The possibility of a random chromatid assortment is confirmed by the occurrence of a low proportion of quadrivalent chromosome pairing in microsporogenesis of *P. simplex* (Caceres, unpublished).

RFLP mapping

Of the 192 rice clones from the New Landmarker Set, 112 were screened for polymorphisms between the parental lines of the mapping population, together with 16 *Paspalum* clones previously selected for their capacity to reveal SDRFs in the apomictic parent (Pupilli et al. 1997). The rice clones screened were chosen to leave uncovered no more than 25 cM along the rice map. Of these probes, 28 were not homologous with *Paspalum* DNA, 41 were not polymorphic between parents with any of the restriction enzymes used, 19 showed specific bands only in the sexual parent and 24 probes showed 30 informative fragments, i.e. bands present in the apomictic parent and absent in the sexual one. Of all enzymes tested, *Eco*RI and *Hind*III proved to be the most effective in revealing informative fragments and therefore were used for mapping purposes. The 24 informative rice probes were present in all rice chromosomes, though in variable numbers, from a minimum of 1 (chromosomes 3, 6, 8, 9) to a maximum of 4 (chromosome 1). Non-homologous clones were in general interspersed at random among those showing homology with the exceptions of the telomeric part of the short arm of chromosome 2 and of the centromeric region of chromosome 6 for which none of the available markers was homologous. Of the 30 informative fragments detected with the 24 rice probes, 21 segregated in the ratio of 1:1 revealing SDRFs, 3 segregated in the ratio of 5:1 indicating DDRFs, and 6 showed segregation ratios significantly distorted from either 1:1 or 5:1. Similarly, *Paspalum* clones revealed 12 fragments of which 10 were SDRFs and 2 showed distorted segregation ratios.The few fragments segregating from the sexual parent were all DDRFs as expected from a colchicine induced tetraploid. Curiously enough, some of the fragments which were present in the sexual parent were missing in the mapping population. This fact was not imputable to chromosome loss since a checked subset of 10 plants of the mapping population showed the expected chromosome set of $2n = 4x = 40$, but more likely to chromosome rearrangements caused by treatment with colchicine. As a matter of fact, RFLPs and mixoploidy were noted among cuttings of the original colchicine-treated plant (unpublished, and Quarín, personal communication) and therefore the plant used as female parent could be slightly different from that used as parent control even though they both originated from tillers of the same colchicine-treated genotype.

The segregation of both rice and *Paspalum* markers was initially analysed irrespective of apomixis, as the first progeny test data became available one year and a half after sowing the mapping population. After the apo/sex phenotypes could be attributed to each plant, the apomixis trait was treated as a SDRF and included in the data set. Linkage analysis showed 3 rice markers linked in coupling with apomixis: one, R202 was mapped on rice chromosome 8, and the other two, C1069 and C901, were both located on the telomeric region of the long arm of rice chromosome 12. R202 was mapped 11.4 cM distant from apomixis while C1069 and C901 were linked 100% in coupling phase with apomixis. No other markers of the New Landmarker Set nor *Paspalum* probes detected any significant linkage with apomixis, indicating that a single locus located in a well defined chromosome area is responsible for conferring the potentiality to express the apomixis trait in *P. simplex*. Therefore an additional set of rice markers, mapped on the long arm of the rice chromosome 12, was obtained from the NIAR, and screened for parental polymorphism and co-segregation with apomixis. Of these, 4 probes were not homologous, 4 were not polymorphic between the parental lines, another 4 could not be tested for different reasons, and 3 showed fragments linked 100% in coupling phase with apomixis. Similarly, 5 mark-

ers mapped in close vicinity to R202 were tested but found not informative. Figure 1 shows (1) the alignment of the telomeric region of the long arm of rice chromosome 12 (f) with the homoeologous chromosome region of *P. simplex* related to apomixis (g), (2) a *Paspalum* linkage group identified by the same probes showing linkage with apomixis (h), and (3) the hybridizing banding pattern of each of the 5 rice markers, showing one or two bands (arrowheads in a–e) co-segregating with apomixis. These markers were mapped in an area spanning 14.5 cM in rice, but they were clustered together in the corresponding *Paspalum* area with no recombination recorded among the 87 individuals of the mapping population. Since fragments at different molecular weights were revealed by the five probes to be tightly linked to apomixis, cross-hybridization can be excluded. None of the probes used detected fragments that were linked in repulsion phase with apomixis. One linkage group, derived from the apomictic parent, was formed by 3 fragments unrelated to apomixis, but revealed by the same probes that showed SDRFs linked to apomixis (Figure 1h). One of these fragments, C901b (arrow in a), was revealed by the telomeric clone C901 and mapped at a distance of 22.2 cM from the other two fragments C1069b and C454b (arrows in d and e respectively) which were hardly detectable in the autoradiographs. These two fragments were linked 100% in coupling in *Paspalum*, as they were in rice. Although no direct allelic relationships nor even repulsion-phase linkage could be detected between these fragments and those linked with apomixis, we believe that this linkage group must have evolved together with that related to apomixis in this species. None of the other tested probes located on rice chromosome 12 showed any fragment specific to the apomictic parent with the exception of the probe R642, mapped at the short arm end, which showed a SDRF unlinked with apomixis. Two markers, C955 and C885, mapped at a distance of around 10 cM in rice chromosome 1, were also linked in *Paspalum* though at a shorter distance (1.6 cM). No other syntenic relationships between rice and *Paspalum* were detected. To sum up, we can conclude that the potentiality of *P. simplex* for apomictic reproduction is given by the presence of a relatively large gene block characterized by lack of recombination.

Discussion

The tetrasomic inheritance of a natural tetraploid apomictic genotype of *P. simplex* was previously ascertained on the basis of a 5:1 segregation (presence/absence) of DDRFs (Pupilli et al. 1997). The same plant gave 22 apomictic and 5 sexual F_1 individuals when crossed with a sexual colchicine-treated diploid (Caceres et al. 1999), and one of the apomictic F_1s gave 34 apomictic and 53 sexual individuals when backcrossed with the same sexual mother plant (this study). The overall picture is consistent with a model postulating apomixis under the control of a single locus segregating in a tetrasomic manner and with the alleles conferring the apomixis acting dominantly. A single allele A is necessary (but not sufficient) to confer the trait, but the original natural apomict probably had two doses of A in duplex condition. Random chromatid assortment should be assumed, with or without linkage to a recessive lethal gene. These data are consistent with those reported for *Pennisetun ciliare* (Sherwood et al. 1994), *Pennisetum squamulatum* (Ozias-Akins et al. 1998), *Panicum maximum* (Savidan 1981), *Brachiaria* (do Valle et al. 1994) and, at least for the parthenogenetic component, for *Poa* (Matzk 1991). Our data fit in particularly well with those of Nogler (1984b) who provided evidence that the allele A conferring bipolar apospory (*Hieracium*-type) in *Ranunculus* behaves as a lethal factor when it is present in the gametes as A or AA, in such a way as the viable gametes from a AAaa genotype would be in the ratio of 4Aa:1aa. *Hieracium*-type embryo sacs have been observed in apomictic *P. simplex* (Caceres et al. 1999). However, the possibility that the chromosome segment we identified as being present in all plants expressing apomixis is one of two closely linked loci cannot be ruled out. For example, if the two components of apomixis in *P. simplex*, apospory and parthenogenesis, though closely linked have conserved a limited possibility to recombine, then we can expect some sexual plants to display occasional aposporic or parthenogenetic phenomena. Research is in progress to test this hypothesis.

Data reported here provide strong evidence that the rice homoeologous counterpart of the locus controlling apomixis in *P. simplex* is located in the telomeric region of the long arm of chromosome 12. Five markers, located in this area and spanning around 15 cM were linked in coupling phase with apomixis without any recombination event being detected across the 87 plants of our mapping population. Lack of re-

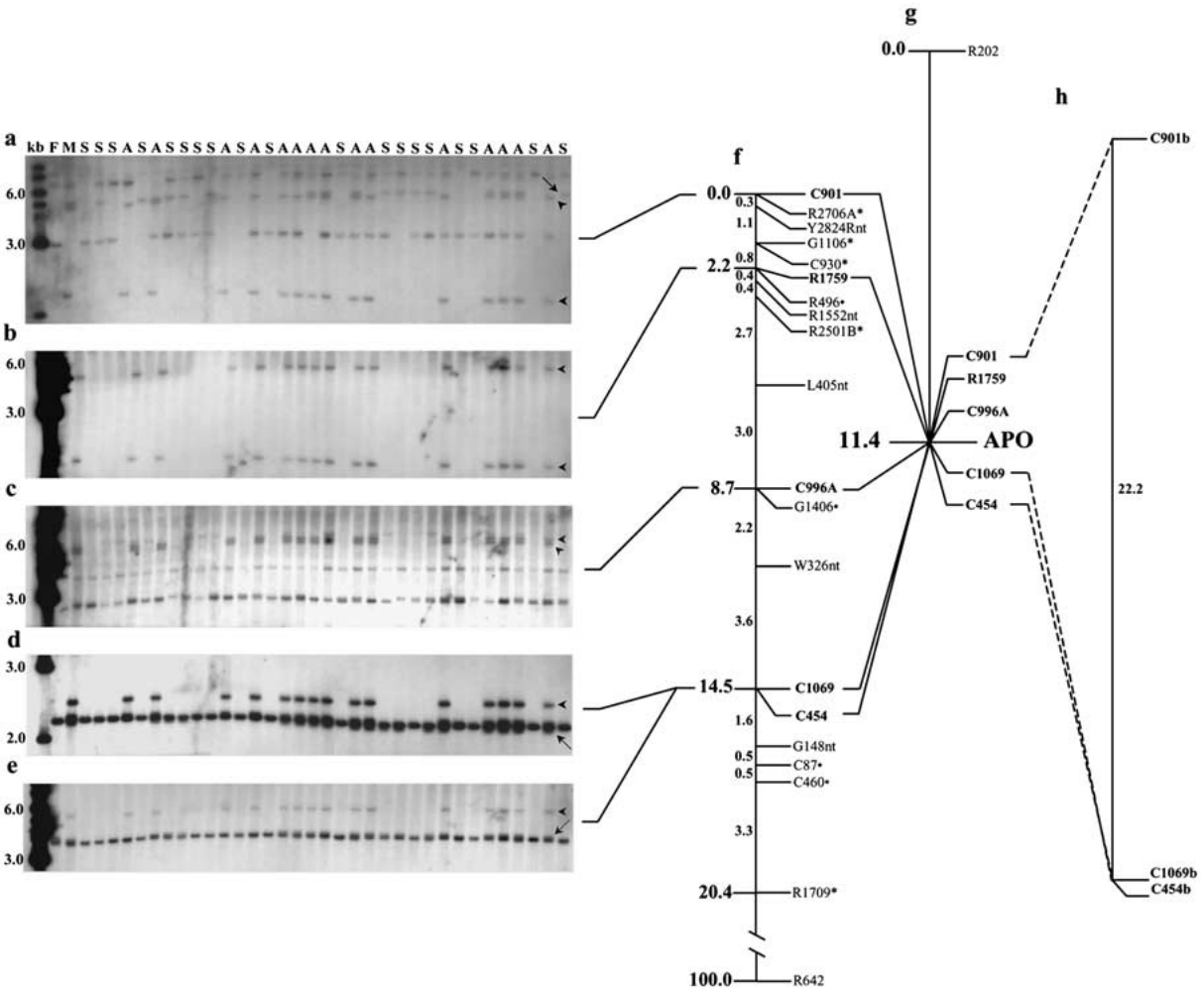

Figure 1. Alignment of the chromosomal region related to apomixis in *P. simplex* with its homoeologous area corresponding to the distal part of the long arm of rice chromosome 12 (Kurata et al. 1994). Rice markers showing fragments tightly linked to apomixis are reported in bold type. The hybridizing banding patterns of one blot containing the DNA digests (*Eco*RI) of sexual (F) and apomictic (M) parental lines and 34 apomictic (A) or sexual (S) BC individuals, probed with each of these markers are shown on the left. Vertical lines represent: f, the long arm end of rice chromosome 12; g, the apomixis-related region of *P. simplex*; h, a *P. simplex* linkage group identified by fragments putatively allelic to those related to apomixis. Arrowheads indicate apomixis-linked fragments, arrows indicate fragments putatively allelic to the apomixis-related ones; nt, not tested; *, not polymorphic; •, not homologous. The marker R642 was polymorphic between parents but unrelated to apomixis. Distances are given in cM.

combination around the apomixis locus seems to be a recurrent feature among apomictic grasses, whether they are diplosporous or aposporous variants. Grimanelli et al. (1998) reported a strong restriction of the recombination rate in the apomixis-controlling area of diplosporous *Tripsacum* using maize heterologous markers. Similarly, Ozias-Akins et al. (1998) found 12 homologous RAPD markers strictly co-segregating with aposporous apomixis in *Pennisetum squamulatum*, and a subset of these markers were also linked to apospory and showed no recombination in *Cenchrus ciliaris* (Roche et al. 1999). Similar observations were made in *P. simplex* by using both heterologous anchor markers and homologous random AFLPs, indicating that lack of recombination is not a matter of which marker is used but a characteristic of the apomictic reproduction itself. Lack of recombination could be due to the location of the apomixis locus on centromeric or other heterochromatic regions that are known to be characterized by low recombination (Wu and Tanksley 1993). However, comparative mapping data in *P. simplex* as well as in *Brachiaria* (Pessino et al. 1997) sug-

gest a telomeric location of the apospory locus on the homoeologous chromosome area of rice and maize, respectively. The presence of one RFLP (this study) and one AFLP (Labombarda, unpublished) markers showing a low capability of recombination with apomixis at both sides of the apomixis-related chromosome segment may suggest that this segment could have translocated, during speciation of *Paspalum*, from the original telomeric position to centromeric or other heterochromatic chromosome regions. Translocation or inversion of the apomixis-related region could explain the absence of allelism or even repulsion-phase linkage relationships between apomixis and the fragments C901b, C1069b and C454b of Figure 1h.

The formation of a low percentage of sexual embryo sacs has been observed in aposporous apomicts (Sherwood et al. 1980; Savidan 1982; Nogler 1984a). In our material it was impossible to distinguish reduced from unreduced embryo sacs morphologically, but the occurrence of multiple embryo sacs in the same ovule (Caceres, unpublished) and the fact that 2 plants carrying the apomixis-controlling allele retained their capability of sexual development are further evidence, in *P. simplex*, supporting the view expressed by Harlan et al. (1964) that 'apomixis and sexual reproduction are not alternative modes of reproduction, either genetically or operationally but are independent and simultaneous phenomena'. Depending upon how the balance between reduced and unreduced embryo sacs is shifted towards the formation of unreduced embryo sacs we have variable penetrance of apomixis. It is possible that the seeds utilized for the progeny tests were formed in environmental circumstances that favoured sexual reproduction in the first year and apomictic reproduction in the second. Seasonal changes in the incidence of apomixis in natural populations have already been reported in *Paspalum* (Quarín 1986).The apomixis-conferring allele A triggers the mechanisms leading to apospory and suppresses the development of sexual embryosacs. It would be interesting to investigate whether the suppression of sexual gametophyte development depends upon a higher competitiveness of the unreduced embryo sacs for nutrients, compared to the reduced ones, or upon the *trans*-inactivation of the genes or part of the genes involved in sexuality. It has been reported (Bennett 1997) that the nuclear cycle of unreduced nuclei is shorter than that of reduced cells; this could imply higher mitotic activity, and therefore a higher growth rate, of unreduced versus reduced embryo sacs. Finally, gene silencing, through transcriptional or post-transcriptional mechanisms may be functioning in the two individuals that changed phenotype along the two-year evaluation. There are in plants a number of examples involving endogenous genes in which a sensitive allele can be inactivated by an inducing allele as a result of several mechanisms of gene silencing (Matzke and Matzke 1995, and references therein). If the apomixis locus can suppress, or be suppressed by, other loci (i.e. those involved in sexuality), its incomplete penetrance could be accommodated. Evidence for apomixis suppression has been reported in *Cenchrus ciliaris* (Taliaferro and Bashaw 1966) and *Pennisetum squamulatum* (Ozias-Akins et al. 1998).

Recently, Moore et al. (1995) speculated that all those grasses in which collinearity of genes on chromosomes is ascertained, could have evolved from a common ancestor whose genome was formed by linkage segments that broke and reorganized like 'lego blocks' and, being interspersed with repetitive and non coding sequences, gave rise, during speciation, to the chromosomes of the different species. All of these blocks can be related to rice linkage segments (Moore et al. 1997) of which the one carrying the apomixis-related allele of *P. simplex* is identified as 12a. Synteny studies showed that the apospory region in *Brachiaria* is colinear with markers mapped on the short arm of maize chromosome 5 (Pessino et al. 1997), and diplosporous apomixis in *Tripsacum* showed linkage with markers located on the long arm of maize chromosome 6 (Leblanc et al. 1995). Our data disclose a new homoeologous group that has never before been found to carry apomixis-related markers. This finding is of interest for two main reasons: (1) it may contribute to clarify how apomixis has evolved during the speciation of the grass family, by comparing the structure of rice block 12a and its correlation with apomixis in the different species, and (2) it may be of help in directly inducting apomixis in rice in which a modification of this chromosome area at the structural and/or functional levels, coupled with polyploidization, could trigger at least some components of apomixis in this important crop.

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