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Z.-W. Liu · R. L. Jarret · S. Kresovich · R. R. Duncan

Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz)

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Abstract A size-fractionated *Taq*I genomic library of seashore paspalum (*Paspalum vaginatum* Swartz) was screened for the presence of $(GA)_n$ and $(CA)_n$ simple sequence repeats (SSRs). A total of 54 clones with a positive signal were detected among 13,000 clones screened. Forty-seven clones having repeats of $n \geqslant 3$ were identified, of which 85% were perfect, 13% were imperfect and 2% were compound repeat sequences. Five of ten primer pairs synthesized to amplify selected loci resulted in a product in the expected size range and were subsequently used to examine SSR polymorphisms among 46 ecotypes of *P. vaginatum*. The number of alleles resolved on agarose or polyacrylamide gels were similar and ranged from 6 to 16 with an average of 14 per locus. Phenetic analysis of SSR polymorphisms revealed genetic relationships among the *P. vaginatum* ecotypes that were in general agreement with relationships determined previously by RAPD analysis of the same plant materials. Further screening of the genomic library did not identify $(AT)_n$, trimeric or tetrameric repeats. Hybridization of an $(ATT)_8$ oligonucleotide probe to genomic DNA isolated from *I. batatas*, *E. coli*, *Citrullis lanatus* and *P. vaginatum* suggested that the *P. vaginatum* genome contained significantly fewer ATT repeats than either the *I. batatas* or *C. lanatus* genome.

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Z.-W. Liu[1] · R. L. Jarret (✉) · S. Kresovich
USDA/ARS, Plant Genetic Resources, 1109 Experiment Street, Griffin, GA 30223, USA

R. R. Duncan
Department of Crop and Soil Sciences, University of Georgia, Georgia Experiment Station, 1109 Experiment Street, Griffin, GA 30223, USA

Present address:
[1] Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

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Introduction

Simple sequence repeats (SSRs), also referred to as microsatellites, short tandem repeats (STRs) or variable number tandem repeats (VNTRs), are tandemly repeated DNA sequences with core motif repeat lengths of 6 bp or less (Wang et al. 1994). SSRs, for example, $(CA)_n$ or $(TG)_n$, were first shown to be abundantly present in human and other eukaryotic genomes (Miesfeld et al. 1981; Hamada et al. 1982; Litt and Luty 1989; Tautz 1989; Weber and May 1989). In the past several years, surveys of DNA sequence databases have revealed an abundance of SSR loci in plants, and subsequent studies have demonstrated the informativeness of these markers in several genera (Weising et al. 1989; Condit and Hubbell 1991; Akkaya et al. 1992; Zhao and Kochert 1992; Wu and Tanksley 1993; Saghai Maroof et al. 1994). Database searches indicate that $(AT)_n$, $(A)_n$, $(GA)_n$, $(TAT)_n$ and $(CA)_n$ repeats are the most frequently occurring SSRs among the plant species examined (Lagercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994). While the function of SSRs remains to be determined, several authors have reported the ability of specific SSR sequences to adopt a Z-DNA conformation (e.g. Hamada et al. 1982; Nordheim and Rich 1983) and to stimulate recombination (e.g. Stringer 1985; Gaillard and Strauss 1994).

SSRs have several positive attributes as genetic markers when compared to other DNA-based marker systems. SSRs are "sequence tagged sites" (STS, Olson et al. 1989), as opposed to random amplified polymorphic DNA (RAPD), and thus can be amplified reproducibly by the polymerase chain reaction (PCR). This makes their analysis less technically demanding than, for example, the analysis of restriction fragment length polymorphisms (RFLPs). Secondly, they appear to be

ubiquitously dispersed throughout the genomes of all investigated eukaryotic organisms (Weber 1990a), as compared to the nonrandom distribution of minisatellites in humans. Random dispersion of SSR loci in the genome makes these markers suitable for genetic and mapping studies. Finally, SSR loci are highly polymorphic as compared, for example, to the relatively low informativeness of most RFLPs (Botstein et al. 1980). In barley as many as 37 alleles have been observed at a single SSR locus (Saghai Maroof et al. 1994).

We report here on the abundance and allelic diversity of $(GA)_n$ and $(CA)_n$ SSRs in seashore paspalum (*Paspalum vaginatum* Swartz). *Paspalum vaginatum* is a warm-season, fast-growing and morphologically variable grass. The species has many desirable turfgrass characteristics, such as salt- and drought-tolerance, low water and fertility requirements and tolerance to mowing at a range of heights (Dudeck and Peacock 1985; Lakanmi and Okusanya 1990). Genetic diversity within *P. vaginatum* has recently been examined using RAPD markers (Liu et al. 1994).

The objectives of this study were to (1) isolate and characterize SSR markers in seashore paspalum for plant germ plasm characterization, (2) investigate the presence and degree of simple sequence repeat DNA length polymorphism among a collection of *P. vaginatum* accessions and (3) compare estimates of genetic relationships among these plant materials by analysis of SSRs with a previous analysis of these plant materials using RAPDs.

Materials and methods

Construction of a *P. vaginatum* genomic library

Total DNA was isolated from *P. vaginatum* cv 'Excalibre' using a modified CTAB (hexadecyltrimethylammonium bromide) procedure (Liu et al. 1994). DNA was purified by cesium chloride purification and digested with *Taq*I in order to generate a high proportion of low-molecular-weight DNA fragments suitable for automated DNA sequencing. The digested DNA was size-fractionated on 1.5% Sea-Plaque agarose (FMC, Rockland, Me.), and fragments 200–500 bp in length were isolated from the gel by digestion with β-agarase (FMC). DNA fragments were cloned into the plasmid vector pGEM-7zf(+) (Promega, Madison, Wis.), which had been digested with *Cla*I, an enzyme generating *Taq*I-compatible ends. The ligation products were used to transform *E. coli* JM109 high efficiency competent cells (Promega) according to the manufacturer's directions. Transformation mixtures were spread onto LB plates containing ampicillin, X-gal and IPTG.

Library screening for clones containing SSRs

A total of 13,000 colonies were screened by colony hybridization (Sambrook et al. 1989). White colonies were picked onto Duralon-UV filters (Stratagene, La Jolla, Calif.) overlain on LB plates (15% agar). Replica plates were prepared as described by Sambrook et al. (1989), and the original filters were transferred to LB containing 15% glycerol and stored at −20 °C.

Six oligonucleotide probes were used: $(GA)_{10}$, $(CA)_{10}$, $(AT)_{10}$, $(ATT)_8$, $(CTT)_{10}$ and $(GATA)_6$. The probes were labeled with gamma-[^{32}P] ATP using T4 polynucleotide kinase. The hybridizations of probes to filters were carried out according to the protocols provided by the manufacturer (Stratagene), with the exception that hybridization temperatures were 5 °C below the calculated melting temperature (T_m). For example, hybridizations with $(GA)_{10}$ probes were conducted at 55 °C. Filters were screened twice, and only those positive colonies detected in both screens were sequenced.

Sequencing clones containing SSRs and designing PCR primers flanking SSRs

DNA sequencing was performed using an Applied Biosystems International (ABI) 370 A DNA Sequencer with either the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit or the Prism Sequenase Terminator Double-Stranded DNA Sequencing Kit. Plasmid DNA of positive clones was isolated using either the QIAwell 8 plasmid kit (Qiagen Inc., Chatsworth, Calif.) or protocols provided by ABI. Both strands of DNA were sequenced over the repeat region.

Sequence data of DNA fragments containing SSRs were analyzed for primer selection using Oligo (NBI, Plymouth, Minn.) or Designer PCR (Research Genetics, Huntsville, Ala.). Primers were designed with a T_m of about 65 °C and a primer pair T_m variance of less than or equal to 1 °C. Primers were synthesized using an ABI 392 nucleic acid synthesizer and were purified by either HPLC or OPC purification cartridges.

Amplifying and detecting SSR alleles

DNA samples for SSR analysis were isolated from 46 ecotypes of *P. vaginatum*, which were collected from various geographic regions (Liu et al. 1994). These plant materials included cultivars and Plant Introductions (PIs). Of the 46 ecotypes included in this study, 1 was collected from California (cv 'Excalibre'), 15 from Ft. Myers, Florida (AP-1 to AP-11 and AP-13 to AP-16), 1 from Parrish, Florida (cv 'Parrish'), 2 from Sea Island, Georgia (SIPV-1 and SIPV-2), 1 from Tybee Island, Georgia (AM-3554), 10 from Hawaii (HI-1 and K-1 to K-9), 2 from Galveston, Texas (GAL-1 and GAL-2), 2 from Temple, Texas (Temple-1 and Temple-2), 1 from Australia (cv 'Adalayd'), 9 from Argentina (310-79, 561-79, PI 509018-1, PI 509018-2, PI 509018-3, PI 509020, PI 509021, PI 509022, and PI 509023), 1 from South Africa (PI 377709) and 1 from Zimbabwe (PI 299042).

PCR reactions contained 50 ng of template DNA, 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 3.0 m*M* Mg^{2+}, 0.25 m*M* each dNTP, 20 pmol each oligonucleotide primer and 1.0 U of Perkin Elmer *Taq* DNA polymerase in a reaction volume of 20 µl. PCR was carried out in a Perkin Elmer Cetus 480 thermal cycler using a "touchdown" amplification program (Don et al. 1991; Mellersh and Sampson 1993). The thermocycling parameters were 94 °C for 1 min denaturation, 72 °C extension for 1 min. Annealing (30 s) temperatures were decreased 1 °C every second cycle from beginning from 70 °C (or 67 °C) to 61 °C (or 58 °C). After reaching the final annealing temperature, the reactions were subjected to 30 additional cycles of PCR.

SSR alleles were detected using two electrophoretic separation matrices: agarose and polyacrylamide.

1) Agarose gel electrophoresis. PCR products were separated electrophoretically on 4% MetaPhor agarose gels (FMC) using TBE or TAE buffer. The gels, containing 0.3 µg/ml ethidium bromide, were run at 5 V/cm for 4 h. PCR products were visualized over a transilluminator and photographed under UV light. MetaPhor agarose has been reported to be effective in resolving low-molecular weight (LMW) DNA fragments and has been successfully used to separate SSR alleles (Senior and Heun 1993).

2) Polyacrylamide gel electrophoresis. PCR reactions separated on polycrylamide were modified to contain 50 ng of template DNA, 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 2.5 m*M* Mg^{2+}, 150 µ*M* each of dCTP, dGTP and dTTP, 5 µ*M* dATP, 1 µCi (37 KBq) α-[^{32}P] dATP, 0.1 µ*M* each of the oligonucleotide primers and 1.25 U of Perkin Elmer *Taq* DNA polymerase in a reaction volume of 20 µl. PCRs were carried out as described previously. PCR products (2 µl/lane) were denatured for 5 min at 94 °C and separated on 6% denaturing polyacrylamide gels containing 8 *M* urea at 55 W (constant power) for 2 h using a DNA sequencing system (Model S2, GIBCO/BRL,

Gaithersburg, Md.) Gels were immediately covered with plastic wrap and exposed to X-ray film at −70 °C for several hours.

Southern hybridization

DNA samples used for Southern analysis were obtained from *E. coli* O_{157}:H_7 strain 932 (provided by Dr. S. Zhao at the Department of Food Science and Technology, University of Georgia), *P. vaginatum* cv 'Excalibre', sweetpotato (*Ipomoea batatas* L.) PI 508506 and watermelon (*Citrullus lanatus* L.) cv 'New Hampshire Midget'. Three micrograms DNA per lane was digested to completion with *Eco*RI, separated on 0.8% agarose, and transferred to nylon membrane (MSI, Westboro, Mass.) as described by Sambrook et al. (1989). Membranes were baked at 75 °C for 2 h and then hybridized with a ^{32}P-labeled $(ATT)_{10}$ probe. Membranes were washed once with 6 × SSC/0.1% SDS at room temperature for 15 min, and then twice with 2 × SSC/0.1% SDS at 50° C for 15 min. Membranes were wrapped in plastic wrap and exposed to X-ray film (Kodak X-Omat AR).

Phenetic analysis

To compare the patterns of genetic variation among accessions of *P. vaginatum* as revealed by SSR polymorphisms with the genetic relationships among these plant materials as determined previously using RAPD markers (Liu et al. 1994), we conducted a phenetic analysis of the SSR data sets obtained from the separation of PCR products on both agarose and polyacrylamide gels using NTSYS-pc version 1.70 (Rohlf 1992). DNA fragments on gels were scored as 1 (present) or 0 (absent). Simple matching similarity coefficients (Sokal and Michener 1958) were calculated and subjected to cluster analyses using unweighted pair-group method analysis (UPGMA).

Results and discussion

Characterization of dinucleotide repeats in *P. vaginatum*

A total of 13,000 clones were screened with $(CA)_{10}$ and $(GA)_{10}$ oligonucleotide probes. Twenty-three CA-positive and 31 GA-positive clones were detected, which represent approximately 0.1% and 0.2% of the library, respectively. Condit and Hubbell (1991) observed a similar preponderance of GA repeats. The 47 of the 54 total sequences with repeat numbers $\geqslant 3$ were further classified into the categories described by Weber (1990b) (Table 1). The majority of the repeat sequences were perfect: 65% for CA and 81% for GA, respectively. Length distributions of the clone sequences are shown in Fig. 1. SSR length for imperfect and compound sequences was defined by the total runs of uninterrupted repeats plus the internal nonrepeated bases. As illustrated in Fig. 1, repeat numbers of most of the CA repeat sequences were in the range of 3–10, and CA repeats longer than 14 were not detected. In contrast, for most of the $(GA)_n$ repeats, n was $\geqslant 7$. Seven sequences and repeats of 15 or longer.

Table 1 Numbers of SSRs in different sequence categories

Category	Repeat motif	Number of sequences	Percentage of total[a]
Perfect	CA	15	65
	GA	25	81
Imperfect	CA	2	9
	GA	4	13
Compound	CA	1	4
	GA	0	0

[a] Among the total positive clones screened, 5 CA-positive and 2 GA-positive clones contained 0–2 repeats, respectively

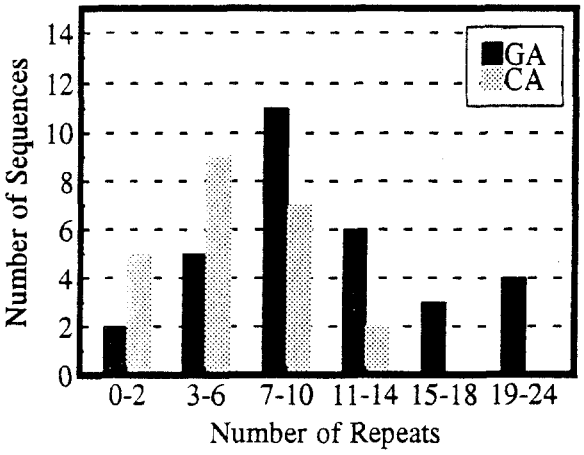


Fig. 1 Distribution of CA and GA SSRs in *Paspalum* in various length classes

The approximate DNA content of *P. vaginatum* is about 1 pg/1 c = 965,000 kb (R. L. Jarret, unpublished). Since the average insert size of our library was about 350 bp and we screened approximately 13,000 clones (= 4,550 kb), our screening procedure covered about 0.47% of the haploid genome of *P. vaginatum*. We detected, on average, 1 CA per 270 kb and 1 GA repeat per 160 kb of *P. vaginatum* genomic DNA. We estimate that the *Paspalum* genome contains approximately 3.5×10^3 and 6.0×10^3 $(CA)_n$ and $(GA)_n$ repeats, respectively, assuming that our library construction procedures resulted in an unbiased sampling of the genome for these repeats. Condit and Hubbell (1991) estimated that the frequency of occurrence of $(AC)_n$ and $(AG)_n$ repeats in the genomes of five tropical tree species ranged from 5×10^3 to 3×10^5. Our estimates of the frequency of occurrence of $(CA)_n$ and $(GA)_n$ in *P. vaginatum* are in general agreement with those of Condit and Hubbell (1991) who also observed that libraries constructed from smaller inserts leads to higher estimates of SSR abundance.

Polyacrylamide versus agarose for seperation of SSR alleles

Ten CA- and GA-positive sequences with repeat numbers $\geqslant 13$ were selected for primer design. PCR products were separated on 4% Metaphor agarose gels as well as on 6% denaturing polyacrylamide gels. Of the ten primer pairs designed, five generated PCR products with either many bands or only a smear. These were excluded from further analysis. The remaining five primer pairs (Table 2) produced amplification products in the expected molecular weight size range (Fig. 2) and

Table 2 Sequences of *P. vaginatum* SSR primers

Locus	5′ Flanking sequence	Repeat sequence	3′ Flanking sequence	Length (bp)
P.v. 3	5′ TATGGACCGACTGCATGATTCTT	$(CA)_{14}$	5′ GTAGCTAGGTGAGAGGCATTC	152
P.v. 11	5′ AGGTTTGTAGGTTGGGTGCAACTGA	$(GA)_{13}$	5′ TTGGGCGGCGGAGGGTAAT	104
P.v. 35	5′ TCGAAATCGAAAAAGAAGATCGTTC	$(GA)_3T(GA)_{21}$	5′ GGCGCCAGCTACAAGGTTAG	132
P.v. 51	5′ TCCCATCATCAGTTCTTCCAATC	$(GA)_{13}$	5′ GCCCTGTGCTATTATTCATCATCTT	120
P.v. 53	5′ CTCGGAAACCGCAGCTCA	$(GA)_{15}G$	5′ GCTCCGCCTCCTCTATTCCA	112

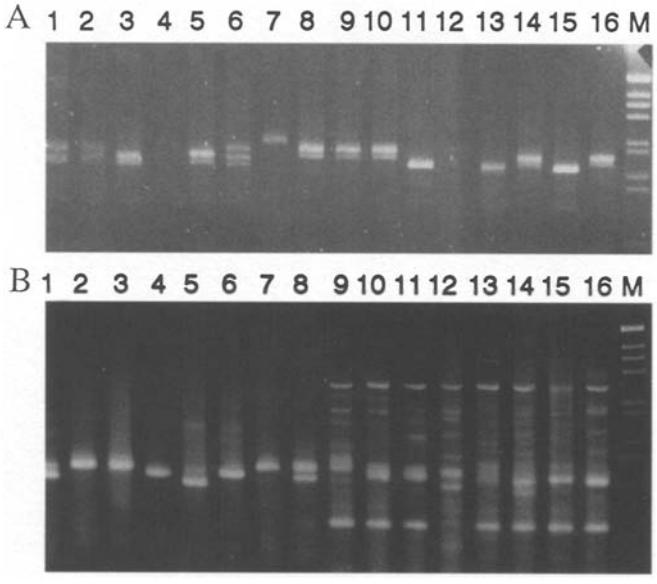


Fig. 2A,B Metaphor agarose gel electrophoresis of PCR products amplified with three primer pairs. **A** Alleles produced by primer pair P.v. 3 in 16 ecotypes of *P. vaginatum*, **B** alleles produced by primer pair P.v. 51 (*lanes 1–8*) and by primer pair P.v. 53 (*lanes 9–16*) in 8 ecotypes of *P. vaginatum*, respectively. *M* BRL 1-kb ladder

were used to profile the 46 ecotypes of *P. vaginatum*. These five primer pairs flanked 1 CA repeat sequence (P.v.3) and 4 GA repeat sequences (P.v.11, P.v.35, P.v.51 and P.v.53).

The number of alleles identified within the 46 ecotypes of *P. vaginatum* after separation of PCR products on either Metaphor agarose or denaturing polyacrylamide gels are presented in Table 3. The numbers of alleles resolved on agarose gels ranged from 6 to 16 for the five putative loci, with an average of 13 alleles per locus (Fig. 2; Table 3). The number of alleles resolved on polyacrylamide gels were similar to those detected on agarose gels, with an average of 14 alleles per locus (Fig. 3; Table 3). However, the apparent strand slippage (Luty et al. 1990) during PCR amplification of our dimer repeats occasionally precluded the accurate scoring of alleles and may have resulted in an underestimation of the number of alleles present.

As noted by Cregan (1992) the requirement for separation of SSR alleles of near-identical length on acrylamide gels remains an impediment to the more widespread use of SSR length polymorphisms in plant genetics. However,

Table 3 SSR allelic diversity among 46 ecotypes of *P. vaginatum* as determined by separation on agarose or polyacrylamide gel electrophoresis

Locus	Size range of alleles (bp)[a]	Gel type	Number of alleles
P.v. 3	145–235	Agarose	16
		Polyacrylamide	17
P.v. 11	75–140	Agarose	14
		Polyacrylamide	17
P.v. 35	95–130	Agarose	14
		Polyacrylamide	14
P.v. 51	90–130	Agarose	6
		Polyacrylamide	7
P.v. 53	95–135	Agarose	16
		Polyacrylamide	17
Average		Agarose	13.2
		Polyacrylamide	14.4

[a] Approximate, as determined using a BRL 1-kb DNA ladder

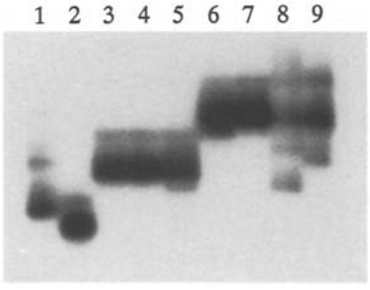


Fig. 3 Autoradiograph of a denaturing polyacrylamide gel with PCR-amplified alleles in 9 ecotypes of *P. vaginatum* using primer pair P.v. 51

Senior and Heun (1993) utilized agarose gels to separate segregating SSR alleles in a population of *Zea mays*. Our data indicate that separation of SSR alleles on acrylamide gels may be advantageous in efforts to accurately determine dimer repeat numbers or to clearly resolve polymorphisms when repeat lengths are near-identical.

Polymorphisms and genetic relationships of a collection of *P. vaginatum*

We have previously investigated genetic relationships and variation within the 46 ecotypes of *P. vaginatum* included in this study using RAPD markers (Liu et al. 1994). We also phenetically analyzed the SSR data of these ecotypes. The resultant phenogram, based on

allelic diversity as detected by agarose gel separation of PCR products, is shown in Fig. 4. Genetic relationships among the ecotypes as revealed by SSR analysis were very similar to the relationships revealed by analysis of RAPD marker data (Liu et al. 1994).

In our previous study (Liu et al. 1994) we noted that RAPD analysis is direct, cost effective and does not requre sequence data. RAPD fragments are easily separated on agarose gels. In contrast, analysis of SSRs requires sequence data, and SSR alleles differing by only a few repeat units may be difficult to separate on agarose. This necessitates the separation of similarly sized alleles using [^{32}P]-labeled PCR products on denaturing polyacrylamide gels. Utilization of SSR markers does, however, eliminate concerns for an inability to ascertain the homology of similarly sized RAPD fragments. In addition, although sequence data for many crop plants remains limited, we have observed that sufficient homology exists between genomes in the regions flanking SSR loci that primers pairs designed on the basis of sequence data obtained from one crop (e.g. *Paspalum*) may be used to detect SSR polymorphisms in another crop (e.g. *Sorghum* – data not shown). Sequence homology in the regions flanking SSR loci extends the utility of these markers to crop genera for which little, or no sequence data are available.

Survey of other nucleotide repeats

A number of reports have indicated that $(AT)_n$ repeats are the most frequent class of SSRs among the plant species examined (Lagercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994). Although less abundant than dinucleotide repeats, tri- and tetranucleotide repeats are seen as desirable since variations in the number of core repeat units between loci are somewhat easier to detect due to the larger number of basepairs differentiating sequential increases in the SSR. This has permitted less ambiguous allele sizing and reduced stuttering when compared to dinucleotide repeats (Edwards et al. 1991).

In addition to the $(CA)_n$ and $(GA)_n$ repeat sequences, we screened the library for $(AT)_n$, $(ATT)_n$, $(CTT)_n$ and $(GATA)_n$ repeats. However, we failed to identify any positive clones after hybridization of the library with these four probes, even though the experimental conditions were carefully controlled to ensure low stringency hybridization and filter washes. The apparent absence of $(AT)_n$ repeats in our library appears to contradict the findings of Morgante and Olivieri (1993), who noted that $(AT)_n$ repeats are the most frequently occurring SSRs in plants. However, the failure of this screening procedure to detect $(AT)_n$ repeats in *P. vaginatum* may have resulted from the self-annealing of the $(AT)_n$ oligonucleotide immediately before, or during, hybridization. It also appeared that $(ATT)_n$, $(CTT)_n$ and $(GATA)_n$ repeats occurred at much lower frequencies in our library than the dimeric repeats $(GA)_n$ or $(CA)_n$ detected earlier. A relatively lower abundance of trimeric repeats in comparison to dimeric repeats has been reported previously (Morgante and Olivieri 1993; Wang et al. 1994).

In order to examine the frequency of occurrence of $(ATT)_n$ repeats in *P. vaginatum*, the $(ATT)_8$ probe was hybridized to Southern blots of genomic DNA extracted from four different taxa; *P. vaginatum*, sweetpotato (*Ipomoea batatas*), watermelon (*Citrullus lanatus*) and *E. coli*. The results (Fig. 5) showed that the *P. vaginatum* genome appears to possess fewer ATT repeats than the genomes of the other taxa examined under our experimental conditions. A strong hybridization signal to the *I. batatas* DNA indicated that this repeat is abundant in *I. batatas*, and $(ATT)_n$ was subsequently found to be the predominant microsatellite repeat in an *I. batatas* genomic library screened with the oligonucleotide probes discussed previously (R. L. Jarret, unpublished).

Fig. 4 Phenogram of *P. vaginatum* ecotypes based on SSR data with the five primer pairs using Metaphor agarose gel electrophoresis

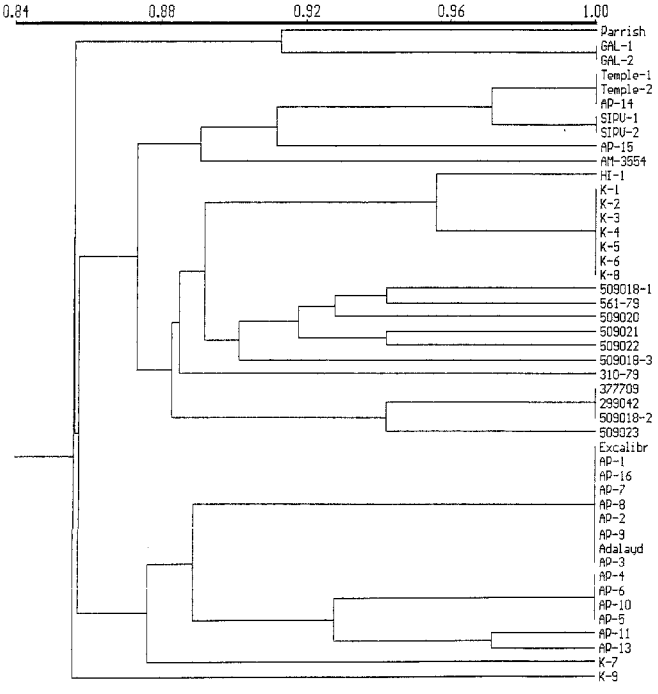
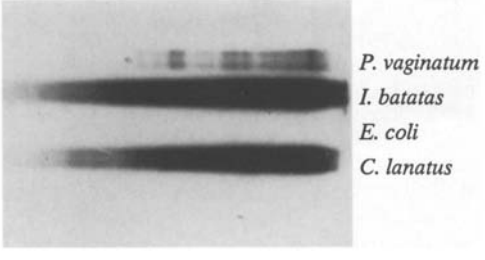


Fig. 5 Hybridization of a [^{32}P]-labeled $(ATT)_8$ probe to a Southern blot containing DNA from four taxa



Wang et al. (1994), using data obtained from the EMBL and GenBank databases, found that tri- and tetranucleotide repeats were relatively abundant in plant genomes. As noted by Cregan (1992) and Wang et al. (1994) strand slippage ('stutering') diminishes as repeat length increases, therefore we expect that tri- and tetranucleotide repeats will be used increasingly in plant genetic studies. Trinucleotide repeats in humans are unstable and their amplification has been found to be associated with hereditary diseases (Kremer et al. 1991). The relationship of the occurrence of SSRs and genetic instability in plants has yet to be investigated.

In summary, 47 SSR loci with repeats of $n \geqslant 3$ were isolated from a genomic library of *P. vaginatum*, a turfgrass of economic importance in the Southeastern United States. Allelic variation detected by these genetic markers was utilized to examine genetic relationships among 46 ecotypes of *P. vaginatum*, and results were in general agreement with a previous evaluation of genetic relationships among these same plant materials using RAPD genetic markers. No $(AT)_n$, $(ATT)_n$, $(CTT)_n$ or $(GATA)_n$ repeats were detected under our experimental conditions. Our data indicate that SSRs are useful for the characterization of *P. vaginatum* ecotypes.

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References

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Condit R, Hubbell SP (1991) Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome 34:66–71

Cregan PB (1992) Simple sequence repeat DNA length polymorphisms. Probe 19–22 (Spring)

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008

Dudeck AE, Peacock CH (1985) Effects of salinity on seashore paspalum turfgrass. Agron J 77:47–50

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 59:746–756

Gaillard C, Strauss F (1994) Association of poly (CA). poly(TG) DNA fragments into four-stranded complexes bound by HMG1 and 2. Science 264:433–436

Hamada H, Petrino MG, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc Natl Acad Sci USA 79:6465–6469

Kremmer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, Schlessinger D, Sutherland GR, Richards RI (1991) Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)$_n$. Science 252:1711–1714

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115

Lakanmi OO, Okusanya OT (1990) Comparative ecological studies of *Paspalum vaginatum* and *Paspalum orbiculare* in Nigeria. J Trop Ecol 6:103–114

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Liu Z-W, Jarret RL, Duncan RR, Kresovich S (1994) Genetic relationships and variation of ecotypes of seashore paspalum (*Paspalum vaginatum*) determined by random amplified polymorphic DNA (RAPD) markers. Genome 37:1011–1017

Luty JA, Guo Z, Willard HF, Ledbetter DH, Ledbetter S, Litt M (1990) Five polymorphic microsatellite VNTRs on the human X chromosome. Am J Hum Genet 46:776–783

Mellersh C, Sampson J (1993) Simplifying detection of microsatellite length polymorphisms. BioTechniques 15:582–584

Miesfeld R, Krystal M, Arnheim N (1981) A member of a new repeated sequence family which is conserved throughout eucaryotic evolution is found between the human delta and beta globin genes. Nucleic Acids Res 9:5931–5947

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Nordheim A, Rich A (1983) The sequence (dC-dA)$_n$. (dG-dT)$_n$ forms left-handed Z-DNA in negatively supercoiled plasmids. Proc Natl Acad Sci USA 80:1821–1825

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245:1434–1435

Rohlf, FJ (1992) NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exeter Software, New York

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884–889

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. Univ Kans Sci Bull 38:1409–1438

Stringer JR (1985) Recombination between poly[d(GT).d(CA)] sequences in simian virus 40-infected cultured cells. Mol Cell Biol 5:1247–1259

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463–6471

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6

Weber JL (1990a) Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. In: Davies KE, Tilghman SM (eds) Genome analysis, vol 1: genetic and physical mapping, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp 159–181

Weber JL (1990b) Informativeness of human (dC-dA)$_n$.(dG-dT)$_n$ polymorphisms. Genomics 7:524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weising K, Weigand F, Driesel AJ, Kahl G, Zischler H, Epplen JT (1989) Polymorphic simple GATA GACA repeats in plant genomes. Nucleic Acids Res 17:10128

Wu K, Tanksley SD (1993) Abundances, polymorphisms and genetic mapping of microsatellites in rice. Mol Gen Genet 241:225–235

Zhao X, Kochert G (1992) Characterization and genetic mapping of a short, highly repeated, interspersed DNA sequence from rice (*Oryza sativa* L.). Mol Gen Genet 231:353–359