Theor Appl Genet (1997) 95: 850–856

J. P. A. Ortiz · S. C. Pessino · O. Leblanc
M. D. Hayward · C. L. Quarín

Genetic fingerprinting for determining the mode of reproduction in *Paspalum notatum*, a subtropical apomictic forage grass

Received: 5 March 1997 / Accepted: 13 May 1997

Abstract *Paspalum* is an important genus of the family Gramineae that includes several valuable forage grasses. Many of the species are polyploid and either obligate or facultative apomicts. Cyto-embryological observations of several tetraploid genotypes of *P. notatum* were performed to determine their mode of reproduction. Afterwards, selfed progenies of the genotypes F131, Q3664 and Q4117 were analysed using RFLP and RAPD genetic fingerprints to identify maternal and non-maternal (aberrant) plants, and to establish the degree of apomictic reproduction. Five maize clones and six primers were used for detecting genetic deviations from the maternal profile. Maize clones umc379, umc384 and umc318 and primers OPG10 and OPI4 were the most informative for discriminating between maternal and aberrant individuals within the progenies of F131 and Q3664. The combined results of three RFLP clones or 4–6 RAPD primers were necessary to ascertain the mode of reproduction in plants F131 and Q3664. The results obtained with the RFLP and RAPD markers were in agreement with the cyto-embryological studies in ascertaining the mode and degree of apomictic reproduction. Plant F131 showed a completely sexual reproductive behaviour, Q3664 an elevated expression of sexuality, while Q4117 was highly apomictic. A fingerprint analysis of an outcrossing population, aimed at the identification of hybrid plants, was also performed. Maize clones um318 and umc379 and primers OPC2 and OPC9 were used. The presence of specific bands belonging to the male parent permitted a rapid and easy detection of hybrids. The methodology described here can be applied both for the characterisation of *P. notatum* populations and to identify hybrid progenies in *Paspalum* breeding programs.

Key words Apomixis · DNA fingerprinting · Molecular markers · *Paspalum* · Progeny test

Introduction

Paspalum is a large genus of the family Gramineae with nearly 400 species mostly native to the warm regions of the Western Hemisphere (Chase 1929). It is one of the most economically important genera of the tribe Paniceae because many species are valuable forage grasses. *Paspalum notatum* Flugge (bahiagrass) is a rhizomatous species widely distributed from Central Eastern Mexico to Argentina and throughout the West Indies (Burton 1946). Cytotypes with chromosome numbers of $2n = 2x = 20$, $3x = 30$, and $4x = 40$ have been described (Burton 1946; Saura 1948; Gould 1966). *P. notatum* var. *saurae* Parodi ($2n = 2x = 20$) was accidentally introduced into the United States prior to 1926 and has become one of the major forage grasses in south-eastern US (Burton 1967).

P. notatum has an extremely versatile reproductive system with sexual self-fertile diploids and pseudogamous apomictic autopolyploids (Burton 1948; Forbes and Burton 1961). Occasional triploids are apomictic (Burton and Hanna 1986). Since apomixis means asexual reproduction through seeds, leading to maternal offspring that are genetically an exact copy of the mother plant (Nogler 1984), segregation analysis of obligate apomicts is impossible unless sexual germplasm is available. In facultative apomicts,

Communicated by G. Wenzel

J. P. A. Ortiz (✉) · S. C. Pessino · O. Leblanc · M. D. Hayward
Institute of Grassland and Environmental Research (IGER),
Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK
Fax: + 44 1970 828357
e-mail: juanpa.ortiz@bbsrc.ac.uk

C. L. Quarín
Instituto de Botánica del Nordeste (IBONE) – Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, CC 209,
3400 Corrientes, Argentina

progenies generally segregate into apomictic (maternal) and non-apomictic (aberrant) classes.

Cytological and embryological analysis of the mother plant and screening for morphologically aberrant progenies are the methods most commonly employed for assessing the mode of reproduction in *Paspalum* and other apomictic grasses (Young et al. 1979; Quarín and Hanna 1980; Quarín et al. 1982; Norrmann et al. 1989). However, these are time-consuming tests and often limited in their application.

Progeny analysis attempts the identification of every aberration from the maternal genotype as the result of a sexual reproduction event (Marshall and Brown 1974). Molecular markers, like restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD), can be used for the identification of non-maternal offspring when individual DNA fingerprints reveal a deviation from the maternal profile (Mazzucato et al. 1995). Genetic fingerprints can also help in the identification of hybrid plants in breeding programs (Bellamy et al. 1996). Molecular markers have been used to discriminate between sexual and apomictic progeny in *Rubus* (Antonius and Nybom 1995) and to establish the degree of apomixis of mother plants in *Poa* (Mazzucato et al. 1995). RAPD markers have been combined with flow cytometry to determine the genetic origin of aberrant plants within progenies of *P. pratensis* (Huff and Bara 1993). Moreover, RFLP and RAPD markers have been used to analyse transmission of the apomictic mode of reproduction in *Pennisetum* (Ozias-Akins et al. 1993) and in maize-*Tripsacum* hybrids (Leblanc et al. 1995) as well as to identify a linkage group including the apomixis gene in *Brachiaria* (Pessino et al. 1997).

The objective of the present work was the determination of the mode of reproduction of tetraploid *P. notatum* genotypes with the aid of RFLP and RAPD genetic fingerprints of selfed progenies. Cyto-embryological studies of the mother plants were also carried out to compare the degree of apomictic reproduction assessed by both methods. Additionally, a genetic fingerprinting study of an outcrossed population was performed to test the efficiency of the molecular-marker analysis for the identification of *P. notatum* hybrids.

Materials and methods

Plant material

Tetraploid *P. notatum* accessions from IBONE's germplasm collection (Argentina), were established from seed in a glasshouse at IGER (UK). Accession Q4117 was collected in the State of Rio Grande do Sul, Brazil. Accessions Q3775 and Q3778 were collected in Tamaulipas (Municipio Gómez, Mexico) and Palmar Grande (Corrientes, Argentina), respectively. Material of experimental origin was also studied: tetraploid plant Q3664 was generated at Tifton (Georgia, USA) through controlled crosses between a sexual colchicine-induced tetraploid *P. notatum* var *saurae* and a white-stigma apomictic tetraploid form of *P. notatum*, followed by several generations of backcrossing to the white-stigma parent plant (Quarín et al. 1984). Q3664 has white stigmas and it is highly sexual. The plant F131 was produced from a cross between Q3664 as pistillate parent and the tetraploid accession Q3853 as pollen donor. Selfed progenies from Q3664 and F131 plants and from accession Q4117 were analysed using RFLP and RAPD markers to identify maternal and non-maternal individuals. An F_1 hybrid population from a cross between Q3664 and Q4117 was used for hybrid-identification analysis.

Cyto-embryological analysis

Inflorescences at anthesis were collected and fixed in FAA (70% ethanol, glacial acetic acid, formaldehyde, 90:5:5) for 24–48 h. Pistils were dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Samples were sectioned at 12–15 µm and stained with safranin and fast green. Observations were carried out with a light transmission microscope.

DNA extraction

Genomic DNA was extracted essentially as described by Dellaporta et al. (1983) from 3–5 g of leaf tissue. Plant material was frozen in liquid nitrogen, powdered with a mortar and pestle and transferred to 15 ml of extraction buffer (100 mM Tris-HCl pH 7.5, 50 mM EDTA pH 8, 500 mM NaCl, 2% SDS and 1% polyvinylpirrolidone MWt 360000). The mixture was incubated at 68°C for 30 min followed by the addition of 4 ml of 5 M potassium acetate. Samples were incubated on ice for at least 30 min and centrifuged for 20 min at 4°C and 11 000 g. The supernatant was collected through a cotton mesh (Miracloth, Calbiochem Catalogue No. 475855) and 1 volume of isopropanol was added. Samples were incubated overnight at − 20°C and then centrifuged (20 min, 11 000 g, 4°C). Pellets of DNA were re-suspended in 700 µl of TE, treated with 5 µl of RNAase (10 mg/ml) for 20 min at room temperature, phenolized, and ethanol-precipitated at − 20°C. Samples were finally centrifuged (20 min, 15 000 rpm), washed in 70% ethanol, dried, and dissolved in 400–500 µl of sterile distilled water.

RFLP analysis

Genomic DNA (25 µg) was digested with *Eco*RI or *Hin*dIII, electrophoresed overnight at 25 V in 1% agarose /1 × TAE gels and blotted onto nylon membranes (Hybond N, Amersham) using 10 × SSC buffer. DNA was UV-fixed with a Stratagene UV crosslinker and filters were baked at 80°C for 2 h. Sixteen maize clones from the University of Missouri-Columbia series (umc 16, 31, 81, 84, 128, 130, 304, 318, 321, 327, 338, 342, 346, 379, 384 and 389), kindly donated by CIMMYT, Mexico, were screened to identify informative markers. Non-radioactive hybridisation and detection procedures were performed according to Boehringer-Mannheim instructions (DNA Labeling and Detection Kit Non-radioactive, Catalogue No. 1 093 657). Probes were PCR-labelled in a 100-µl reaction, containing 5 ng of plasmid DNA, 15% glycerol, 1 × *Taq* buffer (Boehringer-Mannheim), 1.5 mM $MgCl_2$, 50 mM of each dNTP, 0.2 mM oligonucleotides, 5% dig-dUTP or 15% fluorescein-dUTP and 1 U of *Taq* polymerase (Boehringer-Mannheim). Hybridisation were carried out at 63°C, by adding 25 µl of the probe-labeling reaction to 10 ml of hybridisation solution. Detection was carried out using 5 µl/ml of CSPD, disodium 3{4-methoxyspiro [1,2-dioxetane-3,2′-(5′-chloro) tricyclo $(3.3.1.1^{3,7})$ decan]-4-yl} phenyl phosphate (Boehringer-Mannheim), as a chemiluminescent substrate. Hyperfilm-ECL (Amersham) films were exposed for between 2 and 12 h.

RAPD analysis

Sixty six 10-mer arbitrary oligonucleotides from Operon Technologies Inc. (Alameda, Calif., USA), series C1-10, F1-12, G1-12, I1-12, L1-10 and M1-10, were screened to detect primers giving multiple band-amplification patterns. PCR amplifications were performed in 25-µl total-volume reactions containing 20 ng of genomic DNA, 1 × *Taq* reaction buffer (Boehringer-Mannheim), 12.5 µM of each dNTP, 1.5 mM $MgCl_2$, 30 ng primer and 1 U of *Taq* polymerase (Boehringer-Mannheim). Reactions were carried out using either a Perkin-Elmer GenAmp PCR System 9600 thermocycler or a MJ Research, PTC-100 Programmable Thermal Controller. Cycles began with 2 min at 95°C followed by 40 cycles of 1 min at 95°C, 1 min at 36°C and 2 min at 72°C. A final incubation of 5 min at 72°C was included. Amplification products were analysed by adding 5 µl of loading buffer and running 15 µl of the mixture in 2% agarose/1 × TBE gels at 65 V during 3–4 h. Gels were stained with ethidium bromide and fragments were visualised under ultraviolet light. Photographs were taken using Polaroid 667 films.

Selection of informative markers

RFLP clones and RAPD primers detecting polymorphic loci were considered informative for discriminating between maternal and non-maternal (aberrant) plants. Screening of informative markers was carried out using DNA samples of the maternal parents and 5–10 individuals of their progeny. This scheme was used to allow the detection of fragments segregating in a 1 : 1 or 3 : 1 Mendelian ratio. Thereafter, segregation analysis was performed on 25–32 individuals of each progeny family.

Results

Embryo-sac analysis

Observations of ovaries from tetraploid *P. notatum* revealed the presence of three different types of ovules:

(1) Ovules bearing a single meiotic embryo sac characterised by the egg apparatus (egg cell and two synergids), a large two-nucleated central cell and a mass of proliferated antipodals at the chalazal end (Fig. 1 A).

(2) Ovules with one to several aposporous embryo sacs (Fig. 1 B). These sacs showed a different size (30–60 µm), constitution, shape and orientation. The largest ones usually contained two large polar nuclei in a widely vacuolated central cell, the egg cell, and one or two synergids. The absence of antipodals characterised the aposporous sacs.

(3) Ovules with one meiotic embryo sac surrounded by one to several aposporous sacs.

The frequency of the different types of ovules observed in each accession is presented in Table 1.

The three natural tetraploid accessions, Q4117, Q3775 and Q3778, were shown to be facultative apomicts because both aposporous- and meiotic-sacs were observed in ovules of the same individuals. Q4117

Table 1 Embryo-sac types in different accessions and experimental plants of tetraploid ($2n = 4x = 40$) *P. notatum*. M, one meiotic embryo sac; A, one to several aposporous embryo sacs

Accessions[1] or plants[2]	Scored ovules (number)	Percentage of ovules bearing			
		M	A	M + A	Aborted
Q4117[1]	100	3	95	2	0
Q3775[1]	100	3	87	5	5
Q3778[1]	85	24	61	9	6
Q3664[2,a]	88	70	15	15	< 1
F131[2]	82	94	0	0	6

[a] From Quarin et al. (1984)

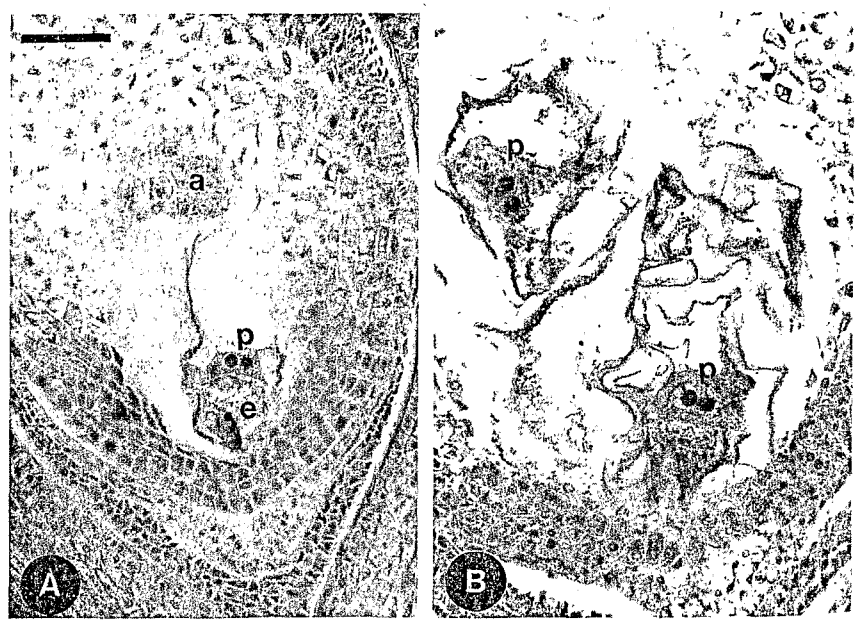

Fig. 1A, B Photomicrographs of sectioned ovules of tetraploid *P. notatum* genotypes. **A** Ovule bearing a single meiotic sac from plant F131 (Two synergids and more antipodals are not shown because they are in an adjacent section of the ovule). **B** Ovule bearing two aposporous embryo sacs from highly apomictic accession Q4117 (other nuclei in adjacent sections). References: *a* antipodals; *e* egg cell; *p* polar nuclei; *bar* = 33µm

and Q3775 showed a higher proportion of aposporous sacs than Q3778. The plant Q3664 showed mostly meiotic sacs. No aposporous sacs were observed in 82 ovules of F131. These observations suggest that Q4117 and Q3775 are highly apomictic accessions and Q3778 is a facultative intermediate. Plant Q3664 has a high expression of sexuality and F131 appears to be completely sexual. In accordance with these results, plants F131 and Q3664 and accession Q4117 were selected for the progeny test.

Screening of RFLP and RAPD informative markers

Although a genetic map of *Paspalum* is not available, the co-linearity between grass genomes (Moore et al. 1995) allowed us to employ the information of maize linkage groups (Heredia-Díaz et al. 1994) for testing different genomic regions of *Paspalum*. The 16 maize probes selected were assumed to detect loci distributed over various sectors of the *Paspalum* genome. Screening of maize clones on filters containing DNA of the maternal genotype and 5–10 individuals of their progeny showed that 5 of the 16 probes tested (umc 16, 81, 130, 321 and 338) produced smearing with all three population samples and were thus rejected. The rest yielded 3–5 fragments, although clone umc 379 produced between 7 and 11 bands (Fig. 2). All successfully hybridised probe showed segregating fragments in at least one of the five individuals of F131 offspring. Similar results were obtained with family Q3664, but four clones (umc 304, 327, 346 and 389) did not reveal any segregation. None of the probes showed segregating fragments in the screening filters of the Q4117 family. Clones umc 31, 304, 318, 379 and 384 were selected for the progeny test because they produced good hybridisation patterns and 1–3 segregating bands, which allowed the identification of variation in the maternal profile. The screening of 66 RAPD oligonucleotides, led to the identification of 16 primers that produced good amplification signals. The rest did not amplify or else showed smearing. All selected primers generated between three and eight bands in the three populations assayed. Primers OPC2, OPC9, OPG6, OPG10, OPI4 and OPL7 were chosen for the progeny analysis because they produced reproducible and easily readable amplification patterns with 1–2 segregating bands among the progeny of the F131 abd Q3664 genotypes (Fig. 3).

Aberrant-plant identification

Offspring plants were classified as maternal when both RFLP and RAPD genetic fingerprints were identical to the maternal profile, or aberrant when any deviation was observed. Molecular analysis of 32 individuals from each progeny family of F131, Q3664 and Q4117 allowed an investigation of the reproductive mode of the maternal genotypes. This sample size is adequate to detect at least one representative of a recessive genotype (RAPDs) or a homozygote of one allelic form when co-dominance is found (for RFLPs) with a probability of at least 0.999 (see Mather 1951).

In the F131 population, hybridisation with umc 379 showed that 25 out of 32 individuals (78%) presented deviations from the maternal profile. Likewise, clone umc 384 detected 23 aberrants (72%). Probes umc 318, 304 and 31 recognised 21 (67%), 20 (62%) and 14 (44%) non-maternal plants, respectively. In family Q3664 clone umc 379 detected 24 aberrants (75%) out of the 32 individuals assayed. Umc 318, 31 and 384 detected 17 (52%), 10 (32%) and 9 (29%) non-maternal plants in the offspring, respectively. In family Q4117 only one individual out of the 32 tested showed a non-maternal profile when clones umc 384 and umc 318 were used. The combination of fingerprints obtained with the five selected clones revealed no maternal individuals in the offspring of F131, five maternal plants (15.6%) in family Q3664, and only one aberrant (96% maternal plants) in the Q4117 progeny.

RAPD fingerprints of F131 progeny showed 15 (60%) and 13 (52%) non-maternal plants out of 25 assayed when primers OPG10 and OPC2 were used. Primers OPI4, OPG6 and OPC9 displayed a similar

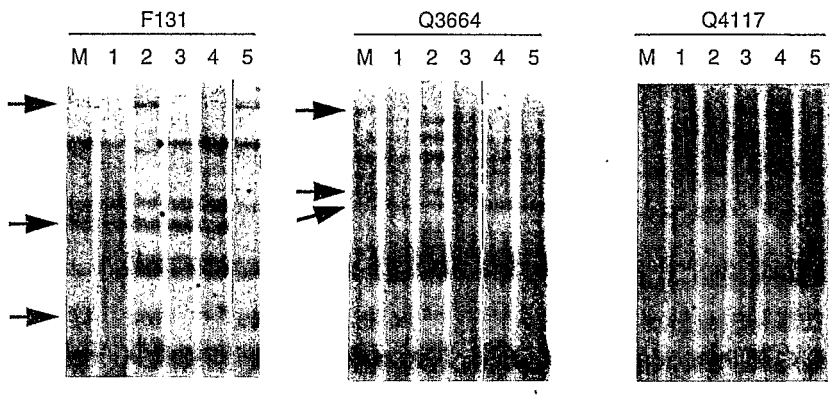

Fig. 2 RFLP fingerprints of plants F131, Q3664 and Q4117 and some of their offspring. DNA was digested with *Hin*dIII and hybridised with probe umc 379. *M* maternal genotype. *Lanes 1–5* individuals of the corresponding progeny. *Arrows* indicate the segregating fragments used in the discrimination of maternal and non-maternal plants

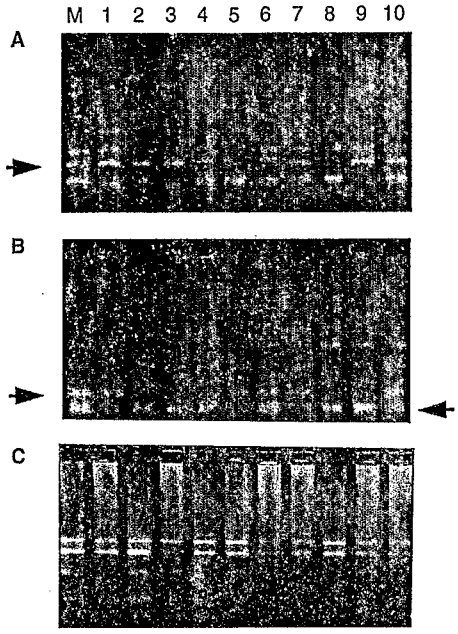

Fig. 3 RAPD fingerprints produced by primer OPI4 in F131 (**A**), Q3664 (**B**) and Q4117 (**C**) progeny family. *M* maternal genotype, *lanes 1–10* individuals of their progeny. *Arrows* indicate the segregating amplification fragments used in the progeny analysis

discriminatory capacity detecting 10, 9 and 8 non-maternal plants, respectively. Only four aberrants were detected by OPL7. In the Q3664 population, primer OPI4 detected 12 non-maternal plants (48%) while OPC2, OPC9, OPG6, OPL7 and OPG10 detected 7, 6, 5, 5 and 4 aberrants, respectively. In the Q4117 progeny, primers OPC9, OPG10 and OP14 each detected two aberrant plants. The combination of results obtained with the six selected oligonucleotides showed non-maternal progeny in F131 five maternal plants (16%) in Q3664, and 23 maternal plants (92%) in the Q4117 family.

The combined result of RFLP and RAPD fingerprints and the cumulative detection of non-maternal plants with both types of molecular markers is shown in Fig. 4. In the F131 population all non-maternal individuals could be detected by the combination of three RFLP probes or four RAPD primers (Fig. 4 A). Likewise, in the Q3664 population three RFLP clones (umc 379, 318 and 31) allowed the detection of all aberrants, but a six-primer combination was necessary to detect 96% of them (Fig. 4 B). The degree of apomixis determined by both RFLP and RAPD markers was similar and agreed with the embryological studies (Table 2).

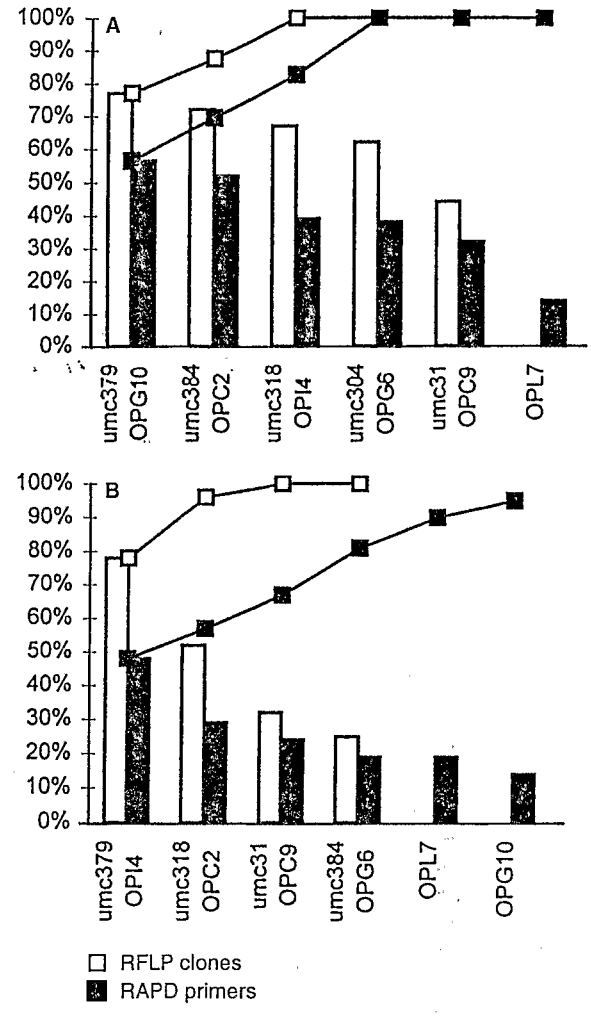

Fig. 4 Discriminating efficiency of RFLP clones and RAPD primers in F131 (**A**) or Q3664 (**B**) progenies. Efficiency was calculated as the percentage of aberrant plants detected in each population (indicated by *bars*). Cumulative percentage is indicated by *lines* (□ RFLPs, ■ RAPDs)

Table 2 Degree of apomixis (%) of *P. notatum* plants estimated by cytological observations and molecular analysis

Accessions	Cytological observations	RFLPs[a]	RAPDs[b]
F131	0	0	0
Q3664	15–30	15.6	16
Q4117	97	96	92

[a] Results were obtained using a combination of four maize probes
[b] Results were obtained using a combination of six primers

Hybrid-plant identification

As the female parent used to generate the F_1 population (Q3664) had white stigmas and the male parent (Q4117) had purple stigmas, the F_1 plants with purple stigmas were all considered to be hybrids. Genetic fingerprinting was carried out on 32 randomly chosen hybrid plants. Hybridisation with clones umc 318 and umc 379 showed segregating markers from both progenitors. The presence of specific bands belonging to

the male parent allowed the rapid identification of a large proportion of the hybrid plants. Using umc 318 and 379, 72% and 94% of total hybrids were detected, respectively. The combination of results with both clones allowed the molecular identification of 98% of them. Similar results were produced by RAPD markers. The combined results obtained with OPC2 and OPC9 allowed the detection of 96% of the total hybrid plants. In both types of genetic tests maternal bands segregated in a 1:1 ($\chi^2_1 < 0.125$, $P > 0.7$) or 3:1 ($\chi^2_1 < 0.168$, $P > 0.5$) Mendelian ratio. No homozygous markers belonging to the male parent were detected. The segregation of parental bands in the F_1 progeny also resulted in 1:1 ($\chi^2_1 < 0.32$, $P > 0.8$) or 3:1 ($\chi^2_1 < 0.166$, $P > 0.5$) ratios.

Discussion

Our results indicate that both RFLP and RAPD molecular markers are appropriate for investigating the mode of reproduction in *P. notatum* and identifying hybrid plants in outcrossing populations. Maternal and non-maternal progeny can also be detected at an early stage of development. Due to the high level of heterozygosity present in natural populations of *P. notatum*, selfed progenies showed a high number of segregating markers useful for fingerprinting. According to our results a complete analysis of the degree of apomixis in tetraploid *P. notatum* genotypes should include a combination of at least three informative RFLP clones or 4–6 informative PCR primers (Fig. 4). However, considering the lower amount of DNA required, the simplicity of the methodology and the short time needed to produce the results, RAPD analysis would be an appropriate system for application to breeding programs. RAPD markers also have a high discriminatory capacity since a single primer can at the same time amplify several different genomic regions, so giving a wide coverage of the genome (Williams et al. 1990). Nevertheless, informative primers should be screened on the different genotypes because the efficiency of "aberrant identification" depends on the population studied. Natural tetraploid *P. notatum* strains had been previously reported to be completely apomictic (Burton 1948; Bashaw et al. 1970) or facultatively apomictic (Burton and Hanna 1986). Our cytoembryological studies indicated that natural tetraploid accessions reproduce mainly by means of apospory. Nevertheless, the three natural tetraploid strains that were analysed showed some potential for sexual reproduction. The progeny tests carried out with the aid of molecular markers demonstrated that this potential was effectively expressed, even in the progeny of strain Q4117 which showed the highest degree of apospory (97%). This is an important verification that occasional sexual reproduction should be a decisive mechanism for the production of genetic variation and for evolution in apomictic tropical grasses. On the other hand, our embryological observations of genotype F131 revealed that it had only meiotic embryo sacs. Moreover, the lack of maternal individuals within its progeny supports its completely sexual reproductive behaviour. This sexual tetraploid genotype is of considerable potential utility for future breeding programs. It can be used as a female parent, while the apomictic forms can be used as pollen donors. Crosses with obligate and facultative apomictic plants will allow the production of segregant populations for the reproductive mode, which could be of use in strategies for tagging the gene/genes controlling apomixis in this species.

The agreement between the cytological and molecular studies in determining the degree of apomixis was high. Therefore, we consider that genetic fingerprints with both RFLP or RAPD markers provide an important tool for studying the level of apomixis expressed by a single genotype. Likewise, an accurate identification of aberrants and hybrid plants can be speedily performed. The methodology described here can also be applied to study the genetic relationships among different ecotypes of *P. notatum* and to characterise new cultivars of commercial value.

Acknowledgements This work was financed by a grant from the Commission of the European Communities-STD3 program (TS3-CT93-0242). J.P.A.O. was supported by IBONE-Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina. S.C.P. received a grant from The British Council/Fundación Antorchas, Buenos Aires, Argentina. O.L. was funded by a Lavoisier Fellowship from the French Government (Ministère des Affaires Etrangères). C.L.Q. is a member of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina).

References

Antonius K, Nybom H (1995) Discrimination between sexual recombination and apomixis/automixis in a *Rubus* plant-breeding programme. Hereditas 123:205–213

Bashaw EC, Hovin AW, Holt EC (1970) Apomixis, its evolutionary significance and utilization in plant breeding. In: Norman JT (ed) Proc 11th Int Grasslands Congr, University of Queensland Press, St. Lucia, pp 245–248

Bellamy A, Vedel F, Bannerot H (1996) Varietal identification in *Cichorium intybus* L. and determination of genetic purity of F_1 hybrid seed samples, based on RAPD markers. Plant Breed 115:128–132

Burton GW (1946) Bahiagrass types. J Am Soc Agron 38:273–281

Burton GW (1948) The method of reproduction in common Bahiagrass, *Paspalum notatum*. J Am Soc Agron 40:443–452

Burton GW (1967) A search for the origin of Pensacola Bahiagrass. Econ Bot 21:379–382

Burton GW, Hanna WW (1986) Bahiagrass tetraploids produced by making apomictic (tetraploid × diploid) × diploid hybrids. Crop Sci 26:1254–1256

Chase A (1929) North American species of *Paspalum*. Cont US Natl Herb, Vol 28, Part 1

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Forbes I, Burton GW (1961) Cytology of diploids, natural and induced tetraploids, and intraspecific hybrids of Bahiagrass, *Paspalum notatum* Flugge. Crop Sci 1:402–406

Gould FW (1966) Chromosome numbers of some Mexican grasses. Can J Bot 44:1683–1696

Heredia-Díaz O, Gardiner J, Hoisington D, Chao S, Coe E, Musket T, Xu G (1994) Combined F2 and IF2 RFLP map. Maize Genet Coop Newslett 68:30–34

Huff DR, Bara JM (1993) Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow-cytometry and silver-stained RAPD markers. Theor Appl Genet 87:201–208

Leblanc O, Grimanelli D, González-de-León D, Savidan Y (1995) Detection of the apomictic mode of reproduction in maize-*Tripsacum* hybrids using maize RFLP markers. Theor Appl Genet 90:1198–1203

Marshall DR, Brown AHD (1974) Estimation of the level of apomixis in plant populations. Heredity 32:321–333

Mather K (1951) The measurement of linkage in heredity. Methuen, London

Mazzucato A, Barcaccia G, Pezzotti M, Falcinelli M (1995) Biochemical and molecular markers for investigating the mode of reproduction in the facultative apomict *Poa pratensis* L. Sex Plant Reprod 8:133–138

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Nogler GA (1984) Gametophytic apomixis. In: Johri BM (ed) Embryology of angiosperms. Springer-Verlag, Berlin, pp 475–518

Norrmann GA, Quarín CL, Burson BL (1989) Cytogenetics and reproductive behavior of different chromosome races in six *Paspalum* species. J Hered 80:24–28

Ozias-Akins P, Lubbers EL, Hanna WW, McNay JW (1993) Transmission of the apomictic mode of reproduction in *Pennisetum*: co-inheritance of the trait and molecular markers. Theor Appl Genet 85:632–638

Pessino SC, Ortiz JPA, Leblanc O, do Valle CB, Evans C, Hayward MD (1997) Identification of a maize linkage group related to apomixis in *Brachiaria*. Theor Appl Genet 94:439–444

Quarín CL, Hanna WW (1980) Effect of three ploidy levels on meiosis and mode of reproduction in *Paspalum hexastachyum*. Crop Sci 20:69–75

Quarín CL, Hanna WW, Fernandez A (1982) Genetic studies in diploid and tetraploid *Paspalum* species: embryo-sac development, chromosome behaviour, and fertility in *P. cromyorrhizon*, *P. laxum*, and *P. proliferum*. J Hered 73:254–256

Quarín CL, Burson BL, Burton GW (1984) Cytology of intra- and inter-specific hybrids between two cytotypes of *Paspalum notatum* and *P. cromyorrhizon*. Bot Gaz 145:420–426

Saura F (1948) Cariologia de gramineas en Argentina. Rev Fac Agron Vet Buenos Aires 12:51–67

Young BA, Sherwood RT, Bashaw EC (1979) Cleared-pistil and thick-sectioning techniques for detecting aposporous apomixis in grasses. Can J Bot 57:1668–1672

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

THEORETICAL AND APPLIED GENETICS

International Journal of Plant Breeding Research

Volume 95 Number 5–6 October 1997

Diwan N, Cregan PB: **Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean** 723

Calderini O, Pupilli F, Paolocci F, Arcioni S: **A repetitive and species-specific sequence as a tool for detecting the genome contribution in somatic hybrids of the genus *Medicago*** 734

Roa AC, Maya MM, Duque MC, Tohme J, Allem AC, Bonierbale MW: **AFLP analysis of relationships among cassava and other *Manihot* species** 741

Fukunaga K, Domon E, Kawase M: **Ribosomal DNA variation in foxtail millet, *Setaria italica* (L.) P. Beauv., and a survey of variation from Europe and Asia** 751

Bommineni VR, Jauhar PP, Peterson TS, Chibbar RN, Almouslem AB: **Analysis of hybrids of durum wheat with *Thinopyrum junceiforme* using RAPD markers** 757

Spooner DM, Ugarte ML, Skroch PW: **Species boundaries and interrelationships of two closely related sympatric diploid wild potato species, *Solanum astleyi* and *S. boliviense*, based on RAPDs** 764

Rahman SM, Takagi Y, Kinoshita T: **Genetic control of high stearic acid content in seed oil of two soybean mutants** 772

Mohan M, Sathyanarayanan PV, Kumar A, Srivastava MN, Nair S: **Molecular mapping of a resistance-specific PCR-based marker linked to a gall midge resistance gene (*Gm4t*) in rice** 777

Milach SCK, Rines HW, Phillips RL: **Molecular genetic mapping of dwarfing genes in oat** 783

Wang Y-H, Thomas CE, Dean RA: **A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers** 791

Zhuang J-Y, Lin H-X, Lu J, Qian H-R, Hittalmani S, Huang N, Zheng K-L: **Analysis of QTL×environment interaction for yield components and plant height in rice** 799

Liu KD, Wang J, Li HB, Xu CG, Liu AM, Li XH, Zhang Qifa: **A genome-wide analysis of wide compatibility in rice and the precise location of the S_5 locus in the molecular map** 809

Nielsen KM, Gebhard F, Smalla K, Bones AM, Elsas JD van: **Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium *Acinetobacter calcoaceticus* BD413** 815

Mingeot D, Jacquemin JM: **A wheat cDNA coding for a thaumatin-like protein reveals a high level of RFLP in wheat** 822

Taguchi-Shiobara F, Lin SY, Tanno K, Komatsuda T, Yano M, Sasaki T, Oka S: **Mapping quantitative trait loci associated with regeneration ability of seed callus in rice, *Oryza sativa* L.** 828

Joshi CP, Klueva NY, Morrow KJ, Nguyen HT: **Expression of a unique plastid-localized heat-shock protein is genetically linked to acquired thermotolerance in wheat** 834

Galotto FA, Bramucci M, Grattapaglia D: **Estimation of outcrossing rate in a breeding population of *Eucalyptus urophylla* with dominant RAPD and AFLP markers** 842

Ortiz JPA, Pessino SC, Leblanc O, Hayward MD, Quarin CL: **Genetic fingerprinting for determining the mode of reproduction in *Paspalum notatum*, a subtropical apomictic forage grass** 850

Giroux MJ, Morris CF: **A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch-surface friabilin** 857

Chan KF, Sun M: **Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*** 865

Rouppe van der Voort J, Wolters P, Folkertsma R, Hutten R, Zandvoort P van, Vinke H, Kanyuka K, Bendahmane A, Jacobsen E, Janssen R, Bakker J: **Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers** 874

(Continued on page A3)