Available online at www.sciencedirect.com
SCIENCE DIRECT®
Scientia Horticulturae 102 (2004) 375–386
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

New microsatellite markers developed from reported *Ipomoea trifida* sequences and their application to sweetpotato and its related wild species

Jingjie Hu [a,b,*], Makoto Nakatani [c], Antonio Garcia Lalusin [d], Tatsuhito Fujimura [e]

[a] *Doctoral Program in Agricultural Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[b] *Division of Life Sciences and Technology, Ocean University of China, Qingdao 266003, China*
[c] *National Institute of Crop Science, Tsukuba, Ibaraki 305-8518, Japan*
[d] *Doctoral Program in Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan*
[e] *Institute of Agricultural and Forest Engineering, University of Tsukuba, Tsukuba 305-8572, Japan*

Received 6 October 2003; received in revised form 18 March 2004; accepted 19 April 2004

Abstract

Ipomoea trifida belongs to the family Convolvulaceae and is closely related to sweetpotato. In this study, we reported the microsatellites in *I. trifida* sequence database, and tested their transferability and polymorphisms for both sweetpotato and its related wild species.

In the DNA database, 1425 sequences were registered for *I. trifida*. Sixty-one independent sequences were found to have microsatellite motifs and PCR primers were designed to amplify 15 microsatellites loci identified. Twelve primer pairs could amplify the expected product size and nine primer pairs showed polymorphisms among the three genotypes of *I. trifida*. These 12 functional primer pairs were used to assess the transferability and the level of polymorphism between sweetpotato cultivars and its related wild species. The transferability showed, 100% for *I. batatas*, 83.3% for *I. tiliacea*, 75% for *I. triloba* and 66.7% for *I. Lacunosa*, respectively. These markers also revealed high level of polymorphism between wild species and sweetpotato cultivars.

Keywords: Microsatellites; *Ipomoea trifida*; Sweetpotato; Database; EST

* Corresponding author. Tel.: +86-532-203-2276; fax: +86-532-203-2276.
E-mail address: hujingjie@hotmail.com (J. Hu).

0304-4238/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2004.04.014

1. Introduction

Microsatellites, also called simple sequence repeats (SSRs), are present in all plants and animals. The polymorphism of microsatellites is caused by slippage of DNA polymerase during replication and/or unequal crossing-over, resulting in variation in the number of repeated DNA motifs (Schlotterer and Tautz, 1992). As DNA marker, microsatellites show high level of polymorphism (Akagi et al., 1997) and co-dominant inheritance.

Morgante et al. (2002) reported the analysis of the density and distribution of microsatellites in plant genomes. They compared the genomes of *Arabidopsis thaliana*, rice (*Oryza sativa*), soybean (*Glycine max*), maize (*Zea mays*) and wheat (*Triticum aestivum*), which have genome size ranging from 125 to 5660 Mbp. They found that microsatellites were abundant in transcribed regions, particularly in untranslated regions. Analysis of 5000 full-length cDNA sequences of *Arabiposis* showed highly significant differences among the regions in the frequencies of all microsatellite types, with 3′-UTRs had a higher microsatellite frequency than the whole genome, while 5′-UTRs had an almost threefold higher frequency than any other genomic fraction. They also found that microsatellite frequency is not a function of overall genome size and microsatellites were more frequent in single- or low-copy DNA fractions than in repetitive DNA.

Microsatellites have recently been applied successfully to plant genetic analysis and breeding programs (Powell et al., 1996; Gupta et al., 1999). However, establishment of microsatellite marker system has lagged for many plants because it is a considerably complex, costly and time-consuming task. At present, microsatellites are developed by two main strategies: DNA library construction and public DNA database search. The intensive labor of screening small insert libraries and low efficiency of microsatellite-enrichment approaches illustrate the importance of searching for microsatellite from published DNA database. Wang et al. (1994) searched for all possible microsatellites on GenBank and EMBL sequence databases and identified 130 microsatellites in 54 different plants. Scott et al. (2000) described 10 polymorphic microsatellites derived from a grape expressed sequence tags (EST) database. They found that microsatellites located in both untranslated regions (5′-UTR and 3′-UTR) as well as in coding sequences and the microsatellites in 3′-UTR showed the most polymorphic at cultivar level. Silva (2001) analyzed sugarcane EST database and identified 402 microsatellites. PCR primers were designed for 20 microsatellite loci and were tested on eight sugarcane genotypes for detecting polymorphism. Hackauf and Wehling (2002) established 157 microsatellite markers from 8000 reported cDNA sequences for rye.

Ipomoea trifida belongs to the family Convolvulaceae, having different ploidy levels such as diploids ($2n = 30$), tetraploids ($2n = 60$), and hexaploids ($2n = 90$). Austin (1988) pointed that *I. trifida* is one of the most likely ancestors of the cultivated sweetpotato, *I. batatas*. The ISSR, RFLP and RADP data also supported that *I. trifida* is related most closely to sweetpotato (Jarret et al., 1992; Jarret and Austin, 1994; Huang and Sun, 2000; Hu et al., 2003). Wild species of *Ipomoea* are a potential reservoir of useful genes and play an important role in sweetpotato varietal improvement. Interspecific hybridization between *I. trifida* and sweetpotato, produced new sweetpotato cultivars having high yield of storage roots and high dry matter content have been developed in China and Japan (Komaki, 2001).

The development of molecular markers as a tool for *I. trifida* characterization is highly desirable, which will contribute to knowledge of the genetic relationships among the wild and cultivated accessions, and hence facilitate the breeding of sweetpotato cultivars to satisfy market needs. Despite the importance and efficiency for genetic diversity and mapping study, no information on microsatellite markers in *I. trifida* is available to date. In this paper, we describe the identification of microsatellites from *I. trifida* sequence database, and report our findings on the transferability and the polymorphism of these microsatellites for both sweetpotato and its related wild species.

2. Materials and methods

2.1. Plant materials and DNA isolation

A total of 12 accessions, including six sweetpotato cultivars or breeding lines, three accessions of *I. trifida* and other three related species of *Ipomoea* were selected for analysis of the polymorphisms and transferability of the designed primers (Fig. 1).

Total DNA was isolated from about 2.0 g of young leaves, following the CTAB method described by Gawel and Jarret (1991).

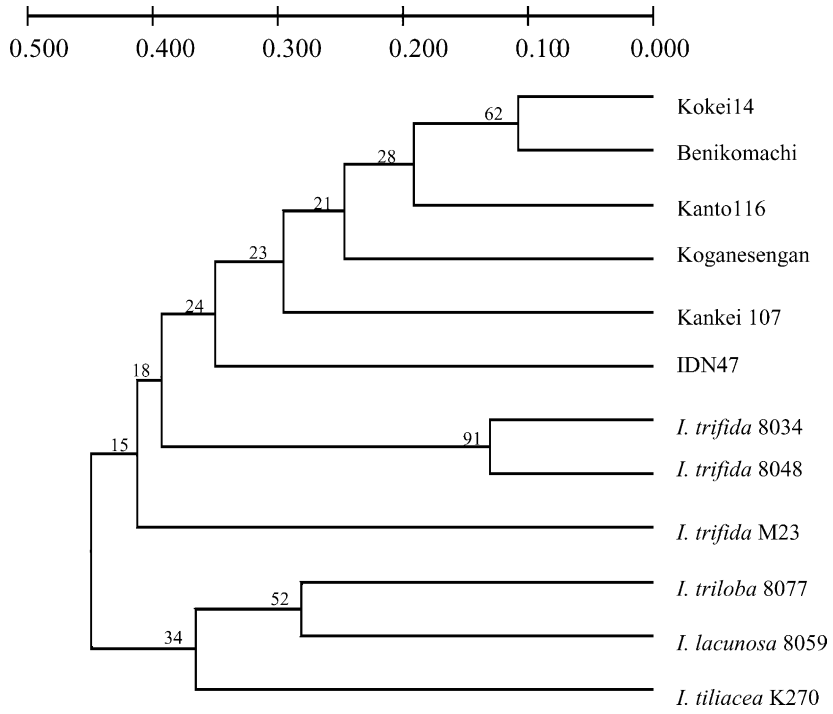

Fig. 1. Dendrogram of 12 accessions of sweetpotato and its related wild species generated by UPGMA cluster analysis with SSR markers. The numerical scale indicates genetic distance. Bootstrap values are shown on the corresponding node for each cluster.

2.2. Microsatellites mining

All *I. tridifa* DNA sequences were obtained from the GenBank (http://www.ncbi.nlm.nil.gov) database. The resultant sequences were screened for all theoretically possible tandem-repeated sequences. A repeat was identified as a microsatellite if the number of repeats (*n*) was more than seven for dinucleotide repeats, or more than five for trinucleotide repeats, or more than four for tetra-nucleotides repeats, or more than three for penta- and hexa-nucleotide repeats with home made program in PC.

2.3. Primer design, PCR amplification and electrophoresis

Sets of PCR primers, between 18 and 25 bp in length and located in the flanking regions of the repeated sequences, were designed using MacVector (Oxford Molecular Group, 1996). Primers were selected to give an expected fragment size between 100 and 250 bp based on GC content, melting temperature and the lack of a secondary structure.

PCR amplifications were performed in a thermal cycler (MJ Research PTC-200, Peltier, USA). Reaction mixtures contained 20 ng of DNA, 0.2 μM of each primer, 200 μM of each dNTP and 1 U GeneTaq (Nippon Gene) with 1× PCR universal buffer (Nippon Gene) in a total volume of 20 μl. The amplification condition included an initial denaturation of 94 °C for 5 min, followed by 35 cycles of amplification with a 30 s denaturation at 94 °C, 30 s annealing at 50–64 °C (depending on primers used), and 30 s extension at 72 °C. A final extension was performed at 72 °C for 5 min. In the case of ITSSR09 locus, touchdown PCR amplification was conducted, programmed with an initial denaturing step at 94 °C for 5 min, followed by 10 touchdown cycles of 30 s at 94 °C, 30 s at 58 °C, and 60 s at 72 °C, decreasing 0.5 °C per cycle down to 53 °C; ending with 30 cycles of 30 s at 94 °C, 30 s at 53 °C, and 30 s at 72 °C. The amplified PCR products were resolved in 12% non-denaturing polyacrylamide gels at 200 V for 1–2 h, stained with ethidium bromide and visualized under UV light.

2.4. Sequencing amplified microsatellite alleles

PCR products amplified with ITSSR02 were excised from polyacrylamide gels and ligated into pGMEM-T easy vector following manufacturer's instruction (Promega). Plasmid DNA was isolated and sequenced using an ABI Prism 310 Genetic analyzer. Several different independently clones were sequenced for each allele to derive a consensus sequence. Sequences were compared with each other using the multiple sequence alignment procedure with Genetyx-Mac (Software Development Co. Ltd., 1999).

2.5. Relationship analysis

Each unambiguous amplified fragment was treated as a character and scored manually for the presence or absence. Nei's (1978) genetic distance was calculated using TFPGA program (Miller, 1997) and dendrogram was constructed using UPGMA (Unweighted Pair-Group Method with Arithmetical averages). Bootstrap resampling (n = 1000) was performed to test the robustness of the dendrogram topologies.

Table 1
Number of sequences containing SSRs from reported *I. trifida* sequences

Motif (bp)	mRNA (18[a])	EST (1407[b])	
		Anther (561)	Stigma (846)
2	1	6	5
3	1	7	14
4	1	2	4
5	0	3	10
6	0	2	8
Total	3	20	41

The numbers within parentheses are the number of sequences in the database.
[a] Data downloaded on 31 October 2002.
[b] Data downloaded on 21 February 2003.

3. Results

3.1. SSRs in published database

The NCBI database contained 1425 *I. trifida* sequences when the data mining analysis was performed. The database sequences included 18 individually deposited cDNA sequences and 1407 sequences from two EST libraries, stigma and anther EST libraries (Table 1). The downloaded data were used to investigate for all 2–6 bp combination as repeat motif unit and 64 sequences were found to contain microsatellites. The sequences containing microsatellites were 4.5% of the total population in the published sequences. Out of the 64 sequences, three were duplicates and were removed. The proportions of all types of microsatellite motifs with more than 14 bp were shown in Table 2. The repeat unit number of microsatellite loci ranged from 3 to 18 and most (83.6%) showed repeat number of less than 7.

Database-derived microsatellites contained a variety of repeat motif sequences. Di- and trinucleotide repeat motifs presented about 50% of the total microsatellites in the *I. trifida* database sequences. The most abundant dinucleotide microsatellites were TA, followed by TC. The trinucleotide microsatellites TAA, TCT and CCG showed high abundance, as has also been found in other plant species (Gupta et al., 1996; Cordeiro et al., 2001).

3.2. PCR amplification and polymorphism in I. trifida

From the sequences containing microsatellites, 15 were chosen for the design of PCR primers (Table 3). The 15 sequences were chosen to represent di-, tri-, tetra-, penta- and hexa-nucleotides motifs microsatellites in order to test the relationship between the type of repeat motif and the level of polymorphism. Being tested in PCR reaction with *I. trifida* genomic DNA, 12 pairs amplified the products with scorable size (less than 300 bp) under the used conditions. ITSSR05 and ITSSR13 failed to amplify any PCR product. The products amplified by ITSSR10 were too large to detect polymorphism. Out of the 12 functional primers, nine primers revealed polymorphisms for three genotypes of *I. trifida* (Table 4).

Table 2
Occurrence of microsatellites in reported *I. trifida* sequences (non-redundant)

Motif	Number of repeats										Total
	3	4	5	6	7	8	9	12	13	18	
2 bp											
TA					2	1	1		2	2	8
TC						2		1			3
TG								1			1
3 bp											
TCT			4	1							5
TAA			1	2	2						5
CCG			5								5
TAG			2	1	1						4
TAC			1								1
4 bp											
TTAT		1			1						2
GCCT			2								2
AAAC		1									1
TGCA		1									1
5 bp											
AATAA	3	1									4
GATCG	2										2
AAAAC	2										2
CAGAA			1								1
ACAGT	1										1
GATCC	1										1
TATAT	1										1
TTTTC	1										1
6 bp											
AGATTC	2										2
ATGCAT	2										2
TTTTAT		1									1
TGTTTT	1										1
AGGCCT	1										1
AAGCCT	1										1
CAAGGC	1										1
TGATAA	1										1
Total											61

In contrast to reports from a number of plant genomic-derived microsatellite studies (Jones et al., 2001; Kolliker et al., 2001), amplification efficiency and polymorphism did not appear to be influenced by repeat structure or repeat number.

3.3. Microsatellites transferability and polymorphism for sweetpotato and its related wild species

The 12 functional primers were applied to six sweetpotato cultivars and other three related wild species in order to assess the transferability and the level of polymorphism.

Table 3
Sets of primers for detecting SSRs loci developed from the reported *I. trifida* sequences. SSR marker name, accession number, repeat motif and expected size are shown

Name	Accession number	SSR	Forward primer sequence (5′ → 3′)	Reverse primer sequence (5′ → 3′)	Length (bp)[a]
ITSSR01	AU224762	$(TC)_{12}$	GCTCTCAATTCCCATCCAAAC	TCTGCACCATCCTTCTTGG	133
ITSSR02	U51739	$(TA)_6(TG)_{12}$	AGGTCAAGGTGGTTTTGGTTCTG	TTGCCCTCCAACAAGCATTCCC	198
ITSSR03	AU223715	$(AT)_{18}$	AATAACTACAATGCAAGTCG	TCATCCAGGAAACAGCCCAG	158
ITSSR04	AU224072	$(AT)_{13}$	GCGTAACACATAAATTGGACTG	GGAAGTTTGAAAAGGTTAAGCC	223
ITSSR05	AU223720	$(AT)_{18}$	CAAACAACCATGAAACTG	TAAATTCACTCAACTCCTTC	101
ITSSR06	AU224193	$(TCT)_6$	ACACAAAAGAGTTGATCCTCCACC	TCCGGCTCTCGAGCCTATTG	183
ITSSR07	AU223818	$(AAT)_7$	CACCATACCCAATTTTTACAGATGC	GATTGAATGAATGATGCGG	169
ITSSR08	AU224817	$(AAT)_3(TAA)_5$	CAATCTGGGAGGAATGACATC	AAGTGTTTGAAGATCCTGCAAC	158
ITSSR09	AU224328	$(AAAC)_4$	TGTGTCACCTTAGGGACATAACTC	TTGGTATAGGTTAGGGCTTAGAAG	135
ITSSR10	AU224826	$(TTAT)_7$	CCATCCCTAGTGCATCATCCAG	ACAAAGCATCCCGCCTCTATCC	190
ITSSR11	AU224219	$(TGCA)_4$	GGCTCCTTAAAAGGCAAACAC	CCCTATTCGTAAATGGACTACAGC	113
ITSSR12	AU224351	$(TTATT)_4$	CGTTGGTGAGTTTCCCAAACC	AAAACGACGGCGTATCTGAGGCAG	122
ITSSR13	AU224607	$(CAGAA)_5$	TGGTGGTACTAACCTGAAAGGGTC	TTGCTGGAAGCTGGATTCATTC	109
ITSSR14	AU224674	$(TGTTTT)_3$	CTCCATTCAAACAGCGTCTC	CGGGCAATTCAGTTGACTC	151
ITSSR15	AU224600	$(AGATTC)_3$	CGAGATAACCCATTTTTGTGG	ATTTGTGGTGTGAGCATTGG	118

[a] The lengths were determined from the reported sequences.

Table 4
Allele sizes (bp) of database-derived-SSRs for *Ipomoea* species. Polymorphisms (P) and transferability (Trans) between genotypes (G) and species (S) are shown

SSR	*I. trifida*	*I. batatas*	*I. lacunosa*	*I. triloba*	*I. tiliacea*	P	Trans	T_a (°C)
ITSSR01	222, 224, 230	216, 222, 226, 232,	228	222	224	G, S	A	64
ITSSR02	158, 162	167, 192, 194, 198, 200	180	174	167	G, S	A	64
ITSSR03	142, 162, 178	142, 162, 164, 170	n.a.	n.a.	n.a.	G, S	S	60
ITSSR04	266, 272, 276	264, 266, 268, 272	268	268	268	G, S	S	64
ITSSR06	177	177, 179, 183	n.a.	n.a.	181	G, S	S	50
ITSSR07	168, 171	171, 174	171	168	171	G, S	S	57
ITSSR08	175, 181	175, 181	175	175	178	G, S	S	57
ITSSR09	135	111, 135, 156	n.a.	188	129	G, S	S	58–53
ITSSR11	118, 122	122	122	122	118	G, S	S	65
ITSSR12	122, 117	127, 122	117	122	122	G, S	A	64
ITSSR14	175, 163	163	193	151	157	G, S	A	55
ITSSR15	132	132, 136	n.a.	n.a.	n.a.	S	S	64

A: transferable to all accessions in this study; n.a.: no amplification; T_a: annealing temperature.

Because these new microsatellite markers were developed from cDNA, their DNA sequences may be conserved between related species and they can be more transferable to cultivated sweetpotato and its related species (Table 4). All the 12 primer pairs can amplify the genomic DNA of sweetpotato and 10 revealed polymorphisms among six sweetpotato cultivars. Ten primers could amplify the genomic DNA of *I. tiliacea*, nine for *I. triloba* and eight for *I. lacunosa*. Finally, eight primers could amplify all 12 accessions of DNA samples.

The 12 functional primer pairs were applied to test their abilities to discriminate sweetpotato and its related species. Analysis of microsatellites polymorphism across 12 accessions used in this study identified 53 alleles. The SSRs-based Nei's genetic distance ranged from 0.1076 to 0.5245, with an average of 0.3857. A dendrogram was constructed based on the genetic distances (Fig. 1). At the distance of 0.3655, three groups were clustered in the dendrogram. Group 1 contained all six sweetpotato cultivars, 'Kokei 14' and 'Benikomachi' was very close in the dendrogram because 'Kokei 14' is one of the parents in the breeding program. The second group included two *I. trifida* accessions. The third group consisted of *I. triloba*, *I. lacunosa* and *I. tiliacea*. The dendrogram showed that *I. trifida* is closely related to sweetpotato as compared to other wild species.

3.4. Alleles variation of ITSSR02 in sweetpotato

DNA sequence analysis of the amplified products derived from locus ITSSR02 showed that the size of polymorphism resulted mainly from differences in the SSR repeat number. Moreover, differences were observed in the nucleotide composition of SSR arrays that were identical in size (192 bp) from 'Kokei 14' and 'Indonesia 47'. The smallest allele 167 bp included repeat number reduction and two small deletion regions, 7 bp in the 5′ flanking region and 4 bp in the 3′ flanking region of the SSR array. Insertion/deletion (indel) and mutations were also observed in some alleles, for example, 198 bp allele from 'Indonesia 47', 167 bp allele from 'Benikomachi' and 'Kokei 14'.

4. Discussion

We searched the microsatellite loci in *I. trifida* DNA sequences registered in the GenBank. The database-derived microsatellites are more economical than the standard library screening, since they are filtered out computationally for the generation of primers. The few number of published sequence data limits the development of microsatellites from database for many plants. However, the EST projects are undertaken for some important plants, which could enlarge the sequence databases. For instance, more than 549,000 and 393,000 ESTs have been released for wheat and barley till 5 March 2004, respectively. A large number of microsatellites, being the by-product of EST projects, have been found for grape (Scott et al., 2000), rye (Hackauf and Wehling, 2002) and sugarcane (Cordeiro et al., 2001; Silva, 2001). In our study, microsatellites were abundantly found (4.5% of total sequences) in *I. trifida* DNA sequences based on the moderate microsatellite stringency criteria. Although direct comparison between estimates of microsatellite frequencies in different reports is difficult owing to various repeat motif length criteria, the di- and trinucleotide motif microsatellites occupied 2.4% of the total *I. trifida* sequences, which corresponds to the recently described frequencies in other plants, e.g. 2.5% in grape (Scott et al., 2000).

A diverse range of SSRs repeat motif sequences were present in published *I. trifida* DNA sequences (Table 2). The short trinucleotide repeat units were most frequent in the microsatellite loci. It is found that the abundance of different repeat motifs in EST-derived microsatellites vary between plant species, but trinucleotide repeat units, perfect repeat structure, and low repeat numbers were common features of EST-SSRs (Gupta et al., 1996; Scott et al., 2000; Cordeiro et al., 2001). It is well known that $(AT)_n$ repeat is the predominant motif in plant (Powell et al., 1996) and is the most polymorphic microsatellites in the rice species (Akagi et al., 1997). However, $(AT)_n$ motif is difficult to find by using traditional hybridization methods. Searching for database can overcome this limitation.

Out of 15 primer pairs tested in this study, 12 primer pairs can amplify scorable fragments with genomic DNA of *I. trifida*. Some of the amplified fragments were larger than the expected size, indicating the possible presence of introns within the genomic DNA sequence. The presence of long introns between the sequences homologous to the primers in the genomic DNA may explain the very large DNA fragment amplified with ITSSR10. This is also one of the possibilities why there is no PCR product with ITSSR05 and ITSSR13. Failures of amplification may also be due to the fact that the primer sequences contained mutation and/or indels, or primer design across intron–exon boundaries. The mutation and indel were also observed in the flanking regions of microsatellites in *I. trifida* and sweetpotato (Buteler et al., 1999).

Since most of the database-derived microsatellites come from cDNA, it would be expected that these markers are more transferable than anonymous microsatellites. The transferability of microsatellites established here was high, 100% to sweetpotato, 83% to *I. tiliacea*, 75% to *I. triloba* and 66.7% to *I. lacunosa*, respectively. High efficiency of transfer of EST-SSRs across related species has been reported previously in other plants (Scott et al., 2000; Cordeiro et al., 2001). The high transferability indicates that the primer binding sites may have been conserved in genomic DNA over a long evolutionary history. These results imply that EST-SSRs will be useful—along with other DNA-based marker systems—in the comparative genetic mapping of sweetpotato and related wild species and in tracking the

introgression of agriculturally important traits from exotic wild species and/or sweetpotato germplasm sources. Furthermore, the results also demonstrated that cross-species transferability is related to the genetic distance, implying that microsatellites could be powerful markers for synteny analysis. Besides the high transferability, the functional primer pairs revealed a high level of polymorphisms between species and genotypes. The dendrogram constructed in this experiment showed that *I. trifida* is the most close to sweetpotato, which is in agreement with other authors' conclusion as established by RAPD, RFLP and ISSR analysis (Jarret et al., 1992; Jarret and Austin, 1994; Huang and Sun, 2000; Hu et al., 2003).

In the present study, all *I. trifida*-derived primer pairs were able to amplify microsatellite loci in sweetpotato. Buteler et al. (1999) found that only 9 out of 63 (14.2%) genomic library-derived primer pairs amplified clearly scorable banding patterns. The low efficiency of amplifying microsatellite loci was also reported in other polyploidy species such as *Medicago sativa* (5%, Diwan et al., 1997) and wheat (32%, Bryan et al., 1997). The high percentage of *I. trifida*-derived microsatellite loci that were successfully amplified sweetpotato genomic DNA may be attributable to the close relatedness between sweetpotato and *I. trifida*, and the fact that primer pairs were designed in highly conserved regions of the genome (EST sequences). Our results indicated that the microsatellite markers derived from *I. trifida* database provide a unique and efficient source of microsatellites for sweetpotato.

Out of the 12 functional primer pairs, 10 (83%) were polymorphic and useful in the differentiation of sweetpotato cultivars. The number of alleles detected per polymorphic locus ranged from 2 to 8 with a mean of 5.3 alleles, and these values were comparable to those reported by Buteler et al. (1999). It is likely that the large genome size, allopolyploidy, and heterozygosity of sweetpotato are the reasons for its high level of polymorphisms (Hwang et al., 2002). Moreover, He et al. (1995) reported that self-incompatibility and outcrossing nature of sweetpotato favor high gene flow among genotypes, while vegetative reproduction maintains the high level of genetic diversity. In tomato, many microsatellites have been found (Smulders et al., 1997; Areshchenkova and Ganal, 1999; He et al., 2003) and only a small number of polymorphic SSR markers were obtained. Out of 158 SSR primer pairs screened, 41% were polymorphic among 19 tomato cultivars (He et al., 2003), which was lower than that found in this study. The low polymorphic nature of the microsatellite loci was expected because of the narrow genetic base of modern cultivars, combined with the self compatible nature of this crop (Miller and Tanksley, 1990). The low genetic diversity of modern tomato cultivars is also reflected by a low level of polymorphism for protein, isoenzymes, and several DNA markers (Miller and Tanksley, 1990; Van der Beek et al., 1992; Rus-Kortekaas et al., 1994; Broun and Tanksley, 1996). Comparison of the gene diversity values for 500 varieties in the collection with those reported for 16 other varieties showed that these values are a direct result of the choice of the varieties used (Bredemeijer et al., 1998, 2002). In a study on microsatellites in cassava (Chavarriaga-Agurirre et al., 1998), a 27% increase in both the number of alleles was observed and the level of polymorphism for a $(GA)_n$ microsatellite locus was recorded when the number of accessions screened increased from 38 to over 500.

DNA sequence analysis of ITSSR02 locus showed that the polymorphisms detected were predominantly due to the differences in the SSR array. The sequence data also suggests that microsatellite allelic variation is caused by complex genetic mechanisms (Schlotterer and Tautz, 1992; Di Rienzo et al., 1994). Moreover, indel and mutation were observed within

the non-repeated flanking regions, which might be associated with the complex polyploidy nature of sweetpotato (Buteler et al., 1999).

In conclusion, the database-derived microsatellites have some obvious advantages, such as easy to search by electronic filtering, abundant, unbiased in repeat type and present in gene-rich areas. Microsatellite markers derived from reported *I. trifida* sequences are viable sources of polymorphic, highly transferable SSRs for *Ipomoea* species. EST-SSRs derived from *I. trifida* may be a unique and efficient source of microsatellites for sweetpotato.

References

Akagi, H., Yokozeki, Y., Inagaki, A., Fujimura, T., 1997. Highly polymorphic microsatellites of rice consists of AT repeats, and a classification of closely related cultivars with these microsatellite loci. Theoret. Appl. Genet. 94, 61–67.

Austin, D.F., 1988. The taxonomy, evolution and genetic diversity of sweet potato and related wild species. In: Exploration, Maintenance, and Utilization of Sweet Potato Genetic Resources. International Potato Center, Lima, Peru, pp. 27–60.

Areshchenkova, T., Ganal, M.W., 1999. Long tomato microsatellites are predominantly associated with centromeric regions. Genome 42, 536–544.

Bredemeijer, G.M.M., Arens, P., Wouters, D., 1998. The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. Theoret. Appl. Genet. 97, 584–590.

Bredemeijer, G.M.M., Cooke, R.J., Ganal, M.W., Peeters, R., Isaac, P., Noordijk, Y., Rendell, S., Jackson, J., Röder, M.S., Wendehake, K., Dijcks, M., Amelaine, M., Wickaert, V., Bertrand, L., Vosma, B., 2002. Construction and testing of a microsatellite database containing more than 500 tomato varieties. Theoret. Appl. Genet. 105, 1019–1026.

Broun, P., Tanksley, S.D., 1996. Characterization and genetic mapping simple repeat sequences in the tomato genome. Mol. Gen. Genet. 250, 39–49.

Bryan, G.J., Collins, A.J., Stephenson, P., Orry, A., Smith, J.B., Gale, M.D., 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theoret. Appl. Genet. 94, 557–563.

Buteler, M.I., Jarret, R.L., LaBonet, D.R., 1999. Sequence characterization of microsatellites in diploid and polyploid *Ipomoea*. Theoret. Appl. Genet. 99, 123–132.

Chavarriaga-Agurirre, P., Maya, M.M., Boneierbale, M.W., Kresovich, S., Fregene, M.A., Tohme, J., Kochert, G., 1998. Microsatellites in cassava (*Manihot esculenta* Crantz): discovery, inheritance and variability. Theoret. Appl. Genet. 97, 493–501.

Cordeiro, G.M., Casu, R., McIntyre, C.L., Manners, J.M., Henry, R.L., 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) EST cross transferable to erianthus and sorghum. Plant Sci. 160, 1115–1123.

Di Rienzo, A., Peterson, A.C., Garza, J.C., Valdez, A.M., Slatkin, M., Freimer, N.B., 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. U.S.A. 91, 3166–3170.

Diwan, N., Bhagwat, A.A., Akkaya, M.S., Rongwen, J., 1997. Simple sequence repeat DNA markers in alfalfa and perennial and annual *Medicago* species. Genome 40, 887–895.

Gawel, N.J., Jarret, R.L., 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Mol. Biol. Rep. 9, 262–266.

Gupta, M., Chyi, J., Romero-Severson, J., Owen, J.L., 1996. Microsatellites in plants: a new class of molecular markers. Curr. Sci. 70, 45–54.

Gupta, P.K., Varshnet, R.K., Sharma, P.C., Ramesh, B., 1999. Molecular markers and their applications in wheat breeding. Plant Breed. 118, 369–390.

Hackauf, B., Wehling, P., 2002. Identification of microsatellite polymorphism in an expressed portion of the rye genome. Plant Breed. 121, 17–25.

He, C., Poysa, V., Yu, K., 2003. Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. Theoret. Appl. Genet. 106, 363–373.

He, G., Prakash, C.S., Jarret, R.L., 1995. Analysis of genetic diversity in a sweetpotato (*Ipomoea batatas*) germplasm collection using DNA amplification fingerprinting. Genome 38, 938–945.

Hu, J., Nakatani, M., Lalusin, A.G., Kuranouchi, T., Fujimura, T., 2003. Genetic analysis of sweetpotato and wild relatives using inter-simple sequence repeats (ISSRs). Breed. Sci. 53, 297–304.

Huang, J.C., Sun, M., 2000. Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple sequence repeats (ISSR) and restriction analysis of chloroplast DNA. Theoret. Appl. Genet. 100, 1050–1060.

Hwang, S.Y., Tseng, Y.T., Lo, H.F., 2002. Application of simple sequence repeats in determining the genetic relationships of cultivars used in sweet potato polycross breeding in Taiwan. Sci. Hort. 93, 215–224.

Jarret, R.L., Austin, D.F., 1994. Genetic diversity and systematic relationships in sweetpotato (*Ipomoea batatas* (L.) Lam.) and related species as revealed by RAPD analysis. Genet. Res. Crop Evol. 41, 165–173.

Jarret, R.L., Gawel, N., Whittensmore, A., 1992. Phylogenetic relationships of the sweet potato [*Ipomoea batatas* (L.) Lam.]. J. Am. Soc. Hort. Sci. 117, 633–637.

Jones, E.S., Dupal, M.P., Kolliker, R., Drayton, M.C., Forster, J.W., 2001. Development and characterization of simple sequence repeat (SSR) markers for perennial ryegrass (*Lolium perenne* L.). Theoret. Appl. Genet. 102, 405–415.

Kolliker, R., Jones, E.S., Drayton, M.C., Dupal, M.P., Forster, J.W., 2001. Development of and characterization of simple sequence repeat (SSR) markers for white clover (*Trifolium repens* L.). Theoret. Appl. Genet. 102, 416–424.

Komaki, K., 2001. Phylogeny of *Iopomea* species closely related to sweetpotato and their breeding use. Bull. Natl. Inst. Crop Sci. 1, 1–56 (in Japanese with English abstract).

Miller, J.C., Tanksley, S.D., 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theoret. Appl. Genet. 80, 437–448.

Miller, M.P., 1997. Tools for population genetic analysis [TFPGA] 1.3: a windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Morgante, M., Hanafey, M., Powell, W., 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat. Genet. 30, 194–200.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Powell, W., Machray, G.C., Provan, J., 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 7, 215–222.

Rus-Kortekaas, W., Smulders, M.J.M., Arens, P., Vosman, B., 1994. Direct comparison of levels of genetic variation in tomato detected by a GAVA-containing microsatellite probe and by random amplified polymorphic DNA. Genome 37, 375–381.

Schlotterer, C., Tautz, D., 1992. Slippage synthesis of simple sequence DNA. Nucl. Acids Res. 20, 211–215.

Scott, K.D., Eggler, P., Seaton, G., Rossetto, M., Ablett, E.M., Lee, L.S., Henry, R.J., 2000. Analysis of SSRs derived from grape ESTs. Theoret. Appl. Genet. 100, 723–726.

Silva, J.A.G., 2001. Preliminary analysis of microsatellite markers derived from sugarcane expressed sequence tags (ESTs). Genet. Mol. Biol. 24, 155–159.

Smulders, M.J.M., Bredemeijer, G., Rus-Kortekaas, W., Aren, P., Vosman, B., 1997. Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theoret. Appl. Genet. 97, 264–272.

Van der Beek, H., Verkerk, R., Zabel, P., Lindhout, P., 1992. Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: CF9 (resistance to *Cladosporium fulvum*) on chromosome 1. Theoret. Appl. Genet. 84, 106–112.

Wang, Z., Weber, J.L., Zhong, G., Tanksley, S.D., 1994. Survey of plant short tandem DNA repeats. Theoret. Appl. Genet. 88, 1–6.