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2n + n HYBRIDIZATION OF APOMICTIC *PASPALUM DILATATUM* WITH DIPLOID *PASPALUM* SPECIES

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Common dallisgrass (*Paspalum dilatatum*) is an apomictic pentaploid (2n=5x=50) of hybrid origin with irregular meiosis and with the genome formula IIJJX. The I and J genomes are homologous to those of diploid *P. intermedium* and *P. jurgensii,* respectively, but the source of the X genome is unknown. Members of the X genome may have genes of special biological significance, including those controlling apomixis. Common dallisgrass was crossed with several diploid *Paspalum* species in an attempt to identify the source of the X genome. Since common dallisgrass is apomictic, all hybrids produced will be formed by fertilization of an unreduced egg (2n+n). Any hybrid showing 30 chromosome bivalents at meiosis would indicate that the male diploid parent has a chromosome set that is homologous to the X genome of dallisgrass. Over 36,000 spikelets of dallisgrass were emasculated and dusted with pollen of 15 different diploid species (diploid species bearing I or J genomes were excluded). Only five (*P. chaseanum*, *P. equitans*, *P. fasciculatum*, *P. notatum*, and *P. simplex*) produced 2n+n hybrids with *P. dilatatum*. Meiotic chromosome behavior was similar in all hexaploid hybrids showing ca. 20 bivalents and 20 univalents. Results indicated a very low rate of 2n+n hybridization; none of the five diploid species possessed the X genome. Because several diploid species failed to hybridize with 5x dallisgrass, other methods should be attempted. Molecular markers specific for the X genome may help solve the question.

Keywords: dallisgrass, *Paspalum dilatatum*, apomixis, 2n gametes.

Introduction

Common dallisgrass (*Paspalum dilatatum* Poir.) is an important constituent of native pasture grasslands in temperate and subtropical regions of South America. The species includes at least five biotypes, three ploidy levels, and different meiotic chromosome behaviors, morphologies, and methods of reproduction. The common biotype is widely distributed throughout the world's temperate and warm regions. *Paspalum dilatatum* is a pentaploid (2n=5x=50) with 20 bivalents and 10 univalents (Bashaw and Forbes 1958), and it reproduces by obligate apomixis (Bashaw and Holt 1958). Important phylogenetic investigations through inter- and intraspecific hybridization programs concluded that the 5x biotype has the genome formula IIJJX (Burson 1983). Two other biotypes of dallisgrass are meiotically stable tetraploids with 40 chromosomes with 20 bivalents. Genome formula IIJJ has been assigned for both sexual biotypes, the yellow-anthered dallisgrass (*P. dilatatum* ssp. *favescens*) from Uruguay (Burson et al. 1973; Burson 1978) and the "Virasoro" type from northeastern Argentina (Caponio and Quarín 1990). Two dallisgrass biotypes are hexaploid apomictics. The "Uruguayan" type occurs in a small area in northwestern Uruguay and has essentially 30 bivalents and the genome formula IIJJXX (Burson 1991*a*, 1991*b*). The "Uruguaiana" type is endemic in a restricted area in southern Brazil and is not as meiotically stable as the Uruguayan type, having several univalents, an occasional multivalent association during meiosis, and the genome formula $IIJJXX_2$ (Burson 1995). In addition, two tetraploid and sexual taxa closely related to *P. dilatatum* occur in South America, both with the genome formula IIJJ: *P. urvillei* (Burson and Bennett 1972; Burson 1979) and *P. dasypleurum* (Quarín and Caponio 1995). At least two diploid species of the Paniculata group of *Paspalum* possess the J genome: *P. paniculatum* and *P. jurgensii* (Burson 1978, 1979). Both species reproduce sexually and, according to our personal observations, breed true. The I genome was originally assigned to diploid *P. intermedium* (Burson 1978), and slightly different forms of the I genome were later observed in four other diploid species of the Quadrifaria group (*P. quadrifarium*, *P. brunneum*, *P. haumanii*, *P. densum*) and in diploid *P. rufum* of the Virgata group. These five species and *P. intermedium* are outbreeders at the diploid level as a result of pollen-pistil self-incompatibility (Quarín and Norrmann 1990; Caponio and Quarín 1993). The source of the extra genome in pentaploid common dallisgrass as well as the origin of the third genome of hexaploid biotypes are unknown, and each has been arbitrarily assigned the letter X. Since the tetraploid species or the 4x biotypes of *P. dilatatum* with the IIJJ genome formula are sexual and the 5x (IIJJX) and 6x (IIJJXX) biotypes are apomictic, it has been suggested that gene(s) controlling apomixis in common dallisgrass as well as in 6x biotypes are on chromosomes of the X genome (Burson 1995). Thus, the investigation of the origin of this X genome has outstanding significance in terms of the evolution of the

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Table 1

Crosses between Two Apomictic Pentaploid (2n=50) Accessions of *Paspalum dilatatum* (Female Parent) and Several Diploid (2n=20) *Paspalum* Species (Pollen Donors)

Crosses	No. of emasculated and pollinated spikelets [A]	No. of recovered plants [B]	No. of 2n+n hybrids (2n=6x=60) [C]	Crossability [Cx100/A]
Paspalum dilatatum H163:				
× *P. cromyorrhizon* Q3635	420	15	...	...
× *P. chacoense* Q3630	805	7	...	...
× *P. chaseanum* F3377	824	59	...	...
× *P. dedecae* V11796	119	7	...	...
× *P. equitans* Q3683	944	8	...	...
× *P. fasciculatum* V9368	1880	59	2	0.11
× *P. hydrophilum* V10370	93	25	...	...
× *P. modestum* ERAGIA	162	34	...	...
× *P. notatum* Q3658	2922	83	...	...
× *P. notatum* Q4160	2893	82	2	0.07
× *P. notatum* Q4084	827	37	...	...
× *P. plicatulum* H14	2044	46	...	...
× *P. procurrens* Q4060	179	24	...	...
× *P. procurrens* Q4060	74[a]	10	...	...
× *P. redondense* V11370	675	19	...	...
× *P. redondense* V11370	32[a]	5	...	...
× *P. simplex* Q4109	3213	384	2	0.06
× *P. simplex* Q4109	28[a]	1	...	...
× *P. vaginatum* Q4092	3192	12	...	...
× *P. vaginatum* Q4092	333[a]	3	...	...
× *P. vaginatum* Q3690	155	1	...	...
× *P. vaginatum* Q4168	120	8	...	...
× *P. vaginatum* Q4168	510[a]	0	...	...
P. dilatatum Q4106:				
× *P. chaseanum* F3377	1270	108	3	0.23
× *P. equitans* Q3683	1232	187	10	0.81
× *P. fasciculatum* V9368	654	2	...	...
× *P. lenticulare* N188	1098	10	...	...
× *P. notatum* Q3658	2061	44	1	0.05
× *P. notatum* Q4160	2187	35	1	0.04
× *P. notatum* Q4084	770	43	...	...
× *P. redondense* V11370	159[a]	3	...	...
× *P. simplex* Q4109	1007	110	...	...
× *P. simplex* Q4109	56[a]	7	...	...
× *P. vaginatum* Q4092	1255	9	...	...
× *P. vaginatum* Q4092	809[a]	6	...	...
× *P. vaginatum* Q4168	354	11	...	...
× *P. vaginatum* Q4168	848[a]	2	...	...
Total	36,204	1506	21	$\overline{X}$ = 0.058

Note. Most crosses were performed at the time of anthesis.

[a] Crosses were carried out 2–3 d before anthesis.

Dilatata group of *Paspalum* but also in apomixis research in the Gramineae.

The main obstacle to investigating the homology of the X genome of 5x dallisgrass is the difficulty in producing hybrids with the complete set of chromosomes from this genome. The way to achieve such a hybrid might be through the fertilization of the unreduced gamete of 5x biotype. Fertilization of an unreduced egg of an aposporous embryo sac occasionally occurs in grasses, especially when wide crosses are performed (Bashaw et al. 1992). In apomictic 4x *P. notatum*, 2n+n progeny were easily obtained when forced pollination was performed 2 or 3 d before anthesis. In addition, the detection of a diploid species bearing the X genome, if such a diploid species actually exists, may prove useful for subsequent studies on apomixis in the genus.

The objectives of this research were (1) to produce 2n+n hybrids by crossing apomictic 5x dallisgrass (female parent) with diploid *Paspalum* species and (2) to analyze the meiotic

chromosome pairing of the 2n+n hybrids (2n=6x=60) to determine whether the 10 chromosomes from the diploid parent paired with the chromosomes of the X genome.

Material and Methods

Two pentaploid accessions of *Paspalum dilatatum* were pistilate parents: accession AH-163, collected at Posadas, Misiones, Argentina, and accession Q-4106 from Oliveros, Santa Fe, Argentina. Most diploid *Paspalum* species in the primitive natural area of 5x dallisgrass (Uruguay, central Argentina, and southern Brazil) were pollen parents. Diploid species known to have the I or the J genome were not used. A total of 15 species, including 19 accessions, provided the pollen source.

Crosses were made in two different ways, either at the time of anthesis or ca. 2–3 d before anthesis. In the first case, a potted plant of 5x dallisgrass was maintained overnight in a chamber with a humidifier that was turned on ca. 2 h before sunrise to create a fog mist. Around sunrise, anthesis occurred, but dehiscence was delayed as a result of high humidity. Anthers were eliminated with sharp-pointed tweezers, and then the pollen of the desired parent was dusted on fresh stigmas. To perform crosses before anthesis, a potted plant of 5x dallisgrass was brought into the laboratory, the appropriate spikelets were opened under a stereomicroscope, and fresh pollen of the 2x parent was introduced into the spikelets with the aid of a small brush. Usually, only 50 to 100 spikelets were opened and pollinated in one inflorescence. The remaining unpollinated spikelets or entire intact racemes were removed from the inflorescence.

Approximately 30 d after pollination, inflorescences were harvested and the empty spikelets were separated from those with caryopses. Seeds were germinated in flats with sterilized soil in a greenhouse during late winter. Seedlings were grown in small pots. Chromosome numbers were determined for any plant considered to be an off-type, and in this way, most 2n+n hybrids were detected. The remaining plants were space-planted in a field nursery, in which an additional screening searched for off-type plants at the mature stage.

Chromosome numbers were determined in root tips that had been pretreated with an a-bromonaphthalene aqueous-saturated solution for 2 h, hydrolyzed in hydrochloric acid (1 N) for 10 min at 60°C, stained with Feulgen reagent, and squashed in a drop of 1% aceto-orcein.

Material to study meiosis was fixed in a 3 : 1 solution of absolute ethanol : acetic acid for 24 h and stored in 70% ethanol. Pollen mother cells were stained with aceto-carmine.

Results

A total of 36,204 spikelets of two accessions of 5x *Paspalum dilatatum* were emasculated and pollinated with pollen from 15 different 2x *Paspalum* species, including 2849 crosses made 2 or 3 d before anthesis (table 1). Only 1506 plants were recovered. Visual selection of the off-type plants and corroboration by chromosome counts identified 21 plants as 2n+n hybrids with 60 chromosomes. The overall crossability was 0.058%. Considering the different crossing techniques, crossability was 0% when pollinations were made 2 or 3 d before blooming and 0.063% when emasculations and pollinations were made at anther emergence. Interspecific 2n+n hybrids were produced from only five of the 15 diploid species: *P. chaseanum* from Las Lomitas, Formosa province, Argentina; *P. equitans* from Colonia Garabí, Corrientes province, Argentina; *P. fasciculatum* from Cáceres, Mato Grosso, Brazil; *P. notatum* from Tifton, Georgia, U.S.A. (line 2 and cultivar "Tifton 9"); and *P. simplex* from Ranch Los Gatos, Santiago del Estero province, Argentina. The best performance was achieved with the combination of *P. dilatatum* accession 4106 crossed with accession Q3683 of *P. equitans* (crossability = 0.81%). There were some especially recalcitrant crossing combinations. For example, more than 7500 spikelets were pollinated with pollen of *P. vaginatum*, including both accessions of *P. dilatatum* and three accessions of *P. vaginatum*, and no hybrids were produced.

The five hexaploid 2n+n interspecific hybrids were analyzed: (1) *P. dilatatum* × *P. chaseanum*, (2) *P. dilatatum* × *P. equitans*, (3) *P. dilatatum* × *P. fasciculatum*, (4) *P. dilatatum* × *P. notatum*, and (5) *P. dilatatum* × *P. simplex*. The hybrids had similar meiotic chromosome associations at metaphase I with ca. 20 bivalents (^{II}) and 20 univalents (^{I}) (table 2). Hybrids involving *P. equitans* and *P. notatum* always showed $20^{II}+20^{I}$ (fig. 1*a*), whereas some trivalent chromosome associations were occasionally observed in hybrids whose male parents were *P. chaseanum*, *P. fasciculatum*, or *P. simplex* (fig. 1*b*). The entire meiotic process was similar to that described for 5x *P. dilatatum* (Bashaw and Forbes 1958), with the difference that 5x dallisgrass showed 10 univalent lagging chromosomes at anaphase I, whereas in the hybrids, ca. 20 univalent laggards were usually observed (fig. 1*c*).

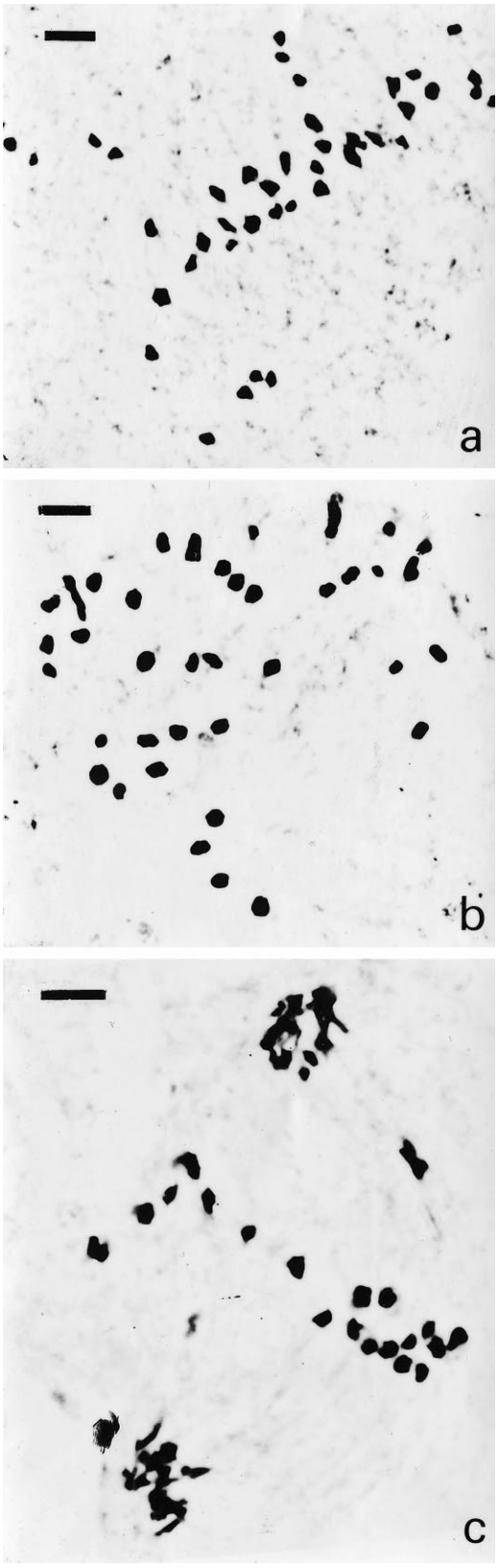
Discussion

The very low general crossability among sexually reproducing *Paspalum* species was ascribed to failure of the sperm

Table 2

Meiotic Chromosome Configurations of 2n + n Hybrids (2n=6x=60) between Two Accessions of Apomictic Pentaploid (2n=50) *Paspalum dilatatum* and Five Diploid (2n=20) *Paspalum* Species

Crosses	2n	No. of analyzed pollen mother cells	Chromosome configurations per pollen mother cell		
			I	II	III
Paspalum dilatatum Q4106:					
× *P. chaseanum* F3377	60	33	19.97	19.97	0.02
× *P. equitans* Q3683	60	46	20	20	...
P. dilatatum H163:					
× *P. fasciculatum* V9368	60	34	18.75	18.75	1.18
× *P. notatum* Q4160	60	32	20	20	...
× *P. simplex* Q4109	60	53	19.89	19.89	0.1



nuclei to fertilize the egg cell in the embryo sac rather than to the action of a cross-incompatible system (Burson 1987). Overall crossability was also low for intraspecific crosses among different cytotypes of *Paspalum dilatatum* (Burson 1991*a*). Thus, low hybridization success was expected from our crosses, chiefly because we used an apomictic plant as the female parent. The complete failure of 2n+n hybridization when pollination was achieved before anthesis may be attributable to the traumatic consequences of handling the inflorescence.

The odd ploidy level of common dallisgrass, pentaploid *P. dilatatum*, has outstanding biological significance as a natural hybrid with unbalanced genomic composition and obligate aposporous apomixis. Tetaploid races of the species are sexual and autogamous, with an allotetraploid origin and regular meiotic behavior with 20 bivalents. The genome formula IIJJ has been assigned for 4x races, whereas IIJJX is the formula for the 5x cytotype (Burson 1983). The I and J genomes are widely distributed among diploid species of *Paspalum* (Burson 1989). On the other hand, the existence of the X genome at the diploid level remains unknown. The hypothesis that the odd X genome is responsible for apomixis in 5x dallisgrass may act as an inducement to speculation that the gene action is dominant and that a single allele may express apomixis in a pentaploid plant. Moreover, it can also be speculated that if an XX diploid plant exists, it might be apomictic. However, since the odd genome does not recombine with II or JJ, it could just as well involve two to many genes acting as a supergene locus that only mimics simple inheritance (i.e., the responsible gene action could be dominant or codominant). The fact that the X genome is recombinationally hemizygous and that it is certainly associated with apomictic reproduction in dallisgrass is consistent with previous reference to hemizygosity as being associated with the gene or the chromosomal region responsible for apomixis. In *Pennisetum,* Ozias-Akins et al. (1998) have been unable to map close to the putative apomixis gene(s). They suggest that tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. Similarly, Grimanelli et al. (1998) observed strong restriction to recombination in the genomic segment responsible for apomixis in *Tripsacum,* as compared with the corresponding segment of sexual plants and the collinear region of maize. This indicates that apomixis may be controlled by a cluster of linked loci and that the genomic region might be hemizygous.

Certainly, the role of the X genome is not well understood, and it could accentuate genetic effects existing in the I or J genomes that are not expressed in tetraploid biotypes. In fact, occasional aposporous embryo-sac development has been de-

Fig. 1 Meiotic chromosome associations of hexaploid 2n+n interspecific hybrids among apomictic pentaploid *Paspalum dilatatum* and diploid *Paspalum* species. *a, P. dilatatum* × *P. notatum*, metaphase I with $20^{II}+20^{I}$. *b, P. dilatatum* × *P. simplex*, prometaphase I with $2^{III}+18^{II}+18^{I}$. *c, P. dilatatum* × *P. chaseanum*, late anaphase I with 20 lagging chromosomes. Bar = 5 mm.

scribed in diploid races of *P. intermedium*, *P. quadrifarium*, *P. haumanii*, *P. brunneum*, and *P. rufum* (Norrmann et al. 1989), five species sharing the basic I genome, which is homologous to the I genome present in 4x and 5x biotypes of dallisgrass.

Independent of the nature of the gene(s) controlling apospory in *P. dilatatum*, the X genome of dallisgrass is undoubtedly of outstanding biological importance. The X genome is certainly associated with apomictic reproduction in dallisgrass, and there is a clear advantage in the ecological versatility of the apomictic 5x biotype in relation to sexual tetraploid races of the same species. That is the reason why the 5x biotype is recognized as the common biotype in the literature.

Our results demonstrate that the efficiency of 2n+n hybridization is extremely low. They also show that none of the five diploid species that produced 2n+n hybrids has genomic homology with the X genome of dallisgrass. Thus, the question remains unsolved: Does a diploid XX species actually exist? Since the question is relevant and the 2n+n hybridization method is inefficient, new methods need to be developed. For example, the identification of molecular markers specific to the X genome may allow for screening the entire range of diploid *Paspalum* species distributed throughout the native area of 5x dallisgrass in South America.

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