Segregation for Sexual Seed Production in *Paspalum* as Directed by Male Gametes of Apomictic Triploid Plants

ERIC J. MARTÍNEZ*, CARLOS A. ACUNA, DIEGO H. HOJSGAARD, MAURICIO A. TCACH and CAMILO L. QUARIN

*Instituto de Botánica del Nordeste, CONICET, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, CC209, (3400) Corrientes, Argentina*

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- **Background and Aims** Gametophytic apomixis is regularly associated with polyploidy. It has been hypothesized that apomixis is not present in diploid plants because of a pleiotropic lethal effect associated with monoploid gametes. Rare apomictic triploid plants for *Paspalum notatum* and *P. simplex*, which usually have sexual diploid and apomictic tetraploid races, were acquired. These triploids normally produce male gametes through meiosis with a range of chromosome numbers from monoploid (*n* = 10) to diploid (*n* = 20). The patterns of apomixis transmission in *Paspalum* were investigated in relation to the ploidy levels of gametes.

- **Methods** Intraspecific crosses were made between sexual diploid, triploid and tetraploid plants as female parents and apomictic triploid plants as male parents. Apomictic progeny were identified by using molecular markers completely linked to apomixis and the analysis of mature embryo sacs. The chromosome number of the male gamete was inferred from chromosome counts of each progeny.

- **Key Results** The chromosome numbers of the progeny indicated that the chromosome input of male gametes depended on the chromosome number of the female gamete. The apomictic trait was not transmitted through monoploid gametes, at least when the progeny was diploid. Diploid or near-diploid gametes transmitted apomixis at very low rates.

- **Conclusions** Since male monoploid gametes usually failed to form polyploid progenies, *eg.* triploids after 4x x 3x crosses, it was not possible to determine whether apomixis could segregate in polyploid progenies by means of monoploid gametes.

**Key words:** Apomixis, monoploid gametes, *Paspalum notatum, Paspalum simplex*, polyploidy, RAPD, SCAR, triploidy.

**INTRODUCTION**

Apomixis is an asexual mode of reproduction through seeds. Embryos are produced without a female haploid reduction phase and without any contribution of the paternal parent. They may originate from unreduced gametophytes, gametophytic apomixis, or directly from the somatic tissue of nucellus or the integument of the ovule (Nogler, 1984). Gametophytic apomixis involves three main components: (1) failure of meiosis, (2) unreduced embryo sac development and (3) parthenogenetic development of the unreduced egg. In general, the process only occurs in the female part, while the male meiosis generates normal haploid pollen. Many apomictic species are pseudo-gamous requiring fertilization to initiate endosperm development. It is well known that gametophytic apomixis is related to polyploidy. Almost all apomictic plants that have been cytologically examined were polyploid, and the majority tetraploid (Savidan, 2000). A few apomictic diploid exceptions are mentioned in the literature, although some remain rather doubtful (Holm and Ghatnekar, 1996). Furthermore, most of the reported apomictic diploids were dihaploids, obtained by haploid parthenogenesis from apomictic tetraploids (de Wet, 1968, 1971; Savidan and Pernés, 1982; Kojima and Nagato, 1997; Naumova et al., 1999), and usually described as weak and sterile (Nogler, 1982; Leblanc et al., 1996; Bicknell and Borst, 1997). Unreduced embryo sacs have been observed in ovules from diploids as well as from artificially induced tetraploids indicating that the apomixis factor(s) was present in diploid races (Savidan, 1982; Quarin and Normann, 1987; Normann et al., 1989; Quarin et al., 2001). However, asexual progeny from those diploids bearing additional aposporous embryo sacs has not been demonstrated.

With a few exceptions, apomixis is poorly represented among crop species. The introduction of apomixis into sexual crops is an important goal in apomixis research (Hanna, 1995). Apomixis has not yet been transferred from a wild species to any sexual relative crop (Savidan, 2000). The diploid status of many crops is an important barrier in achieving this goal. Little is known about the reasons for the absence, or non-expression of this reproductive mode in diploids. However, two hypotheses have been postulated to explain the almost complete absence of apomixis at the diploid level. One assumes that the factor responsible for apomixis could not be transmitted through monoploid gametes; therefore, apomixis could not be recovered in diploids (Nogler, 1982). In *Ranunculus auricomus*, a species with aposporous apomixis, Nogler (1984) has demonstrated that apomixis is monogenic and dominant, and it could only be transmitted in the heterozygous state through diploid or polyploid gametes. The dominant allele responsible for apomixis is lethal in its homozygous

* For correspondence. E-mail eric@agr.unne.edu.ar

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state; therefore, it could not be transmitted through monoploid gametes. The second hypothesis supposes that a dosage requirement is necessary for apomixis to be expressed (Mogie, 1988; Quarin et al., 2001). According to this hypothesis, the absence of apomixis in wild diploids is due to a lack of expression instead of non-transmission. Mogie (1988) suggested that more than two copies of the apomixis allele are necessary for the expression of the trait. Grimanelli et al. (1998) obtained results in maize–Tripsacum hybrids that supported Nogler’s hypothesis (Nogler, 1982). Apomixis in Tripsacum is linked to a segregation distorter-type system that promotes the elimination of the apomixis alleles when transmitted through haploid gametes (Grimanelli et al., 1998). Meanwhile, contrasting evidence has been found in Paspalum both supporting and rejecting Mogie’s hypothesis. A few tetraploids generated by colchicine treatment of diploid tissue were determined to be facultative apomicts indicating the presence, without expression of the apomixis gene(s) in diploid plants (Quarin and Hanna, 1980; Quarin et al., 2001), thus supporting Mogie’s hypothesis. On the other hand, aposporous apomixis in P. notatum (Martínez et al., 2001), and P. simplex (Cáceres et al., 2001) was determined to be inherited as a single Mendelian factor, present in simplex condition (+−−) in autotetraploid accessions, rejecting Mogie’s hypothesis and partially supporting Nogler’s hypothesis. Bicknell et al. (2000) observed in Hieracium that apomixis was transmitted not only by diploid gametes but also by haploid gametes when the recovered progeny were polyploid. They proposed that the absence of apomixis in diploids was caused by selection against the survival of diploid zygotes, rather than against the haploid gametes as had been noted in other systems (Bicknell et al., 2000).

Paspalum is a grass genus native to the New World with several important forage species. Most species are polyploids, and among them, tetraploids are predominant (Quarin, 1992). Species containing apomictic polyploids as well as sexual diploids are frequently found. Apospory is the most common type of apomixis in the genus. Paspalum notatum and P. simplex are the two species of the genus where apomixis has been most extensively studied.

Paspalum notatum is a rhizomatous species widely distributed in native grasslands from central Mexico to Argentina (Chase, 1929). Different ploidy levels were reported for this species (Burton, 1946; Gould, 1966; Tischler and Burson, 1995). Sexual diploids are cross-pollinated due to self-incompatibility (Burton, 1955), while the tetraploid races reproduce by obligate apomixis and are pseudogamous (Burton, 1948). It has been reported that apospory in tetraploid P. notatum is controlled by a major dominant single locus (Martínez et al., 2001). However, the trait was transmitted in a non-Mendelian fashion due to a distortion in the segregation ratio. In addition, several molecular markers completely linked to the apomixis locus were detected (Martínez et al., 2003; Stein et al., 2004).

Paspalum simplex is a warm-season grass found in north-western Uruguay and throughout the phytogeographic

Chaco province in Argentina, Bolivia and Paraguay (Morrone et al., 2000). This species includes sexual diploids (2n = 2x = 20) and triploids (2n = 3x = 30) (Espinoza and Quarin, 1997; Urbani et al., 2002), and tetraploids, tetravalents (2n = 4x = 40) and hexaploids (2n = 6x = 60) that reproduce by pseudogamous aposporous apomixis (Nath et al., 1970; Caponio and Quarin, 1987; Urbani et al., 2002). Genetic analyses of tetraploid intra-specific hybrids of P. simplex revealed that apomixis was under monogenic genetic control (Pupilli et al., 2001). Rice molecular markers linked to apomixis in P. simplex were also detected (Pupilli et al., 2001).

Sexual tetravalent plants of P. notatum and P. simplex have never been collected in the wild. All entirely sexual tetraploids of these species have been obtained experimentally through chromosome doubling of sexual diploid cytotypes (Forbes and Burton, 1961; Cáceres et al., 1999). Crosses of induced tetravalent plants (as the female parent) with pollen of a natural apomictic tetraploid may then produce new sexual tetraploids as well as apomictic tetraploids. New sexual tetraploid genotypes might eventually be obtained through self-pollination of induced sexual tetraploids (Martínez et al., 2001), although the induced 4x plants usually retain the self-incompatibility system of the original diploids.

The aim of this work was to evaluate the patterns of apomixis transmission in Paspalum, in relation to the ploidy levels of gametes, and therefore to determine why apomixis is absent at the diploid level.

MATERIALS AND METHODS

Plant material

Paspalum notatum and P. simplex were used for this study. The plant material and its origin are described in Table 1. All plants were cultivated at IBONE, Corrientes, Argentina. Sexual genotypes with different ploidy levels were used as female parents and apomictic triploid genotypes as pollen donors. Sexual diploid and tetraploid genotypes are expected to produce haploid male and female gametes, while triploid genotypes can generate male gametes with different chromosome numbers. Previous studies have showed that triploid genotypes of both species could generate offspring through monoploid (n = x), diploid (n = 2x) and aneuploid male gametes (E. J. Martínez, unpubl. res.). On the other hand, apomictic triploids of both species form unreduced triploid (3x) female gametes in the aposporous embryo sacs.

Breeding scheme for the transmission of apomixis

Sexual diploid (2n = 2x = 20) and tetraploid (2n = 4x = 40) accessions of P. notatum were crossed with an apomictic conspecific triploid (2n = 3x = 30) genotype. Otherwise, sexual diploid, triploid and tetraploid accessions of P. simplex were crossed with an apomictic triploid plant of the same species. Controlled crosses were made in a fog chamber as described by Burton (1948). Florets from the female parents were emasculated and pollen from the
male parent was harvested in glassine bags and dusted on to the stigmas of the emasculated florets. Emasculation was performed after anthesis, with sharp pointed tweezers, when the three anthers of each floret hung indehiscent due to the high humidity inside the chamber.

Apomictic triploid cytotypes were used as male parents instead of tetraploids, because they were able to produce gametes with different chromosome numbers from \( n = 10 \) (monoploid) up to \( n = 20 \) (diploid). Since the sexual \( 2x \) and \( 4x \) cytotypes only produced haploid reduced gametes, with 10 or 20 chromosomes, respectively, it was possible to estimate the chromosome numbers of the male gametes by chromosome counts in the progeny. Therefore, when an apomictic progeny was detected, the chromosome number of the male gamete that transmitted the trait was estimated. However, it was more difficult to ascertain the chromosome number of the male gametes that gave rise to the progeny from crosses between the sexual \( 3x \) \( P. simplex \) (female parent) and the apomictic \( 3x \) genotype (pollen donor), because either one of the parents could generate gametes with a chromosome range from 10 to 20. However, it can be estimated based on the theory of the endosperm balance number. Johnston et al. (1980) postulate that each species has an effective endosperm balance number (EBN) required for normal endosperm development and seed formation. The EBN must be in a \( 2 : 1 \) maternal to paternal (\( m : p \)) genome ratio. Quarin (1999) demonstrated that the EBN hypothesis is effective in \( P. notatum \) when sexual plants were used as female parents, but pseudogamous apomictic plants set seed regardless of the relationship between \( m : p \) genome input for the endosperm formation. According to this, if a sexual \( 2x \) is pollinated with an apomictic \( 3x \), it would be expected to give rise to progeny with \( 2n = 2x = 20 \), because female input for endosperm (two reduced polar nuclei, \( x + x \)) should be balanced by a ten or near-to-ten chromosome male gamete from the male apomictic triploid. Likewise, the offspring from sexual \( 4x \) by apomictic \( 3x \) crosses should mainly be tetraploid. In this case, the sexual tetraploid plant contributes with two reduced polar nuclei (\( 2x + 2x = 4x \)), while the apomictic triploid should provide a diploid or near-diploid sperm (\( n = 2x \)) for effective endosperm development. It is expected that crosses among two \( 3x \) plants would produce progeny when a male gamete matches a female one with an equal or very similar chromosome number. Fusion between gametes with different chromosome numbers will produce ratios higher or lower than \( 2 : 1 \).

**Molecular markers linked to the apomixis locus (apo-locus) and embryological analyses of mature embryo sacs were used to identify the apomictic plants in the progeny. In \( P. notatum \), two randomly amplified polymorphic DNA (RAPD) markers completely linked to the apo-locus were used (Martínez et al., 2003). Previous analysis confirmed that an apomictic \( 3x \) genotype of \( P. notatum \) used in this study amplified the two RAPD markers linked to the apo-locus (E. J. Martínez, unpubl. res.). Otherwise, a sequence-characterized amplified region (SCAR), which is specific for apomixis in \( P. simplex \) (Calderini et al., 2006), was employed to identify the apomictic progeny of this species, and confirmed by embryological analyses. SCAR markers were obtained according to Paran and Michelmore (1993).**

**Hybrid progeny identification**

Because some female parents produced a few seeds under self-pollination conditions, and because also the emasculation procedure might admit some minimal degree of self-pollination, the diploid offspring derived from the \( 2x \times 3x \) crosses and the tetraploid descendants...
from the $4x \times 3x$ crosses were controlled to guarantee their hybrid origin. Hybridity was examined by progeny tests with molecular markers as it was described in Acuña et al. (2004).

Chromosome numbers and embryological analyses

Chromosome counts were performed for each individual plant that amplified the molecular markers linked to the apo-locus in order to determine the ploidy or chromosome number of the male gamete that produced that plant. In addition, several plants from each cross, that did not amplify such markers, were also counted to assess the chromosome number of gametes that failed to transmit apospory. For chromosome counts, root tips were placed in a saturated solution of bromonaphtalene for 2 h, hydrolysed in $1 \times \text{HCl}$ for 10 min at $60^\circ\text{C}$, stained with Fuelpen reagent, squashed in a drop of 1% aceto-orcein, and observed with a light transmission microscope. Five to ten metaphase mitoses with well-defined spread chromosomes were scored for each individual analysed.

Embryological analyses were based on mature embryo sac observations, carried out only on those plants that amplified the markers associated with apomixis, as described by Martínez et al. (2001).

RESULTS

Transmission pattern of apomixis in Paspalum notatum

Intraspecific crosses were made between eight diploid genotypes of Paspalum notatum as female parents and one apomictic triploid genotype as the male parent. Thirty-seven plants were recovered from a total of 11 532 pollinated florets. The reproductive efficiency (percentage of plants obtained from total number of pollinated florets) was very low in all combinations with a general mean of 0.32% (Table 2). Chromosome counts confirmed 34 diploids ($2n = 2x = 20$), one triploid ($2n = 3x = 30$) and two aneuploids ($2n = 22$ and 29). Then, all diploid descendants were evaluated with several RAPD markers that proved to be specific for the triploid parent in order to rule out self-pollination and verify their hybridity. All thirty-four diploid progeny amplified specific bands derived from the triploid parent, indicating their hybrid origin. The number of male-specific bands observed in each diploid descendant varied from a minimum of three to a maximum of nine. It was determined that monoploid gametes ($n = x = 10$) from the apomictic triploid parent contributed to the formation of $2x$ progeny. None of these diploid hybrids amplified the RAPD markers linked to the apo-locus (Table 2).

The individual cross concerning the diploid genotype Q4084 #2 produced eight diploid, one triploid and one hypotriploid ($2n = 29$) descendants. Both the triploid and the near-triploid progeny amplified RAPD markers linked to the apo-locus (Table 2), and showed a high percentage of mature ovules carrying one or more aposporous embryo sacs (data not shown). These results substantiated the transmission of apospory through diploid ($2x$) or hypodiploid ($2x - 1$) gametes.

Intraspecific crosses were also made between four sexual tetraploid genotypes of P. notatum and the apomictic triploid plant (Table 2). From a total of 11 493 pollinated florets, 154 individuals were recovered. This resulted in an average reproductive efficiency of 1.34% (Table 2). Chromosome counts ranged from $2n = 30$ to 42. However, most plants

Table 2. Crosses performed to determine the type of apomixis transmission between sexual diploid and tetraploid accessions, and an apomictic triploid genotype of Paspalum notatum

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Crossed florets (no.)</th>
<th>Plants obtained (no.)</th>
<th>Reproductive efficiency (%)*</th>
<th>Number of plants with $2n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2x \times 3x$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4084 #1 × Q3686</td>
<td>743</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Q4084 #2 × Q3686</td>
<td>2004</td>
<td>10</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>Q4084 #8 × Q3686</td>
<td>1556</td>
<td>7</td>
<td>0.45</td>
<td>0</td>
</tr>
<tr>
<td>Q4084 #9 × Q3686</td>
<td>395</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Q3658 × Q3686</td>
<td>398</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Q4160 × Q3686</td>
<td>2976</td>
<td>9</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Q4175 × Q3686</td>
<td>1264</td>
<td>7</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>C4-55 × Q3686</td>
<td>1296</td>
<td>4</td>
<td>0.31</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11532</td>
<td>37</td>
<td>0.32</td>
<td>0</td>
</tr>
<tr>
<td>$4x \times 3x$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4188 × Q3686</td>
<td>2038</td>
<td>38</td>
<td>1.86</td>
<td>0</td>
</tr>
<tr>
<td>Q4205 × Q3686</td>
<td>4585</td>
<td>40</td>
<td>0.87</td>
<td>0</td>
</tr>
<tr>
<td>F199 × Q3686</td>
<td>2665</td>
<td>29</td>
<td>1.09</td>
<td>0</td>
</tr>
<tr>
<td>Q3664 × Q3686</td>
<td>2205</td>
<td>47</td>
<td>2.13</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11493</td>
<td>154</td>
<td>1.34</td>
<td>0</td>
</tr>
</tbody>
</table>

* Reproductive efficiency: percentage of plants obtained from the total number of pollinated florets.
† One of them with $2n = 29$ and another with $2n = 30$.
‡ Plant with $2n = 39$. 

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(75%) had 2n = 40 chromosomes and, furthermore, 91% of the progeny ranged from 2n = 39 to 2n = 41. Other aneuploid numbers were less frequently produced, and only one plant had 2n = 30. Several molecular markers (from three to ten) specific of the male triploid parent were generated in each of the tetraploid (2n = 40) progeny by RAPD procedures as a verification of their hybrid origin.

Chromosomes in mitotic metaphase of a progeny plant with 2n = 31 and another with 2n = 39 from sexual 4x/C2 apomictic triploid crosses are illustrated in Fig. 1A and B, respectively. These results indicate a very low recovery of plants from a diploid egg cell (n = 2x) and a monoploid sperm nucleus (n = x). Most descendants originated from the normal 2x egg cell and a male gamete carrying n = 20 or close to 20. Results indicated that in a few cases, female aneuploid gametes with chromosome numbers close to n = 20 (higher or lower than 20) might give rise to some descendants. Although 91% of the progeny originated from diploid or near-diploid gametes of the apomictic triploid parent, only one plant showed the molecular markers linked to the apo-locus. This individual had 2n = 39 and arose from the sexual 4x plant Q4205 and the male 3x parent (Table 2). A total of 153 ovules of this particular progeny were analysed during two flowering periods, and only two ovules showed aposporous embryo sac development. The behaviour indicated that it was a facultative apomictic plant, with a low expression of the trait, and that it was transmitted through a hypodiploid male gamete (n = 2x – 1 = 19).

**Transmission pattern of apomixis in Paspalum simplex**

The transmission of apomixis in *Paspalum simplex* was analysed by crossing a natural apomictic triploid with sexual females differing in ploidy: one diploid, one triploid and two tetraploids used as female parents. One hundred and eighteen plants were recovered when a 2x genotype was used as the female parent (Table 3). The reproductive efficiency was 9.7% from a total of 1210 pollinated florets. From the 118 plants recovered, none amplified the SCAR marker linked to the apo-locus (Table 3 and Fig. 2A). Therefore, chromosome counts were only carried out on a random sample of 28 plants, which were all diploids (2n = 20). The hybrid origin of the whole

**Table 3.** Crosses performed to determine the feasibility of transmitting apomixis and the method of transmission from an apomictic triploid genotype of *Paspalum simplex* to sexual genotypes (diploids, triploids and tetraploids) of the same species

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Pollinated florets (no.)</th>
<th>Plants obtained (no.)</th>
<th>Reproductive efficiency (%)</th>
<th>Number of plants with 2n</th>
<th>Plants with SCAR marker (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Sexual 2x × apomictic 3x</td>
<td>C3-32 × U45</td>
<td>1210</td>
<td>118*</td>
<td>9.7</td>
<td>28</td>
</tr>
<tr>
<td>Sexual 3x × apomictic 3x</td>
<td>U36 × U45</td>
<td>1783</td>
<td>64†</td>
<td>3.6</td>
<td>4</td>
</tr>
<tr>
<td>Sexual 4x × apomictic 3x</td>
<td>C1B2 × U45</td>
<td>817</td>
<td>14</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>C1-2 × U45</td>
<td>1139</td>
<td>23</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Chromosome counting was only performed on 28 randomly selected plants.
† Chromosome numbers were performed on 49 plants because the remaining 15 plants died at seedling stage.
‡ One of them with 2n = 27 and another with 2n = 32.
§ All plants with 2n = 39.
were confirmed to have originated by hybridization and not by self-pollination. From three to six specific markers from the triploid U45 male parent were detected in the tetraploid descendants. Three plants with \(2n = 39\) amplified the SCAR marker (Table 3) and congruently these three exhibited ovules with aposporous embryo sacs, confirming their apomictic reproduction mode (data not shown). All apomictic progenies were generated from C1B2 \(\times\) U45 crosses (Table 3).

**DISCUSSION**

The transmission of aposporous apomixis in *Paspalum notatum* and *P. simplex* was studied with the purpose of determining the mechanism responsible for its absence or effectiveness in diploid forms. In *Paspalum*, all known apomictic species are polyploid. However, most of these apomictic polyploids, especially tetraploids, contain conspecific sexual diploid counterparts. In several species of *Paspalum*, these diploid cytotypes have shown occasional aposporous embryo sacs in their ovules (Quarin et al., 1982, 2001; Quarin and Norrmann, 1987; Norrmann et al., 1989). Similar results were observed in the tropical grass *Brachiaria decumbens* (Naumova et al., 1999). According to Naumova et al. (1999), the diploid plant of *B. decumbens* was a facultative sexual plant, which probably arose as a dihaploid plant from a natural apomictic tetraploid population. By contrast, Quarin et al. (2001) reported that diploid plants of *Paspalum*, with the capacity to form occasional aposporous embryo sacs, have not arisen as dihaploids, but rather they constitute natural diploid populations. Whether these eventual aposporous sacs are able to generate maternal progeny is an issue that has not yet been proved.

The strategy used in this work permitted testing of the hypotheses proposed by Nogler (1982) and Mogie (1988) about the absence of apomixis at the diploid level in *Paspalum*. To achieve this, an attempt was made to produce hybrids, using pollen (from \(n = 20\) to \(n = 2x\)) of apomictic triploids, for crosses with sexual cytotypes of the same species (2x, 3x, 4x).

In both *Paspalum* species, crosses between sexual diploid and apomictic triploid pollen donors generated almost exclusively diploids. Only one triploid and two aneuploids were obtained from \(2x \times 3x\) crosses of *P. notatum*. This indicates that reduced eggs (\(x = 10\)) from diploid mother plants were fertilized by monoploid gametes (\(n = 10\)) from the male parent. None of these diploid plants, however, amplified the molecular markers specific for apomixis. This indicates that monoploid male gametes, carrying the apomixis trait, were not transmitted to the progeny, at least when the progeny was diploid. Intriguingly, in these \(2x \times 3x\) crosses of *P. notatum* the 3x and the near-3x apomictic plants were both generated from a single cross in which the female 2x parent was Q4084 #2. According to previous studies (Quarin et al., 2001), this plant (identified as R2) has revealed a capacity for very occasional aposporous embryo sac development besides the regular meiotic sac. These previous results leave room for speculation about the gametes that gave rise to both the triploid and the hypotriploid (\(2n = 29\))

diploid offspring was verified by progeny tests with molecular markers specific for the male triploid parent. When the sexual triploid U36 was crossed with the apomictic triploid U45, sixty-four individuals were recovered (Table 3). Chromosome counts could only be performed on 49 plants because the remnant seedlings died. In this cross a wide range of chromosome numbers was observed (Table 3), with 73% of the progeny analysed varying from 20 to 24. Chromosomes of an aneuploid plant with \(2n = 22\) are illustrated in Fig. 1C. Two plants amplified the SCAR marker linked to apo-locus and showed aposporous embryo sacs. One of them had 27 chromosomes and the other 32 (Table 3). SCAR amplification patterns for some of the haploids specific for the male triploid parent. When the apomictic triploid male U45 and 14 F1 offspring as a sample of variability in chromosome number from \(2n = 37\) to \(2n = 40\). The arrows point to the apomixis-specific fragment of 650 bp. MW = molecular weight marker (lambda EcoRI/HindIII) expressed in base pairs.

**Fig. 2.** Apomixis-specific SCAR in three progeny of *Paspalum simplex*: (A) SCAR amplification pattern for the sexual diploid mother C3–32 (2x), the apomictic triploid male U45 (3x) and 19 diploid F1 progeny; (B) SCAR amplification pattern for the sexual triploid mother U36, the apomictic triploid male U45, and 19 F1 progeny with varying chromosome numbers from \(2n = 20\) to \(2n = 32\); (C) SCAR amplification pattern for the sexual tetraploid mother C1B2, the apomictic triploid male U45 and 14 F1 offspring as a sample of variability in chromosome number from \(2n = 37\) to \(2n = 40\). The arrows point to the apomixis-specific fragment of 650 bp. MW = molecular weight marker (lambda EcoRI/HindIII) expressed in base pairs.
paternal genome (2m : 1p). Meanwhile, in triploid sperm triploid, receiving two maternal genomes and one

strains of in very low seed set. However, pseudogamous apomictic

numbers, from produce an array of gametes with different chromosome

(Johnston cate 2 : 1 maternal to paternal genome ratio for the devel-

essential for growth of the embryo and production of

could be due to a deviation from the normal 2 : 1

ploidy crosses were not viable. This poor recovery

2

recovered in 2

x/C2

x/C2

x

2

hybrids (2n = 20). When the female parent was a tet-

raploid with expected n = 2x = 20 egg cells, most hybrids

originated from diploid or near-diploid male gametes

(n = 20 or n = 19). The chromosome number of the egg

cell was decisive in selecting the chromosome number of

the effective male gamete. This selection could act

through a precise operation of the endosperm balance

number system, requiring 2 : 1 female:male genome

input for endosperm development. Thus, the aneuploid

hybrids recovered from 3x x 3x crosses originated most

likely from the fusion of an egg cell and a sperm nucleus

with identical or very similar chromosome numbers.

Consequently, the two 3x x 3x hybrids (2n = 27 and

2n = 32) that amplified the SCAR marker linked to the

apo-locus originated from 14 + 13 (or 13 + 14) and 16 +

16 gametes, respectively. Similarly, the three hybrids

(2n = 39) that amplified the SCAR when the female

parent was 4x were probably formed by hypodiploid

sperm nuclei (n = 2x − 1 = 19) matching diploid female

gametes, although inverse fusion, female n = 19 and male

n = 20, could not be excluded from a theoretical standpoint.

The present results indicate that many of the monoploid

male gametes from the triploid parent were functional and

their products viable. However, no diploid progeny

showed the molecular markers completely linked to apo-

mixis, indicating that male monoploid gametes carrying

the apomixis locus were not transmitted to the diploid

progeny. In contrast, when a sexual tetraploid was used as

a female parent, offspring typically arose from diploid or

near-diploid gametes of the apomictic triploid parent.

Thus, formation of polyploid (triploid) progeny by means

of a monoploid male gamete does not occur, concluding

that apomixis would not segregate to polyploid progeny

through a monoploid gamete. Only one triploid plant

arose from a monoploid male gamete of the triploid

parent and it was sexual; most likely the monoploid male

gamete was lacking the factor for apomixis. In Paspalum,

therefore, selection of the male gametes is biased by the

chromosome number of the female gamete. This behaviour

would be strongly associated with the requirement of an

endosperm genomic balance.

Interestingly, the aneuploid apomictic progenies (2n =

27 and 2n = 32) from 3x x 3x crosses of P. simplex

suggest that apomixis could also be transmitted by

gametes carrying chromosome numbers (13, 14 or 16) con-

siderably lower than the diploid condition. Since the parents

3x progenies were obtained in P. almum, P. quadrifarium

and P. rufum (Norrmann et al., 1994).

In P. simplex, the triploid plants are rare members of an

agamic complex that most likely originated by autoploidy

(Urbani et al., 2002). Gametes of these triploids are theoret-

ically expected to have any chromosome number from

n = x = 10 (monoploid) to n = 2x = 20 (diploid). In fact,

the present results from 2x x 3x, 3x x 3x, and 4x x 3x

crosses suggested that triploid plants produced a wide

array of gametes from n = 10 to n = 20. However, the
effectiveness of these gametes when acting from the male

side depended on the chromosome number of the female

gamete: when the egg cell was monoploid (n = 10), the

successful male gamete was also monoploid, giving rise

to 2x hybrids (2n = 20). When the female parent was a tet-

raploid with expected n = 2x = 20 egg cells, most hybrids

originated from diploid or near-diploid male gametes

(n = 20 or n = 19). The chromosome number of the egg

cell was decisive in selecting the chromosome number of

the effective male gamete. This selection could act

through a precise operation of the endosperm balance

number system, requiring 2 : 1 female:male genome

input for endosperm development. Thus, the aneuploid

hybrids recovered from 3x x 3x crosses originated most

likely from the fusion of an egg cell and a sperm nucleus

with identical or very similar chromosome numbers.

Consequently, the two 3x x 3x hybrids (2n = 27 and

2n = 32) that amplified the SCAR marker linked to the

apo-locus originated from 14 + 13 (or 13 + 14) and 16 +

16 gametes, respectively. Similarly, the three hybrids

(2n = 39) that amplified the SCAR when the female

parent was 4x were probably formed by hypodiploid

sperm nuclei (n = 2x − 1 = 19) matching diploid female

gametes, although inverse fusion, female n = 19 and male

n = 20, could not be excluded from a theoretical standpoint.

The present results indicate that many of the monoploid

male gametes from the triploid parent were functional and

their products viable. However, no diploid progeny

showed the molecular markers completely linked to apo-

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Thus, formation of polyploid (triploid) progeny by means

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Interestingly, the aneuploid apomictic progenies (2n =

27 and 2n = 32) from 3x x 3x crosses of P. simplex

suggest that apomixis could also be transmitted by

gametes carrying chromosome numbers (13, 14 or 16) con-

siderably lower than the diploid condition. Since the parents
were autotriploids, the transmission of apomixis would require disomy rather than diplloidy in the transmitting gamete.

No recovery of apomictic diploids through mediation of monoaploid gametes could be explained by a linked lethal system as described by Nogler (1982). However, it was not possible to verify that monoaploid gametes did not transmit the trait to polyploid progeny. Nogler’s hypothesis states that gametes carrying the apomixis factor should be heterozygous because the homozygous condition is lethal. Therefore, monoaploid gametes carrying the control of apomixis would be inviable. The present results cannot be used to support Nogler’s hypothesis if it is not possible to enlarge the polyploid (triploid) population generated by means of monoaploid male gametes. However, the present results do favor the theory that apospory, at least in 4x P. notatum, is controlled by a single dominant gene with a pleiotropic lethal effect with incomplete penetrance (Martínez et al., 2001).

The present results do not fit the hypothesis proposed by Mogie (1988). According to this hypothesis, apomixis arises when the ratio of apomictic vs. sexual alleles is >0.5, meaning that a dose of genetic factor(s) for apomixis should be present in diploids. Therefore, molecular markers completely linked to the apomictic trait should be detected. In turn, markers should also be detected in sexual polyploid plants carrying only one copy of the allele conferring apomixis. None of these markers were amplified in the sexual progeny generated in this work. Bicknell et al. (2000) observed a different apomixis transmission pattern in Hieracium species with aposporous apomixis. Diploid and tetraploid sexual genotypes of H. pilosella were crossed with apomictic triploids of H. piloselloides. When diploid sexual plants were used as the mother plant, very few diploid progeny were recovered. Most of them were triploids or aneuploids and apomixis was only transmitted by diploid gametes. However, when sexual tetraploids were crossed with apomictic triploids, most progeny were triploid and tetraploid. Apomixis segregated among both the triploid and tetraploid progeny as a monogenic dominant trait. This indicates that both monoaploid and diploid gametes were able to transmit the apomixis trait. Non-recovery of diploid apomictic progeny was a consequence of selection against diploid hybrids, acting after fertilization, rather than selection at the gamete level (Bicknell et al., 2000).

In conclusion, the present study showed that the apomixis factor(s) in Paspalum is not transmitted by exact monoaploid gametes. This finding explains why apomictic diploids cannot be generated by hybridization. There was a strong distorted segregation of the apomixis trait when it was transmitted through diploid or higher-than-monoploid gametes. Incomplete penetrance of the lethal effect associated with apomixis, or alternatively, of some factor linked to the apo-locus could be the mechanism causing distorted transmission of the genomic region responsible for apomixis in Paspalum. Future analyses should be carried out to determine why some diploid species of Paspalum show some elements of apomictic reproduction, as the occasional formation of aposporous embryo sacs, and if these sacs are able to generate maternal progeny. In addition it should be determined if this ability by diploids is associated with the generation of apomictic polyploids after colchicine induction from sexual diploids.

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V. Conclusions.