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Sequence characterization of microsatellites in diploid and polyploid *Ipomoea*

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Abstract The objectives of the present study were to evaluate the inheritance and nucleotide sequence profiles of microsatellite genetic markers in hexaploid sweetpotato [*Ipomoea batatas* (L.) Lam.] and its putative tetraploid and diploid ancestors, and to test possible microsatellite mutation mechanisms in polyploids by direct sequencing of alleles. Sixty three microsatellite loci were isolated from genomic libraries of *I. batatas* and sequenced. PCR primers were designed and used to characterize microsatellite loci in two hexaploid *I. batatas* populations, a tetraploid *Ipomoea trifida* population, and a diploid *I. trifida* population. Nine out of the sixty three primer pairs tested yielded a clearly discernible, heritable banding pattern; five showed Mendelian segregation. All other primer pairs produced either smeared banding patterns, which could not be scored, or no bands at all in *I. batatas*. All of the primers which produced discernible banding patterns from *I. batatas* also amplified products of similar size in tetraploid and diploid *I. trifida* accessions. The sequence analysis of several alleles in the three species showed differences due to mutations in the repeat regions consistent with small differences in the repeat number. However, in some cases insertions/deletions and base substitutions in the microsatellite flanking regions were responsible for polymorphisms in both polyploid and diploid species. These results provide strong empirical evidence that complex genetic mechanisms are responsible for SSR allelic variation in *Ipomoea*. Four *I. batatas* microsatellite loci showed polysomic segregation fitting tetraploid segregation ratios. To our knowledge this is the first report of segregation ratios for microsatellites markers in polyploids.

Key words Microsatellite DNA · Polyploid · Repeat instability · Sweetpotato · Simple sequence repeats

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Introduction

An ideal molecular genetic marker for plant genome analysis would disclose multiple alleles (be codominant), have an even distribution throughout the genome, easily differentiate genetically similar individuals, and be relatively easy to score. Microsatellites (Litt and Luty 1989) or simple sequence repeats (SSRs) (Jacob et al. 1991) represent such a class of markers. In short, microsatellite loci consist of varying numbers of tandemly repeated di-, tri-, or tetra-nucleotide DNA motifs. Replication slippage in a stepwise pattern with occasional larger step changes is considered one of the principal mechanisms for generating allelic diversity in microsatellites in animals (Strand et al. 1993; Valdes et al. 1993; Di Rienzo et al. 1994; Jin et al. 1996; Kimmel et al. 1996). However, sequence studies of microsatellites in animals have indicated that allele differences may also involve complex phenomena such as nucleotide substitutions, insertions/deletions (indels) in the repeat flanking regions, and changes in the composition of the repeat element (Grimaldi and Croau-Roy 1997; Macaubas et al. 1997).

In plant genome analyses, microsatellites are frequently used to fingerprint genotypes (Bryan et al.

1997; Plaschke et al. 1995; Rongwen et al. 1995; Provan et al. 1996; Weising et al. 1996), and also employed as genetic markers for linkage mapping (Bell and Ecker 1994; Cregan et al. 1994; Akkaya et al. 1995; Senior et al. 1996; Röder et al. 1998) or marker-assisted selection (Yu et al. 1994). Although microsatellite marker use is widespread in plant genetic studies, there are few reports on the characterization of microsatellite locus variation in polyploids. In allohexaploid wheat, only 36% of the primers designed by Röder et al. (1995) amplified interpretable banding patterns. This contrasts with a near 100% success rate is diploid species (Liu et al. 1995; Morchen et al. 1996). Röder et al. (1995) attributed their low amplification rate to the complexity of the genome, i.e., the three genomes in wheat, and to the large fraction of repetitive DNA that it contains. In tetraploid alfalfa [*Medicago sativa* (L.) L. & L.] and several species from the same genus, Diwan et al. (1997) could only amplify fragments of the expected size in one out of three of the microsatellite sequences obtained from GenBank. They also reported that only three out of 60 SSR loci isolated from an alfalfa genomic library could be successfully amplified.

A number of hypotheses have been extended with supportive cytological data in efforts to elucidate the origin of sweetpotato [*Ipomoea batatas* (L.) Lam.]. Magoon et al. (1970) proposed that sweetpotato consists of three genomes of closely related taxa, in contrast with earlier theories that suggested an allohexaploid origin (Gustafsson and Gadd 1965). Magoon et al. (1970) also presented evidence that appeared to rule out an autopolyploid origin from a single diploid ancestor based on the occurrence of a high frequency of tetravalent and bivalent pairing during meiosis, and the presence of penta- and hexavalent configurations. Nishiyama et al. (1975) proposed *Ipomoea leucantha* (2*x*), *Ipomoea littoralis* (4*x*) and *Ipomoea trifida* as primary, secondary and immediate progenitors of the sweetpotato, based on studies of genome constitution, cross-compatibility, and major plant characteristics. These authors observed greater genetic variability in the hexaploids than in diploids or tetraploids, which they attributed to a gene-dosage effect, suggesting that heterozygosity is promoted by the self-incompatibility of the hexaploids. Austin (1987) claimed that *Ipomoea triloba* (A-group) and *I. trifida* are the species most closely allied with sweetpotato based on morphological studies. Later RFLP analysis indicated a close relationship between *I. batatas* and *I. trifida* (Jarret et al. 1992). Shiotani (1987) suggested that the B genome of autohexaploid sweetpotato is also present in the *I. trifida* complex, based on cytological evidence.

In preliminary studies with hexaploid sweetpotato, we observed numerous incidences of non-Mendelian inheritance of microsatellite markers. Few previous reports exist on the behavior of microsatellites in polyploid genomes. Hence, the objectives of the present study were to: (1) characterize and evaluate the inheritance of microsatellite genetic markers in hexaploid sweetpotato and its putative tetraploid and diploid ancestors; and (2) study possible microsatellite mutation mechanisms in polyploids by the direct sequencing of amplified alleles.

Materials and methods

Microsatellites

Twenty nine primers were designed to flank SSR loci isolated from the small-insert sweetpotato genomic library described by Jarret and Bowen (1994). Thirty four additional primers were designed using Primer3 software (Rozen and Skaletsky 1996, 1997) to flank SSR loci isolated from a $(CT)_n$ repeat-enriched genomic library (Prochazka 1996). Both the enriched and non-enriched libraries were screened for the occurrence of $(CT)_n$ repeats as described by Akkaya et al. (1992). The nucleotide sequences of all primer pairs which produced scorable amplification products are listed in Table 1.

Plant material

Four groups of plant material were used.

The first group consisted of seven *I. batatas* breeding lines or cultivars (Nacional, Huarmeyano, ST87.006, LM87-0045, DPL886, SR68.075) from the germplasm collection of the International Potato Center (CIP), Lima, Peru, and cv Jewel (CIP group). This group also contained 18 F_1 progenies derived from a controlled cross between Jewel and LM87-0045. These 18 offspring were used to characterize the segregation pattern of microsatellites Ib-255, Ib-316 and Ib-318.

The second group consisted of 11 *I. batatas* cultivars or breeding lines (Resisto, 86-33, NC-C75, 90-223, 91-153, Excel, Beauregard, W-151, 82-531, 81-10, and 80-62) from the breeding programs at the Louisiana Agricultural Experiment Station, Baton Rouge, North Carolina State University Agricultural Experiment Station, Raleigh, (LAES group) or the USDA Vegetable Laboratory, Charleston, South Carolina. This group also contained progenies derived from two controlled crosses among three cultivars (Beauregard × Excel, and NC-C75 × Excel). Each segregating population consisted of 42 F_1 individuals that were used to observe microsatellite segregation patterns.

The third and fourth groups were composed of six tetraploid and six diploid *I. trifida* (G.) Don introductions from CIP, respectively. Groups three and four also contained 42 offspring from a cross between two tetraploid individuals, and 12 offspring from a diploid × diploid cross, respectively. These populations were used to evaluate SSR segregation patterns.

DNA extraction

DNA was extracted from about 0.25 g of freeze-dried leaf tissue (Colosi and Schaal 1993) using DNAzol (Gibco BRL, Gaithersburg, Md.). Crude DNA extracts were further purified by repeated extraction with phenol-chloroform and chloroform-isoamyl alcohol. DNA was resuspended in TE.

PCR profiling

PCR reactions were performed in a 25-µl vol containing 12.5 µl of 2 × reaction buffer F (Epicentre Technologies, Madison, Wis.) which

includes 400 μM of each dNTP and 7 mM of $MgCl_2$, 1.5 U of *Taq* polymerase in storage buffer B (Promega Corp., Madison, Wis.), 30 ng of template DNA, and 0.8 μM of each forward and reverse primer. Primers Ib-255F1 and Ib-248 required the use of reaction buffer E (5 mM of $MgCl_2$) and primers Ib-286 and Ib-297 the use of 2 U of *Taq* (Promega Corp., Madison, Wis.) for optimum amplification.

The conditions for PCR were as follows: 3 min denaturation at 95°C; five cycles of 45 s at 94°C, 15 s at 56°C for primers Ib-275, Ib-255, Ib-286, Ib-316 and Ib-318, 15 s at 59°C for Ib-242, Ib-248 and Ib-297, and 15 s at 63°C for Ib-255F1; and 45 s at 72°C; and 20 cycles in which the denaturation conditions were 1 min at 90°C while the annealing and extension (1 min) temperatures remained unchanged from the previous cycles. All reactions contained a terminal elongation step of 72°C for 7 min. PCR reactions were conducted in a Perkin Elmer GeneAmp PCR System 9600 (Perkin-Elmer Corp., Foster City, Calif.). The amplified DNA fragments (2-μl samples) were resolved by electrophoresis in 6.5% non-denaturing polyacrylamide gels (0.4 mm thick, 38.5 cm long) with 1 × TBE buffer (89 mM Tris-Borate and 2 mM EDTA, pH 8.0). The gels were run at 65 W for 2 h. Five lanes with a ϕX174 *Hin*fI marker (Promega Corp., Madison, Wis.) were included. The gels were stained with silver nitrate following the procedure of Bassam et al. (1991) as modified by He et al. (1994). Gels were transferred to blotting paper, dried, and banding pattern images digitized with a flat bed scanner (Scan Jet IIcx Hewlett Packard Corp., Palo Alto, Calif.). Allele sizing was conducted using the PRO-RFLP Molecular Weight software (DNA ProScan, Inc., Nashville, Tenn.).

Microsatellite sequencing

PCR products were isolated from acrylamide gels, ligated into the plasmid vector pCR2.1, and cloned in Top10F′ using the TA Cloning Kit (InVitrogen, Carlsbad, Calif.). Plasmid DNA was isolated using QIAwell 8 (Qiagen, Santa Clarita, Calif.) and sequenced using an ABI 373 automated DNA sequencer (Applied Biosystems, Foster City, Calif.).

Data analysis

Allele frequencies and gene diversity or average heterozygosity (H) ($H = 1 - \sum_{i=1}^{n} p_i^2$) for each population were calculated according to Nei (1987). Heterozygosity (H), as a measure of the degree of genetic variability, is the probability that two randomly chosen alleles with frequencies p_i, the population frequency for the ith allele, are different.

Results

Scored microsatellites

Nine of the sixty three primer pairs tested yielded a clearly discernible, heritable banding pattern. All other primer pairs produced smeared banding patterns which could not be scored, or else produced no amplification product at all. All of the *I. batatas*-derived primers which produced scorable banding patterns amplified similar sized products from diploid and tetraploid *I. trifida* accessions. Sequence data from clones isolated from both genomic libraries, i.e., the orthodox and the enriched libraries, yielded an approximately equal percentage of usable primer pairs (13.8% and 14.7%, respectively) suggesting that the 100-fold increase in positive colonies obtained using the library enrichment procedure of Prochazka (1996) was not the result of an abundance of artifacts. The nine microsatellite loci which were reproducibly amplified included six perfect $(CT)_n$ repeats and three imperfect $(GA)_n$ repeats (Table 1).

Intraspecific variation

Sequencing of microsatellite alleles amplified by primers Ib-242, Ib-255, Ib-316 and Ib-297 in *I. batatas*, 4*x I. trifida*, and 2*x I. trifida*, are presented in Table 2. Microsatellite Ib-255 in tetraploid *I. trifida* and microsatellite Ib-297 in diploid *I. trifida* have repeat polymorphisms that vary in excess of 22 and 20 bp, respectively. An increase in the length of a repeat of this magnitude supports the two-phase model for allelic variation as observed in mammalian dinucleotide repeat loci by Di Rienzo et al. (1994).

Sequence data of amplified allelles revealed a high mutation frequency within the regions flanking the microsatellites in both polyploid and diploid species. Base substitutions resulted in allelic variation that was not detectable based on allele sizing, including: the 136-bp allele from microsatellite Ib-316 in hexaploid *I. batatas*; the 120-bp, 131-bp and 134-bp alleles from microsatellite Ib-242 in tetraploid *I. trifida*; the 133-bp allele from microsatellite Ib-242 in diploid *I. trifida*; and the 153-bp allele from Ib-255 in tetraploid *I. trifida* (Table 2). Insertions/deletions (indel mutations) within the flanking regions were also responsible for changes in allele sizes; namely, the 140-bp and 144-bp alleles from microsatellite Ib-316 in hexaploid *I. batatas* and the 130-bp and 133-bp alleles from microsatellite Ib-242 in diploid *I. trifida* (Table 2). Indel mutations may also result in identically sized amplification products while the microsatellite repeat number varies (Macaubas et al. 1997). For example, the four microsatellite Ib-316 alleles (148 bp, 144 bp, 140 bp, and 136 bp) present in *I. batatas* varied in multiples of two nucleotides. This suggested that differences in amplification-product lengths were due entirely to changes in the repeat length. However, when these products were sequenced, size variation at this locus was found to be due to indel mutations in the flanking regions (homoplasy) as well (Table 2).

Interspecific variation

Interspecific variability was observed to occur at both microsatellites loci and within the microsatellite flanking regions (Table 2, boxed). However, variability within sequenced segments (microsatellite and flanking regions) appeared to be randomly distributed within the between species, and no discernible pattern was

Table 1 Sweetpotato microstallites: observed allele frequencies and heterozygosity in diploid and tetraploid *I. trifida* and hexaploid *I. batatas* populations

Locus	Primer pairs $5' \rightarrow 3'$	Repeat	Expected size [bp][a]	*I. trifida* (2*x*)		*I. trifida* (4*x*)		*I. batatas* (6*x*)	
				Number of alleles	Gene diversity H[b]	Number of alleles	Gene diversity H[b]	Number of alleles	Gene diversity H[b]
Ib-316	CAAACGCACAACGCTGTC CGCGTCCCGCTTATTTAAC	$(CT)_{3C}(CT)_8$	150	m.		7	0.22	4	0.70
Ib-318	AGAACGCATGGGCATTGA CCCACCGTGTAAGGAAATCA	$(CT)_{9C}(CT)_5$	124	m.		m		6	0.51
Ib-242	GCGGAACGGACGAGAAAA ATGGCAGAGTGAAAATGGAACA	$(CT)_3CA(CT)_{11}$	135	3	0.66	5	0.68	5	0.70
Ib-248	GAGAGGCCATTGAAGAGGAA AAGGACCACCGTAAATCCAA	$(CT)_9(CT)_8$	171	n.s. m.l.[c]		8	0.84	10	0.86
Ib-255F1	CGTCCATGCTAAAGGTGTCAA ATAGGGGATTGTGCGTAATTTG	$(CT)_{10}$	242	n.s.m.[c]		n.s. m.l.		n.s. m.l.	
Ib-255	TGGGCATTCTCATATTTTGCT GCCACTCCAACAGCACATAA	$(CT)_{14}$	161	m.m.l.		n.s.		3	0.19
Ib-275	GAGTTCCAAAGAGAAGAGTGGAG AAGCCTACCCGAGAGATAACC	$(CT)_{27}$	263	3 m.l.	0.25	m.		m.	
Ib-286	AGCCACTCCAACAGCACATA GGTTTCCCAATCAGCAATTC	$(CT)_{12}$	105	m.		n.s. m.l.		n.s. m.l.	
Ib-297	GCAATTTCACACACAAACACG CCCTTCTTCCACCACTTTCA	$(CT)_{13}$	134	3	0.61	n.s. m.l.		n.s. m.l.	

[a] Band size in base pairs
[b] Measure of degree of polymorphism, expected heterozygosity ($H = 1 - \sum_{i=1}^{n} p_i^2$)
[c] n.s.: not scorable; m.: monomorphic, m.l.: multiple loci

Table 2 Allele sequences of Ib-316, Ib-242, Ib-255, and Ib-297 sweetpotato microsatellites from hexaploid *I. batatas*, tetraploid *I. trifida*, and diploid *I. trifida*

Locus	Size (bp)	Sequence
Ibt-316[a]	148	GTACACGCACCTCCTCTCTCTCTC--TCTCCCTCTCTCT--AAACTTCAGTTCTGTACATTA----CGCTTCGTATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCAGGAAAC
Ibt-316	144	GTACACGCACCTCCTCTCTCTCTC--TCTCT----------AAACTTCAGTTCTGTACATTAATTACGCTTCGTATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCAGGAAAC
Ibt-316	140	GTACACGCACCTCCTCTCTCTCTC--TCTCT----------AAACTTCAGTTCTGTACATTA----CGCTTCGTATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCAGGAAAC
Ibt-316	136	GTACACNCACTTCCTCTCTCTCTC--T----------AAACTTCAGTTCTGTACATTA----CGCTTCGTATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCATTAAAC
Itr4-316[b]	140	GTACACGCACCTCCTCTCTCTCTC--TCTCT----------AAACTTCAGTTCTGTACATTA----CGCTTCGTATTTCTACCTATTCCTAATTCCTGGGTAGCATACCAGCAGGAAAC
Itr4-316	136	GTACACGCACCTCCTCTCTCTCTC--TCT----------ACTTCAGTTCTGTACATTA----CGCTTCGTATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCAGGAAAC
Itr-316[c]	151	GTACACGCACCTC-TCTCTCTCTCCCTCCTATCTCTCTCTAAACTTCAGTTCTGTACATTA----CGCCTCATATTTCTACCTATTCCTAATTCCTGGATAGCATACCAGCAGGAAAC
Itr-316	150	GTACACGCACCTCCTCTCTCTCTC--TCCTATCTCTCTCTAAACTTCAGTTCTGTACATTA----CGCCTCATATTTCTACCTATTCCTAATTCCTGAATAGCATACCAGCAGGAAAC
Ibt-242	138	AATATCAGATTTC--ACATACAAACTGTGATCTGCGCAAG-CGTCCTTGCTCGCTCTCAATCTCTCAATCTCTCTCTCTCTCTCTCTCTTACTCTCTCTGC
Ibt-242	135	AATATGAGATTTC--ACATACAAACTGTGATCTGCGCAAGGCGTCCTTGCTCGCTCTCAATCCCTCTTTCTCTCTCTCTCTCTC----ACTCTCTCTGC
Ibt-242	129	AATATCAGATTTC--ACATACAAACTGTGATCTGCGCAAGGCGTCCTTGCTCGCTCTCAATCTCTCTTTCTCTCTCTCTC----------ANTCTCTCTGC
Ibt-242	122	---------GTAC--ACATACAAACTACGATCTGCGCAAGGCGTCCTTGCTCGCTCTCAATCTCCTTTTCTCCTCTCTCTC---------ACTCTCTCTGC
Itr4-242	134	--TATCAGATTTC--ACGTACAAAGTGTGATCTGCGCAAG-CGTCCTTGCTCGCTCTCAATCTCTCAATCTCTCTCTCTCTCTCTCTC--ACTCTCTCTGC
Itr4-242	129	AATATCAGATTTC--ACATACAAACTGTGATCTGCGCAAGGCGTCCTTGCTCGCTCTCAATCTCTCTTTCTCTCTCTCTC----------ACTCTCTCTGC
Itr4-242	131	AATATGAGATTTC--ACATACAAACTGTGATCTGCGCAAGGCGTCCTTGCTCGCTCTCAATCTCTCTTTCTCTCTCTCTC--------ACTCTCTCTGC
Itr4-242	120	---------GTAC--ACATACAAACTATGATCTGCGCAAGGCGTCCTTGTTCGCTCTCAATCTCTCTTTCTCTCTCTCTC----------ACTCTCTCTGC
Itr-242	133	AATATCAGATGGTAGACATACAAACTGTGATCTGCGCAAGGCGTCCTTGCTCGTTCTGAATCTCTCTTTCTCTCTCTCTC--------ACTCTCTCTGC
Itr-242	130	AATATCAGATTTC--ACATACAAACTGTGATCTGCGCA-GGCGTCCTTGCTCGCTCTCAATCTCTCTTTCTCTCTCTCTC--------ACTCTCTCTGC
Ibt-255	153	AA-CCATATAAAAAGTAGAGAGAGAGAGAGAGAGA----------TTGGCTAAAATGACAAAAAGAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACGACAGGGGCAAGAAGGAAG
Itr4-255	180	AAACCATATAAAAAGTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGATTGGCTAAAATGACAAAAAGAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACAACAGGGGCAAGGAGGAAG
Itr4-255	157	AA-CCATATAAAAAGTAGAGAGAGAGAGAGAGAGAGAGA----------TTGGCTAAAATGACAAAAAGAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACAACAGGGGCAAGGAGGAAG
Itr4-255	149	AA-CCATATAAAAAGTAGAGAGAGAGAAAGA----------TTGGCAAAAATGACAAAAATAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACAACAGGGGCAAGGAGGAAG
Itr4-255	151	AA-CCATATAAAAAGTAGAGAGAGAGAGAGAGA----------TTGGCTAAAATGACAAAAAGAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACAACAGGGGCAAGGAGGAAG
Itr4-255	152	AA-CCATATGAAAAGTAGAGAGAGAGAGAGAGAGA----------TTGGCTAAAATGACAAAAAGAAGTGAATTGCTGATTGGGAAACCAAAGAAGACTTCACAACAGGGGCA-GGGGGAAG
Itr-297	168	AATCCTNCACTTTTNCACTTCCAAAATTAACTGGTATACATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTATCTATCTATCTATCTATCTATCTATGTATCTATCTATCTATGTTATATGCG
Itr-297	142	GATCCTTTACTTTTTCACTTCCAAAATTAACTGGTATAC--CTCTCTCTCTCTCTCT----------ATCTATCTATCTATCTATCTATCTATCTATCTATC--TC--TGTTATATGCG

[a] Ibt: *I. batatas*
[b] Itr4: *I. trifida* (4*x*)
[c] Itr: *I. trifida* (2*x*)

observed. Indel mutations and base substitutions were present at all ploidy levels, e.g., the 122-bp allele of Ib-316 of *I. batatas* and the 120-bp allele from tetraploid *I. trifida* (Table 2). The assignment of alleles based solely on PCR product size could significantly bias estimates of genetic relatedness among species in the absence of a more detailed sequence analysis.

Inheritance and SSR length polymorphisms

Microsatellite segregation patterns were determined for the nine primer pairs using three *I. batatas* populations and two *I. trifida* populations. Eighteen hexaploid sweetpotato cultivars six tetraploid *I. trifida* accessions, and six diploid *I. trifida* accessions were also genotyped. The results are summarized as follows.

Microsatellites Ib-316, Ib-318, Ib-242 and Ib-248 showed tetrasomic inheritance patterns in the hexaploid test crosses (Table 3, Fig. 1). Caution should be used when interpreting the tetraploid segregation data for microsatellite Ib-248, as the population size employed was small. Although all allele dosage effects could not be accurately determined, in the case of microsatellite Ib-318 the ratios observed where those expected as a result of tetraploid segregation.

In the tetraploid test-cross population, microsatellites Ib-316 and Ib-242 displayed tetrasomic inheritance patterns. Microsatellite Ib-248 gave a hypothetical tetrasomic segregation pattern (it could not be accurately tested for the number of bands present); microsatellite Ib-318 was monomorphic (Tables 1 and 3).

Microsatellites Ib-316 and Ib-318 were both monomorphic in the diploid population. Primers for microsatellite Ib-242 amplified three alleles in the diploid population. These fragments will be called "putative alleles" until further evidence definitely proves their allelic status. The putative alleles in the progeny from the test-cross did not display codominant Mendelian segregation ratios, but did fit a perfect 3:1 ratio. The sequence analysis from the two putative alleles present in the test-cross revealed that base substitutions and insertions/deletions in the repeat flanking regions were the causes of the allelic differences observed (Table 2). Primer Ib-248 amplified multiple bands, which were interpreted as multiple loci, in the diploid population. However, the inheritance pattern was too complex for accurate interpretation.

Microsatellite Ib-255 was monomorphic in the hexaploid and tetraploid test-crosses. In the hexaploid CIP and LAES groups, three alleles were detected. The segregation patterns were non-Mendelian for the progeny population of Jewel × LM87-0045. The observed segregation ratios suggested a possible ancestral chromosome duplications and/or translocation, but this hypothesis could not be tested due to the small sample size (18 individuals). This microsatellite was monomorphic in the diploid population.

Primers for microstallites Ib-255F1, Ib-286, and Ib-297 amplified multiple loci in both the hexaploid and tetraploid progeny and inheritance patterns could not be accurately interpreted. Microsatellites Ib-286 and Ib-255F1 were monomorphic in the diploid population.

Primers for microsatellite Ib-297 amplified three putative alleles in the diploid population. Although the parents used in the test-cross were apparently homozygous for two of these three fragments, the progeny did not show codominant Mendelian segregation ratios for these. Instead, a 3:1 segregation ratio was observed. Sequence analysis revealed variation for repeat length and the occurrence of mutations in the microsatellite flanking regions of these two putative alleles (Table 2). Primers for microsatellite Ib-297 amplified multiple loci in hexaploid genotypes which did segregate, but the inheritance patterns could not be accurately interpreted.

Microsatellite Ib-275 was monomorphic in the hexaploid *I. batatas* population and dimorphic in the tetraploid *I. trifida* population. Two loci in the diploid parental population, each containing two alleles, were amplified; however, only three alleles were found in the segregating population. Alleles at one locus segregated in a 1:1 Mendelian ratio. The other locus was monomorphic.

Primers for three of the nine microsatellites (30%) amplified multiple loci in the hexaploid and tetraploid populations, confounding our ability to discern segregation patterns. The discernment between single and multiple allele loci, as well as between multiple loci, was based on the number of bands observed, the differences in size among bands, the sequencing of some of these bands, and the segregation patterns. The fraction of primers amplifying multiple loci is close to the values reported for wheat, 29% (Bryan et al. 1997), and apple, 25% (Guilford et al. 1997). In the diploid population, primers for microsatellites Ib-255, Ib-275 and Ib-248 amplified more than a single locus, simultaneously. This may reflect ancestral chromosome duplications.

Three to ten alleles were found at each of the five scorable polymorphic loci in sweetpotato, with an average of two alleles/genotype. Heterozygosity values ranged from 0.19 to 0.86 (Table 1). These values are comparable to those reported in the literature for other plant species (Provan et al. 1996; Akagi et al. 1997; Bryan et al. 1997; Guilford et al. 1997).

Discussion

Polysomic segregation ratios were detected at four microsatellite loci (Ib-242, Ib-248, Ib-318 and Ib-316) (Table 3). To our knowledge, this is the first report of polysomic segregation ratios for microsatellites markers in polyploids. The tetraploid segregation

Table 3 Chi-square goodness of fit tests for tetrasomic segregation ratios for sweetpotato microsatellite loci Ib-316, Ib-318, Ib-242, and Ib-248 in *I. batatas* and tetraploid *I. trifida*

Locus	Parental genotypes	Excel × NC-C75[b]	χ^2	*df*	*P* >
Ib-316	$A_2CD \times A_2C_2$ (146, 138, 133 × 146, 138)[a]	O[d] = 5-14-4-18	3.83	3	0.72
	n = 41	E[d] = 3.42-17.08-6.83-13.67			
Ib-318	$A_2B_2 \times A_3B$ (123, 119 × 123, 119)[a]	O = 2-39-1	0.62	2	0.27
	n = 42	E = 1.17-39.67-1.17			
Ib-242	Homozygous monomorphic				
Ib-248	$B_2CE \times ABDE$ (172, 168, 162 × 175, 172, 166, 162)[a]	O = 0-1-5-5-1-0-4-1-6-2-5-0-4-0-3-1	24.87	15	0.06
	n = 38	E = 1.056-1.056-2.11-2.11-5.28-1.056-3.17-1.056-3.17-2.11-5.28-3.17-3.17-1.056-1.056-2.11			
		Beauregard × Excel[b]			
Ib-316	$A_2C_2 \times A_2CD$ (146, 138 × 146, 138, 133)[a]	O = 2-2-14-5-13	7.30	4	0.19
	n = 36	E = 1-1-17-2-15			
Ib-318	$A_3B \times A_2B_2$ (123, 119 × 123, 119)[a]	O = 4-35	0.19	1	0.94
	n = 39	E = 3.25-35.75			
Ib-242	$AB_2C \times A_2C_2$ (132, 128, 119 × 132, 119)[a]	O = 25-16-0-1-0	4.59	4	0.5075
	n = 42	E = 19.8-17.5-1.17-2.33-1.17			
Ib-248	$A_2D_2 \times B_2CD$ (175, 162 × 172, 168, 162)[a]	O = 15-10-2-4-2-1-3	3.90	6	0.81
	n = 37	E = 14.39-8.22-3.08-2.01-3.08-1.03-5.14			
		Jewel × LM87-0045[b]			
Ib-316	$A_3D \times BCD_2$ (146, 133 × 142, 138, 133)	O = 5-4-1-4-2	5.50	4	0.20
	n = 18	E = 6-6-1.5-1.5-3			
Ib-318	$A_3C \times A_2BC$ (123, 117 × 123, 119, 117)	O = 2-6-4-7	0.87	3	0.95
	n = 18	E = 1.58-7.91-3.16-6.33			
		6.1 × 20.1[c]			
Ib-316	$A_3C \times A_3B$ (146, 138 × 146, 142)	O = 16-8-9-8	4.37	3	0.38
	n = 41	E = 10.25-10.25-9-8			
Ib-242	$A_4 \times A_3B$ (132 × 132, 128)	O = 20-22	0.095	1	0.973
	n = 42	E = 21-21			

[a] Allele dosage and allele size (bp) determined on a digitized image
[b] Hexaploid *I. batatas* progenitors
[c] Tetraploid progenitors
[d] O = observed, E = expected

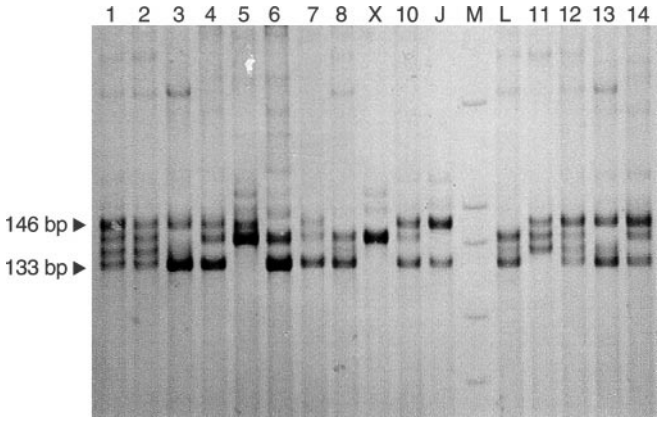

Fig. 1 Microsatellite Ib-316 genotypes for the sweetpotato cross "Jewel × LM87-0045" and 18 offspring. *Lane J* "Jewel"; *lane L* "LM87.045"; *lanes* 1–13, offspring; lane *X* possible discarded contamination; *lane M* molecular marker ϕX174 *Hin*fI

ratios found in hexaploid sweetpotato (Table 3) are not totally unexpected based on previous cytogenetic analysis of this crop. Magoon et al. (1970) proposed that sweetpotato consisted of three genomes; one genome each from two closely related ancestral species and a third genome from a more distant relative. This suggests that microsatellite primers may, or may not, be able to amplify homologous loci located in the different but related genomes of this polyploid. In spite of the complexities of polysomic segregation, it was possible to identify inheritance patterns from segregation ratios. This approach offers new alternatives for genome mapping and analysis. The results of our analysis of microsatellite loci in *Ipomoea* spp. differ from those obtained with wheat microsatellites in which homoeologous loci appear to be genome specific (Röeder et al. 1995; Bryan et al. 1997).

The percentage of primers capable of amplifying microsatellite loci in sweetpotato (14.2%) was lower than that found in hexaploid wheat (32%, Bryan et al. 1997; 22%, Ma et al. 1996; 36%, Röder et al. 1995), tetraploid potato (86.4%, Provan et al. 1996) or diploid species (nearly 100%, Liu et al. 1995; Mörchen et al. 1996). The high percentage of potato microsatellite loci that were successfully amplified may be attributable to the autopolyploid nature of its genome or to the fact that sequence data for primer design were obtained from more highly conserved regions of the genome, in contrast to studies with wheat and sweetpotato where microsatellites were isolated randomly from total genomic libraries.

Our sequence analysis revealed that an additional cause affecting a reduced rate of amplification efficiency could be attributed to the occurrence of mutations in regions flanking some of the microsatellites. Instability in the regions flanking microsatellite loci in humans has been reported previously (Callen et al. 1993; Grimaldi and Crouau-Roy 1997). The frequency of mutations in the non-repeat flanking regions in humans as reported by Calen et al. (1993) was less than the frequency we observed in sweetpotato. Grimaldi and Crouau-Roy (1997) suggested that the instability in the sequences flanking $(CA)_n$ and $(CT)_n$ mammalian microsatellites could either be caused by the repeat region itself or could be only associated with it. Our results are unique given that in animal genomes point-mutation rates in a specific base pair in flanking regions, i.e., 10^{-9}, is two to four orders of magnitude less than that found in repeat regions (Lehmann et al. 1996). The apparently higher mutation rate found in the flanking regions in sweetpotato and tetraploid *I. trifida* might be associated with the complex polyploid nature of these species which could induce chromosome pairing aberrations as was reported for *I. batatas* (Magoon et al. 1970). A high mutation frequency in the microsatellite flanking regions of diploid *I. trifida* was also evident (Table 2) suggesting that this phenomenon might be associated with the phylogeny of this species; *I. trifida* is generally regarded as the nearest relative to sweetpotato (Nishiyama et al. 1975; Shiotani 1987; Jarret et al. 1992).

The sequence analysis of several microsatellite alleles from hexaploid *I. batatas*, tetraploid *I. trifida*, and diploid *I. trifida* suggests that complex genetic mechanisms are responsible for microsatellite allelic variation (Table 2). Changes in allele size involving an increase or decrease in multiples of the dinucleotide repeat fit a simple stepwise mutation model with strand slippage during replication as the most-likely molecular mechanism (Strand et al. 1993; Valdes et al. 1993; Jin et al. 1996; Kimmel et al. 1996). Larger differences in the repetitive region size can be explained by the two-phase model proposed by Di Rienzo et al. (1994). However, insertions/deletions within the non-repeated flanking regions, producing either length polymorphisms (size homoplasy) or base substitutions within these regions, were important and frequent sources of intra- and inter-specific variation. These types of variation result in allelic differences that are not the result of changes in the number of di-nucleotide repeats.

Our data indicate that caution must be used when relying exclusively on band size in the interpretation of SSR length polymorphisms subjected to phylogenetic analysis, and suggest that microsatellite markers might be more appropriately used in studies involving the species from which the markers were originally isolated. Although interspecific differences in the characteristics of microsatellite loci as indicated by the boxed area in Table 2 do occur, the number of sequences sampled in the present study is small. Diwan et al. (1997) reported the inability to amplify a locus in a closely related *Medicago* species. He attributed this to possible primer mismatches, suggestive of interspecific microsatellite flanking-region variation. Also, working with conserved microsatellites in primate species, Blanquer-Maumont and Crouau-Roy (1995) came to

the same conclusion concerning the difficulties of interspecific phylogenetic studies based only on variation in microsatellite allele sizes. Blanquer-Maumont and Crouau-Roy (1995) found changes in the non-repeat flanking regions, and also punctual variations within the microsatellite. In the case of polyploids, differences in allele dosage can also bias estimates of genetic similarity as noted by Provan et al. (1996).

Callen et al. (1993), citing reports of alleles resolved as faint bands, attributed the phenomenon of partial amplification (faint bands) to polymorphisms within the binding sites which partially, but not totally, inhibited the PCR amplification. This might explain the lack of Mendelian segregation ratios in our diploid test populations. That is, when the parental genotypes are homozygous the primers, although not totally homologous, are equally homologous to the region flanking the locus. However, in the heterozygous condition, the region flanking the allele that is most homologous with the primer, is preferentially amplified.

Polymorphic microsatellite loci are a potentially valuable source of genetic markers in sweetpotato. Microsatellite markers follow a polysomic inheritance pattern. However, the utility of microsatellite markers in sweetpotato genetic studies appears to be somewhat limited due to the low percentage of amplifiable loci. Sequence analysis of individual alleles indicated that the mutational mechanisms responsible for the generation of new alleles in allopolyploids are complex and that this may be a factor to be considered when using microsatellite markers in phylogenetic studies.

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