Research Article

Development of microsatellite markers in potato and their transferability in some members of Solanaceae

Atul Grover[1], B. Ramesh[2] and P.C. Sharma[1]

[1]*University School of Biotechnology, Guru Gobind Singh Indraprastha University, Kashmere Gate, Delhi - 110 403, India*
[2]*Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut - 250 004, India*

ABSTRACT

We have developed thirty new microsatellite markers in potato by screening genomic libraries and ESTs. Genomic libraries of potato cultivar Kufri Bahar were screened for sequences containing microsatellite motifs GA, GT, ACA, ATC, GAA, TAA and GATA. Using flanking sequences, PCR primers were designed for microsatellites identified from genomic libraries and ESTs. Sixteen new primer pairs from genomic libraries and fourteen from ESTs along with seven previously published primer pairs amplified PCR products in the selected genotypes comprising of 65 *Solanum tuberosum* lines and 14 other species of the potato gene pool. Neighbor-joining tree based on genetic distance matrix developed using microsatellite markers successfully distinguished all these genotypes in the expected size range. Seventeen microsatellites could also be cross-amplified in at least one of the five members of solanaceae, namely tomato, eggplant, pepper, petunia and tobacco. The new microsatellite markers obtained in this study will be useful in various genetic and taxonomic studies in potato and related genomes. **[Physiol. Mol. Biol. Plants 2009; 15(4) : 343-358]** *E-mail : prof.pcsharma@gmail.com*

Keywords : *Microsatellite markers, genetic diversity, cross-amplification, neighbor-joining tree*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important non cereal crop in the world, and overall it stands fourth in providing food security to mankind (Hawkes, 1990), only after rice, wheat and maize. It belongs to the family solanaceae, which includes many other economically important plants. Members of solanaceae are genetically quite similar to one another (Dognalar *et al*., 2002a; Frary *et al*., 2003), and cross-amplification at genetic loci between different species is well recorded (Livingstone *et al*., 1999; Ashkenazi *et al*., 2001; Dognalar *et al*., 2002a,b). There are about 1500 species of the genus *Solanum*, but only a few of them are cultivated (Knapp *et al*., 2004). Cultivated potato (*Solanum tuberosum* ssp. *tuberosum* and *Solanum tuberosum* ssp. *andigenum*) is a highly heterozygous tetraploid ($2n = 4x = 48$; genome size around 850 Mb) outbreeder. Potato has actually been represented by a number of ploidy levels, ranging from diploid to hexaploid derived from a haploid chromosome number of 12 (Peloquin *et al*., 1999; Raker and Spooner, 2002).

A good number of genetic studies including mapping and tagging of specific traits have been undertaken in potato using RFLP and AFLP markers. For a heterozygous autotetraploid species like cultivated potato, this has been made possible using diploid wild relatives and dihaploid clones. Some of the mapped traits in potato include flesh colour (Bonierbale *et al*., 1988), tuber shape (van Eck *et al*., 1994a), tuber pigmentation (Gebhardt *et al*., 1989; van Eck *et al*., 1994b) and flower colour (van Eck *et al*., 1993). QTL mapping for late blight resistance (Bormann *et al*., 2004; Mayton *et al*., 2009) and resistance against potato cyst nematode (Achenbach *et al*., 2009) have also been undertaken. Potato is one of the first species for which high-density (Tanksley *et al*., 1992) and ultra high density (van Os *et al*., 2006) maps were made available. While, the earlier effort largely relied on RFLP markers using tomato probes (Tanksley *et al*., 1992), the latter map was based on >10,000 AFLP markers (van Os *et*

Correspondence and reprint requests : PC Sharma
Tel. : +91-11-23900220; Fax : +91-11-23900221

al., 2006). Jacobs *et al*. (1995) constructed an integrated map comprising of RFLP, T-DNA, and isozyme loci alongwith ten classical morphological markers. In recent years, linkage disequilibrium approach has also been used for construction of molecular map in tetraploid potato by D'hoop *et al*. (2008).

Techniques like RFLP and AFLP, though successful, suffer from certain shortcomings including lengthy procedures, high cost and use of radioactivity, making these techniques currently unpopular. Among molecular markers, microsatellites are preferred because of the simpler protocol involved and the low DNA requirement. Microsatellite markers being robust, ubiquitous, reproducible, multiallelic, easily transferable and highly polymorphic offer several other advantages. High mutation rates and simple Mendelian inheritance of these loci make them appropriate for investigations on population dynamics, breeding patterns and phylogeny (Ellegren, 2004; Selkoe and Toonen, 2006; Seyfert *et al*., 2008). Moreover, the use of codominant markers like microsatellites allows visualization of four different alleles in potato and may reflect the ploidy of the assayed genotypes in a convincing way.

Relatively few attempts have been made to develop microsatellite markers in potato despite their proven advantages and diverse applications. The development of microsatellite markers in potato has heavily relied upon DNA sequences available in public databases (Veilleux *et al*., 1995; Provan *et al*., 1996; Schneider and Douches, 1997; Ghislain *et al*., 2004; Feingold *et al*., 2005; Tang *et al*., 2008). These markers have been applied to potato genome mapping, gene tagging and germplasm characterization (Milbourne *et al*., 1998; Ashkenazi *et al*., 2001; Ghislain *et al*., 2004; Feingold *et al*., 2005; van Os *et al*., 2006; Spooner *et al*., 2007). Ghislain *et al.* (2004, 2009) have characterized around 1000 cultivars using microsatellite markers, and have developed a genetic identity kit useful in authentication of plant breeders' rights in potato. Similar success in genetic characterization of potato cultivars was obtained by Reid *et al*. (2009) using nine microsatellite markers. Milbourne *et al.* (1998) developed 112 microsatellite markers exploiting different sources that include enriched genomic libraries, cDNA libraries and sequence databases. Of these, 65 microsatellites could be integrated into a pre-existing genetic map of potato. Later, Ashkenazi *et al*. (2001) also utilized these resources in addition to the previously published tomato microsatellites. A high degree of polymorphism was reported in a set of 30 cultivars belonging to New World and Old World collections by Feingold *et al*. (2005) using 94 EST derived primer pairs.

In view of the above, there is an ever existing need to add new markers to the prevailing list of microsatellite markers for this important crop. Higher levels of similarity within the *Solanum* gene pool as reported by Tanksley *et al*. (1992), Ashkenazi *et al*. (2001) and Dognalar *et al*. (2002a,b) raise the possibility of utilization of other solanaceous sequences for microsatellite based studies in potato and *vice versa*. The recent trend of using EST-derived microsatellite markers in comparison to genomic library derived microsatellites is drawing attention of crop scientists (Ghislain *et al*., 2004; Feingold *et al*., 2005; Spooner *et al*., 2007).

The present study was carried out with the primary objective to develop new microsatellite markers originating from both genomic libraries and ESTs in potato and to validate the application of these microsatellite markers in potato germplasm characterization and genome mapping. Transferability of these markers across selected solanaceous genomes has also been investigated.

MATERIALS AND METHODS

Plant material

DNA from an Indian potato cultivar Kufri Bahar (K. Bahar) was used for the construction of genomic library. Thirty three cultivars including K. Bahar, 32 advanced breeding lines developed at Central Potato Research Institute (CPRI), Shimla, India, and 14 accessions of wild *Solanum* species were used to assess the utility of microsatellite markers for genotype characterization (Tables 1a-c). The segregation pattern of amplified products was assayed in a backcross population comprising of 67 lines, developed at Max-Planck-Institut fur Zuchtungsforschung, Koln, Germany (Gebhardt *et al*., 1989). The parental genotypes, P9 (H81.691/1) and P16 (H82.309/5), mainly represented *tuberosum* germplasm, but were introgressed with germplasm belonging to *S. acaule*, *S. andigenum*, *S. demissum*, and *S. spegazzinii*. Diploidization was achieved through parthenogenesis (Gebhardt *et al.,* 1989). During the backcross, P16 was the female parent, while the F_1 i.e., BC916/2 was the male parent. Additionally, microsatellites were also cross-amplified in tomato (cv. Pusa Ravi), eggplant (cv. MHB-80), pepper (cv. Arjun), petunia (cv. Prisma Sunshine) and *in vitro* grown tobacco shoots containing green tissue.

Table 1a. Details of potato cultivars used in genetic diversity analysis

S.No.	Cultivar	Year of release	Parentage
1.	K. Alankar	1968	Kennebec x ON2090
2.	K. Anand	1999	PJ-376 (K. Ashoka) x PN/F-1430
3.	K. Ashoka	1996	EM/C-1021 x CP1468
4.	K. Badshah	1979	K. Jyoti x K. Alankar
5.	K. Bahar	1980	K. Red x Lisneke
6.	K. Chamatkar	1968	Ekishirazu x Phulwa
7.	K. Chandramukhi	1968	Sd. 4485 x K. Kuber
8.	K. Chipsona I	1998	MEX.750826 x MS/78-79
9.	K. Chipsona II	1998	F-6 x Qb/B 92-4
10.	K. Dewa	1963	Craig's Defiance x Phulwa
11.	K. Jawahar	1996	K. Neelamani x K. Jyoti
12.	K. Jeevan	1968	M 109-3 x $(Vtn)^2$ 62.33.3
13.	K. Jyoti	1968	3069d (4) x 2814a (1)
14.	K. Kanchan	1999	SLB/Z-405 (a) x Pimpernal
15.	K. Khasigaro	1968	Taborky x Sd. 698-D
16.	K. Kuber	1958	[*S. curtilobum* x *S. tuberosum* (B-5)] × *S. andigenum* (NA-45)
17.	K. Kumar	1958	Lumbri x Katahdin
18.	K. Kundan	1958	Ekishirazu x Katahdin
19.	K. Lalima	1982	K. Red x AG 14 (Wis. X 37)
20.	K. Lauvkar	1972	Adina x Sarkov
21.	K. Megha	1989	SLB/K-37 x SLB/Z-37
22.	K. Muthu	1971	3046 (1) x M 109-3
23.	K. Naveen	1968	3070 d (4) x 692-D
24.	K. Neela	1963	Katahdin x Shamrock
25.	K. Pukhraj	1998	Craig's Defiance x JEX/B 687
26.	K. Red	1958	Clonal selection of indigenous variety Darjeeling Red Round
27.	K. Safed	1958	Clonal selection of indigenous variety Phulwa
28.	K. Sheetman	1968	Craigs Defiance x Phulwa
29.	K. Sherpa	1983	Ultimus x Adina
30.	K. Sindhuri	1969	K. Red x K. Kundan
31.	K. Sutlej	1996	K. Bahar x K. Alankar
32.	K. Swarna	1985	K. Jyoti x $(vt)^2$ 62.333
33.	K. Thenmalai	Not released	Not known

Table 1b. Details of advanced breeding lines of potato used in genetic diversity analysis

S. No.	Advanced breeding line	S. No.	Advanced breeding line
1.	94P31	17.	J/95-229
2.	94P59	18.	J/95-242
3.	B420(2)	19.	MP/97-583
4.	JEX/A 1270	20.	MP/97-625
5.	HT/93-707	21.	MP/97-644
6.	J/92-13	22.	MP/97-921
7.	J/92-164	23.	MP/98-71
8.	J/93-4	24.	MS/92-1090
9.	J/93-58	25.	MS/93-1344
10.	J/93-77	26.	MS/94-889
11.	J/93-81	27.	MS/94-1118
12.	J/93-86	28.	MS/95-117
13.	J/93-139	29.	MS/95-1309
14.	J/94-90	30.	MS/97-621
15.	J/95-221	31.	MS/97-1606
16.	J/95-227	32.	SM/86-185

Preparation of genomic library

DNA extracted from the potato cultivar K. Bahar using CTAB method (Doyle and Doyle, 1990) was used for the construction of genomic libraries. The genomic DNA was hydro-sheared and 750-1500 bp fragments were eluted from the gel using QIAGEN GenElute kit (Qiagen, USA). Approximately 1 µg DNA fragments were blunt-ended at 37 ºC for 10 min. using 0.2 U T4 DNA polymerase (MBI Fermentas, Lithuania) in the presence of 20 µM of each of the dNTPs. Alternatively, genomic DNA was digested overnight with *Rsa*I (MBI Fermentas, Lithuania) at 37 ºC. Gel eluted 500-1000 bp DNA fragments were ligated to calf intestine alkaline phosphatase (MBI Fermentas, Lithuania) treated linearized pUC18 or pBluescript SK+ vector (Stratagene, USA) at *Sma*I site. Electrocompetent *E. coli* DH10B (Invitrogen, USA) cells (25 µl) were transformed using 1 µl of ligation mix each time in three different sets of experiments using electric shock (1.8 KV/cm; 3.8 ms) in a Biorad Genepulsar (Biorad, USA). Transformed cells were initially grown for 1-2 h at 37 ºC in 1 ml SOC medium. Libraries were later plated on 24 cm x 24 cm Nunc Bioassay plates containing Luria-Bertani (LB) agar medium supplemented with 100 µg/ml ampicillin, 50 µg/ml X-Gal and 0.5 mM IPTG at a density of 20,000 colonies per plate. In a separate

Table 1c. Details of wild species of *Solanum* used in genetic diversity analysis

S.No.	Species	CPRI Accession No.	Ploidy/Chromosome No.
1.	*Solanum sparsipilum* (Bitter) Juz. & Bukasov	1724-6	$2n = 2x = 24$
2.	*S. albicans* (Ochoa) Ochoa	1763-5	$2n = 2x = 24$
3.	*S. berthaultii* Hawkes	1763-11	$2n = 2x = 24$
4.	*S. alandiae* Cardenas	1765-20	$2n = 2x = 24$
5.	*S. berthaultii* Hawkes	1765-24	$2n = 2x = 24$
6.	*S. arnezii* Cardenas	1769-14	$2n = 2x = 24$
7.	*S. avilesii* Hawkes & Hjert.	1774-1	$2n = 2x = 24$
8.	*S. berthaultii* Hawkes	1778	$2n = 2x = 24$
9.	*S. berthaultii* Hawkes	1778-1	$2n = 2x = 24$
10.	*S. bulbocastanum* Dunal	1788	$2n = 2x = 24$
11	*S. demissum* Lindl.	1837-1	$2n = 6x = 72$
12.	*S. demissum* Lindl.	1846-4	$2n = 6x = 72$
13.	*S. tuberosum* L. ssp. *andigenum* (Juz. & Bukasov) Hawkes	2040-15	$2n = 4x = 48$
14.	*S. tuberosum* L. ssp. *andigenum* (Juz. & Bukasov) Hawkes	2045	$2n = 4x = 48$

experiment, 100 µl culture of *E. coli* DH5α competent cells was transformed using 10 µl ligation mix using heat shock at 42 ºC followed by incubation on ice for 5 min. Cells were grown in LB broth medium for 2 h and plated similarly on ampicillin containing LB agar medium on Nunc Bioassay dishes (Nunc, Denmark).

The libraries were screened following the protocol of Ashkenazi *et al*. (2001) using γ^{32}p end labeled $(GAA)_5$, $(GT)_{10}$, $(TAA)_5$, $(GA)_8$, $(GATA)_4$, $(ACA)_5$ and $(ATC)_5$ probes (each 25 µM) in hybridization buffer. The probes were selected on the basis of knowledge of microsatellite abundance in plants (Winter *et al*. 1999; Ashkenazi *et al*. 2001), hybridization temperature and possibility of self-annealing at that temperature. Colonies giving positive signals after primary screening were picked and transferred to 90 mm petri dish plates containing LB medium supplemented with 100 µg/ml ampicillin. Secondary screening was performed using the same procedure as that in primary screening.

DNA sequencing and primer design

Positive clones were scaled up in 5 ml LB broth medium (supplemented with 100 µg/ml ampicillin) for isolation of plasmids. QIAquick plasmid isolation kits (Qiagen, USA) were used for the isolation of plasmids as per manufacturer's protocol. The plasmids were sequenced using M13 universal primers on a MegaBACE DNA sequencer (GE Life Sciences, USA). The sequences were obtained in FASTA format and visually screened for the presence of microsatellites. The primers were designed using online utility Primer3 (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) for the amplification of microsatellites present in the sequences isolated from genomic libraries.

Additionally, primers were also designed for the microsatellite sequences isolated from ~29,000 ESTs downloaded from EST database of Solanaceae Genome Network (SGN) (Grover and Sharmam 2004). These EST sequences can now be mined from Solanaceae Genomics Resource (http://sol-blast.plantbiology.msu.edu/) database of Michigan University. Additionally, seven primer pairs used earlier by Veilleux *et al*. (1995), Milbourne *et al*. (1998) and Ashkenazi *et al*. (2001) were also explored for their use in genetic diversity analysis. The primers used in this study were custom synthesized by Microsynth, Switzerland or Oligator, USA.

Primers were designed atleast 30 bp away from either side of the microsatellite. An effort was made to keep the annealing temperature (T_m-5) ºC of primers near 55 ºC and % GC close to 60. T_m was calculated following the formula- [2 x (A or T) + 4 x (G or C)] ºC. Most of the primers were 20 bp long. Repetitiveness within the primer sequences was tolerated as long as they did not contribute to self-annealing three dimensional structures. Majority of the microsatellite positive ESTs failed in meeting one or more of these criteria.

PCR analysis and allele detection

The PCR reaction mixture contained 1X *Taq* polymerase buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 U *Taq* DNA polymerase (Biotools, Spain), 10 µM of each primer and 40-50 ng template DNA in a total reaction volume of 25 µl. PCR reactions were performed with an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 94 °C (30 s), annealing temperature [(T_m-5) °C; 45 s] and 72 °C (1 min) and a final extension step at 72 °C for 10 minutes. The amplified products were run on 2.0 % agarose gels or whenever necessary on 12.0 % non-denaturing polyacrylamide gels in 1X TBE buffer. The gels were stained with ethidium bromide.

Data analysis for heterozygosity and genetic diversity estimations

Polymorphism information content (PIC; Botstein *et al*., 1980) was estimated as a measure of informativeness of each of the markers. A distance matrix was also generated by infinite allele model (Nei, 1972) using NTSysPC 2.1 (Rohlf, 1992) to construct a neighbor joining tree (Saitou and Nei, 1987).

RESULTS AND DISCUSSION

Potato is a distinguished crop with the most comprehensive genome map available (van Os *et al*., 2006). This map is based on more than 10,000 markers, majority of which are AFLP markers. Much earlier, Tanksley *et al*. (1992) constructed a high density RFLP map of potato with 1400 markers. Microsatellites, otherwise considered to be highly robust markers, are under-expolited for genetic studies, especially mapping, in potato. However, a few attempts have been made towards development of microsatellite markers in potato (Milbourne *et al*., 1998; Ashkenazi *et al*., 2001; Feingold *et al*., 2005; Tang *et al*., 2008) generating around 170 microsatellite markers, which are certainly not sufficient to cover a large genome like potato.

There are three major ways of isolating microsatellites from genomes- using random genomic and enriched genomic libraries, and mining from sequence databases. One can also use microsatellites developed for a related genome with varying degree of success. In the recent years, development of microsatellite markers in plant genomes through *in silico* mining of ESTs and genomic sequences has gained immense popularity owing to some unmatched advantages (Varshney *et al*., 2005; Sharma *et al*., 2007). However, many of the EST-derived markers fail as functional markers, understandably because length polymorphism in conserved regions like genes is not as common as in hypervariable regions of the genome. Despite the relative advantages and disadvantages of different methods, each of these methods has been found useful for development of markers in plants. In this study, we have attempted to develop microsatellites by screening genomic libraries and potato ESTs.

Preparation of genomic libraries and frequency of microsatellites

Electroporation method of *E. coli* transformation expectedly proved better with 90 % efficiency as against heat shock method with only 50 % efficiency. Each plate prepared following electroporation and heat shock transformation represented nearly 0.88 % and 0.35 % of the potato genome, respectively, estimated according to Ashkenazi *et al*. (2001). GT and GAA probes generated strongest signals, while TAA showed remarkable non specific binding when libraries were blotted on to a membrane and hybridized to several microsatellite probes.

Sequence analysis of the putative 81 positive clones obtained following radioactive screening of genomic libraries revealed that some of the clones had no microsatellites while some others contained more than one microsatellite repeat (Fig. 1). Sequence analysis showed 94 microsatellites in the 65 clones out of the 78 clones sequenced in the present study (Fig. 1). Nearly 35 % of all the repeats were compound microsatellites. The general trend of microsatellite mining from genomic libraries resembled to that in monocots, typically showing shorter repeats and fewer complex repeats (Röder *et al*., 1995). In wheat, barley, rice and maize, complex microsatellite repeats isolated from genomic libraries represent less than 50 % of the total isolated

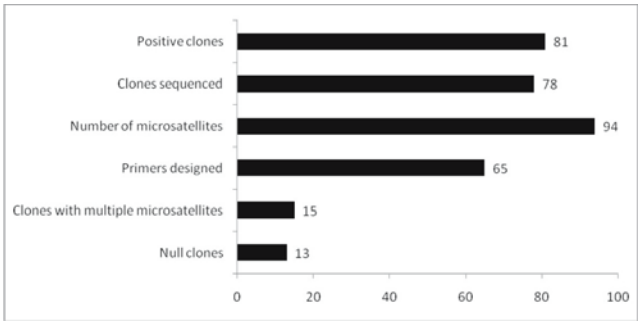

Fig. 1. Details of microsatellite positive clones obtained after secondary screening of potato genomic libraries

Table 2. Frequency of positive clones after primary and secondary screening with different oligonucleotide probes

Probe	Colonies screened (library plated)	Primary screening		Secondary screening	
		Positive clones	Frequency (%)[a]	Positive clones	Frequency (%)[b]
$(GAA)_5$	20,000 (1.1)	173	0.86	17	9.83
$(GT)_{10}$	20,000 (3.1)	362	1.81	25	6.91
$(TAA)_5$	20,000 (3.2)	454	2.27	13	2.86
$(ATC)_5$	24,000 (3.2.2)	57	0.24	5	8.77
$(ACA)_5$	24,000 (3.2.2)	47	0.20	4	8.52
$(GATA)_4$	24,000 (3.2.2)	94	0.39	15	15.96
$(GA)_8$	24,000 (3.2.2)	61	0.25	2	3.28
Total	**84,000**	**1248**	**1.49**	**81**	**6.49**

[a]Frequency for primary screening = (no. of positive clones)/(Total clones screened) × 100,
[b]Frequency for secondary screening = (no. of positive clones after secondary screening)/(no. of positive clones after primary screening) × 100.

repeats (Röder *et al*., 1995). The variation reported for potato microsatellites was not directly dependent on the length of the microsatellite array or its simple/complex composition. However, in some plant species a bias has been reported, for example, in soybean, GT microsatellites were more informative than GA (Powell *et al.,* 1996). In contrast, GT repeats were less polymorphic than GA in *Arabidopsis* and the complexity of microsatellite structure decreased with the level of length variability (Bell and Ecker, 1994).

Since only 81 microsatellite positive clones could be isolated from 84,000 genomic clones screened (Table 2), total microsatellite repeat loci were estimated as 21,857 loci with an average spacing of 81 Kb in the potato genome compared to Ashkenazi *et al*. (2001), who estimated a total of 32,800 repeats with motifs A, GT, CT, ATT and CTT with an average distance between two loci being 52 Kb. On analysis of the actual number of clones carrying microsatellites, and the total number of microsatellites identified from the library, the estimate of the total number of microsatellites of these seven types in potato genome was corrected to 25,365 repeats, spaced apart every 65 Kb. Microsatellite frequency in the potato genome was estimated (using probe hybridization) to be as high as one GT repeat per 600 Kb to as low as one GA repeat per 9 Mb. However, on the actual sequence analysis of the clones, GAA repeats were estimated to be most frequently occurring at every 607 Kb, while ATC and GATA to be most scarce at every 9 Mb in the genome. The maximum number of loci was five that could be found in any of the cloned sequence. Motifs A and ATT are reported to be abundant in plant genomes (Katti *et al*., 2001; Morgante *et al*., 2002; Grover *et al*., 2007). Frequencies of GA (or CT or GA or TC) and GT microsatellites (estimated on the basis of hybridization of probe) range widely from every 17 to 500 Kb and from 86 to 800 Kb, respectively (Bell and Ecker, 1994; Röder *et al.,* 1995; Liu *et al*., 1996). Such low estimates of microsatellite richness in genomes arise due to procedural and technical limitations in cell lysis, probe hybridization and washing steps during colony hybridization. Bioinformatic studies, wherever possible overcome such limitations. In fact, prevalence of GA over GT repeats is a general feature of the plant genomes, as predicted by genome-wide bioinformatic analyses (Morgante *et al*., 2002; Grover and Sharma, 2007; Grover *et al*., 2007). Considering these estimates, it is clear that, to produce a saturated microsatellite map covering the whole potato genome, it is necessary to screen thousands of inserts in search of more and more microsatellites.

Database search

EST sequences downloaded from the ftp site of The Institute of Genome Research (now J Craig Venter Institute; ftp:\\ftp.jcvi.org) were scanned for the presence of microsatellites and the results pertaining to microsatellite abundance and frequency in these sequences have been published elsewhere (Grover and Sharma, 2004). Though more than 1,400 ESTs were found positive for microsatellites, primers were designed only for those microsatellites whose lengths were found more than 20 bp. Among them too, many of the tags were discarded, as primers could not be designed due to the terminal position of the microsatellite or the overall low quality sequence of the EST. Further, properties of the primers designed from many other of these ESTs failed to meet the criteria of desired Tm and % GC content.

Analysis of microsatellite polymorphism among cultivars and species

A total of 130 primer pairs were designed following screening of genomic libraries and EST sequences. These primers are available on request from the corresponding author. Of these, forty four primer pairs were randomly picked up to be employed in genetic diversity analysis. Thirty primer pairs (sixteen from genomic libraries and fourteen from EST sequences) amplified PCR products in the expected size range in the first set of three cultivars namely K. Bahar, K. Ashoka and K. Chandramukhi (Table 3). Additionally, seven previously published primer pairs also amplified products in the expected size range (Table 4). Expected size range refers to the 50 bp up and down from the length predicted by primer design software (or published range in case of previously published primer pairs). This range was based on the published reports of microsatellites on potato (Milbourne *et al*., 1998; Ashkenazi *et al*., 2001; Ghislain *et al*., 2004; Feingold *et al*., 2005). Any band appearing within this range was considered as allele, and a maximum of four alleles per genotype were expected. Any band occurring outside this range was considered as a separate locus.

Thirty seven primer pairs selected as above were used to fingerprint a panel of 79 potato genotypes (Tables 1a-c), of which 67 were tetraploid potato. Most of the cultivars and advanced breeding lines belonged to *Solanum tuberosum* ssp. *tuberosum*. The fingerprint data revealed a total of 110 alleles with a mean of 2.96 alleles in the range of 1-5 alleles per locus (Table 3). The presence of more than one PCR band for a given

Table 3. Data on PCR primers, microsatellite motif and polymorphism displayed by microsatellites markers developed in the present study from the genomic sequences (S. No. 1-16) and potato ESTs (S. No. 17-30). Markers (S. No. 31-37) are from earlier published literature.

S. No.	Micro-satellite ID	GenBank Accession/ EST ID	Microsate-llite motif	Forward primer (5'-3') Reverse primer (5'-3')	Allele number	Allele size range (bp)	PIC	Reference
1	A001	GQ415689	$(T)_{22}...(G)_{15}$	TAT CGG GAA GGA CAA GTT GG TTG GTT GTT TTT CCC CTC AC	5	260-285	0.57	
2	A002	GQ415690	$(T)_{13}...(C)_{15}...$ $(G)_{13}$	GTG TGG GTG TGG GGT TTT C CGC ACA CGA AAC AGA AAA AG	5	225-250	0.70	
3	A003	GQ415691	$(T)_{31}$	TCG GCA CGA GGA GAG AAC TA AGC CAG AGC TTG TGG AAA AA	4	210-240	0.71	
4	ACA001	GQ415692	$(ACA)_4$	CTT GGC GGT TAA CCC ATT TA CAA AAC CGA CCA TCC CAT AA	2	237-250	0.84	
5	ACA002	GQ415693	$(ACA)_2G(AC)_2$ $GC(G)_5TCA$ $(C)_5$	GGG AAC ACA AGC GCA AGA AGT GTG GGC GGT CTT ACT A	2	235-248	0.51	
6	AG001	GQ415694	$(AG)_7$	CGG GTG ACA CAC GAG ATT TT CCG CGA AAA GAA GTG GTA AG	2	150-162	0.56	
7	GAA001	GQ415695	$(GAA)_5$	GGA CCA AAA CCC TAG CGA AG GGG TCA TAA CCT GAC GGA AA	2	162-175	0.34	
8	GAA002	GQ415696	$(AGA)_4...$ $(CAC)_4$	CGA TGG TGG GAT GGA AAT AC ACG GTC CAG AGG AGA ACA GA	3	210-225	0.72	
9	GAA003	GQ415697	$(AAG)_6$	TCT CCC CCT CCA TCT CAA C AGA AAG AGA GGA GAA CAG A	2	180-197	0.45	
10	GAA004	GQ415701	$(AGA)_4$	CTA ATC GAT GGT GGG ATG GA ACG GTC CAG AGG AGA ACA GA	2	210-222	0.73	
11	GAA005	GQ415699	$(AGA)_5...$ $(GAA)_3N$ $(GAA)...$ $(GGGAA)_3$	GCT TAG ATG CCG CAG GTT AC TTC CCC CTG GTT TTG AGT AG	4	225-260	0.72	
12	GAA007	GQ415700	$(GAA)_3GGA$ $(GAA)_3$	TGG AAC CTT TGA CCT TCG AC GGT GCC AAA AGG TGC AGT AT	2	322-340	0.64	
13	GAA008	GQ415698	$(AGA)_4$ $A(ATT)_3$	CAA CCA AAC CCC TGC AAA G GTA GCT GTG CTG CGC CTT C	3	160-180	0.50	
14	GAA010	GQ415702	$(AAG)_{14}$	GGC ACG AGG AGA GAA AGA GA AAC CAA AGA TTT TGC TGA AAA CA	5	210-260	0.82	
15	GAA011	GQ415703	$(AAG)_8$	ACC CAG ACA GCA AGA CGA AA CTA CAA ACC CAG CTG CCA AT	3	201-230	0.89	
16	GT001	GQ415703	$(GT)_9$	CAG GTC AGC AGT GCC TAA GA GGT CCG AAT CAT CAG GAA AG	3	180-200	0.77	

Table 3. Continued .....

S. No.	Micro-satellite ID	GenBank Accession/ EST ID	Microsate-llite motif	Forward primer (5'-3') Reverse primer (5'-3')	Allele number	Allele size range (bp)	PIC	Reference
17	eAG006	PSTBN32TH *cSTA10F16	$(AG)_4(A)_3GC$ $(GA)_9...(GA)_2$ $(AG)_2$	GGA AGA CTC CTT CCT TCC ATC T TCT GCA ACT CAC TCC TTT ACG A	5	220-260	0.63	
18	LSS002	PSHAC75TH *cSTB1N5	$(CAA)_3(CAG)$ $(CAA)_2(CAG)_4$ $(CAA)_2$	CCA TGG AGT TTC AGG ACC AC TGG CGA ACT GAC TGT GTT GT	2	192-205	0.14	
19	LSS005	PSTEK76TH *cSTA29N7	$(TA)_{17}(CA)_4$	CCA GCA ACA GCT CCA CTT TA ATG GGG TTC TTG GGG ATA CT	2	210-219	0.40	
20	LSS009	PSTEH05TH *cSTA28B10	$(AAG)_{14}$	GCT GCT AAA CAC TCA AGC AGA A GCT CAG CTT TCA GAA G AA ACC A	5	200-250	0.79	
21	LSS010	PSTFE78TH *cSTA34N11	$(GGAGAA)_5$ $(GAA)_3GA$ (GAA)	AGC TCA AGG CTT CTG TTG GA ACC ACC TCA GGC ACT TCA TC	2	212-225	0.92	
22	LSS011b	PSTCJ86TH *cSTA16O4	$(CAA)_6$ $(CAG)_4$	AAC CAG ATC CCC AAT CAA CA TGC TTC ATA GTC TGC CAC CA	2	210-228	0.49	
23	LSS012	PSTCQ19TH *cSTA18C13	$(CAACAG)_2$ $(CAG)_3$ $(CAA)_3$ $(ACC)_5$	CCA AGT TCA GTC ATC GCT CA ACT GCA ATC TCG CCT GAA CT	4	210-250	0.68	
24	LSS017	PSTDK17TH *cSTA23C9	$(CTT)_9$	GAA TTG CGG ATC AGT TCA CC AAA CAT TTG GGT AGC CGT TG	2	235-255	0.68	
25	LSS019	PSTAC36TH *cSTA1F23	$(CCT)_4(CCG)_5$ $(TCT)_3CT$ (TCT)	GCG ACC TCC CTT CTT CTC CTT ATA AAA CCC GGG CCT TTG A	2	220-229	0.66	
26	MISC012	PSTDX49TH *cSTA26I2	$(GGC)_5GA$ $(CTT)_2..$ $(CTC)_2CTT$ $(CCT)_4$	TTG TCT GCT TTC CAC TTC ACA TGA GAG GCT TAA GAC GAT GGT	3	150-175	0.74	
27	MISC013	PSTDZ53TH *cSTA26J10	$(CGA)_4G$ $(GAT)_3AAC$ $(GAG)_4$	CAG GTT CTT CGC CTC ATA GC CAT CAT CAT CGT CGT CAT CC	2	230-244	0.66	
28	MISC033	PSTGD05TH *cSTA40B10	$(CTTT)_5$ (GTTT) $(CTTT)_2$ T(CTTT)	CAA AAT ACC TTC TCC CCC TCT AAA ACG TTT AGG GCT GCT TG	2	210-221	0.63	
29	USE102	PSTDT13TH *cSTA25C2	$(CAA)_5...$ $(CAA)_6...$ $(CAA)_5$	TCA ATT TCA GCA GCA TCA GC GGT CTG CCC ATT TGG TAC TG	2	195-206	0.66	

Table 3. Continued .....

S. No.	Micro-satellite ID	GenBank Accession/ EST ID	Microsate-llite motif	Forward primer (5'-3') Reverse primer (5'-3')	Allele number	Allele size range (bp)	PIC	Reference
30	USE103	PSTAM27TH *cSTA4E5	$(ACC)_6$ $(ACA)_2$... $(CAC)_5$	GGA ATT TCA CAG CCC ATG TC AGA GGA AGC GTC AGC TTG AA	2	240-247	0.66	
31	L0506		$(TC)_{12}(TA)_{18}$	CTT GCA ACT TGT TAG TAC CCC C AAA TCC TTT GTG ACC TCC CC	3	180-210	0.73	Veilleux *et al.* (1995), Ashkenazi *et al.* (2001)
32	L3738		$(C)_{14}(CT)_8$ $(AT)_9$	ATT GTT TTC TTT CTT CTT GC AAT TCT AGC CCT TCA CTT TG	2	190-220	0.80	Ashkenazi *et al.* (2001)
33	STM0046		$(GC)_4(AC)_{17}$ $(AT)_4$	TGA CAA GGA TAA CAT CAG CAT AAG GCA TTT AAG TTA GGA GTT CAT GCT	2	100-115	0.60	Milbourne *et al.* (1998)
34	STM0050		$(AC)_{18}$	TCA GAG GTT TTG TCA CGT T TAT ATG GGA CAC ACG TGC	2	117-125	0.62	Milbourne *et al.* (1998)
35	STM3011		$(CT)_{19}$... $(CT)_{16}$... $(AC)_5$	GTG TGG TTG ATT CAT TGA ATT TAG GTT TTT AGG CAG TTC TTG GGG	4	115-145	0.78	Milbourne *et al.* (1998)
36	STM3016		$(GA)_{27}$	TCA GAA CAC CGA ATG GAA AAC GCT CCA ACT TAC TGG TCA AAT CC	3	140-160	0.78	Milbourne *et al.* (1998)
37	STM3023		$(GA)_9$... $(GA)_8$... $(GA)_4$	AAG CTG TTA CTT GAT TGC TGC A GTT CTG GCA TTT CCA TCT AGA GA	3	190-210	0.83	Milbourne *et al.* (1998)

locus indicated level of heterozygosity in the germplasm. PIC values ranged between 0.14 using the primer pair LSS002 to 0.84 using primer pair ACA001 (Table 3). However, in nine cases, additional bands, smaller or larger than the expected bands were also observed, often well conserved across the species, indicating amplification of more than one locus. Null alleles were common at expected length loci, as well as with additional band lengths (Fig. 2). Nearly 50 % of the screened primer pairs successfully amplified products across different species and genera, also in the same size range as they amplified in potato (Table 4). False positives were discarded after repeating the experiments with a subset of genotypes selected at random.

Previous genetic analyses involving EST-derived microsatellite markers in solanaceae indicated that a high number of primer pairs derived from EST sequences amplify PCR products in the expected size range (He *et al*., 2003; Feingold *et al*., 2005). Present study also confirm to such previous findings. In general, the drawback of using cDNA sequences (using cDNA library and ESTs) for microsatellite development is the unknown final size of the amplicon (Scott, 2001; Feingold *et al*., 2005). The success of such markers very much depended on their occurrence in 5'-UTRs and 3'-UTRs (Milbourne *et al*., 1998; Feingold *et al*., 2005).

The level of microsatellite polymorphism reported earlier by Milbourne *et al*. (1998), Ghislain *et al*. (2004) and Feingold *et al*. (2005) was higher than reported in our study. However, Ashkenazi *et al*. (2001) reported a lower average polymorphic level of the newly developed

Table 4. Transferability of potato microsatellites in some members of solanaceae

Micro-satellite ID	Tomato	Egg-plant	Pepper	Petunia	Tobacco
A001	+	-	+	-	-
A002	+	-	+	-	-
A003	-	+	-	-	-
ACA002	-	+	+	-	-
GAA001	+	+	+	+	+
GAA003	+	+	+	+	-
GAA004	+	-	-	-	-
GAA005	+	-	-	-	-
GAA007	+	+	-	-	-
GAA008	+	+	-	-	-
GAA010	+	+	+	+	+
GAA011	+	+	+	-	-
GT001	+	-	-	-	-
L3738	+	+	-	-	+
LSS002	+	-	+	+	-
LSS010	+	+	-	-	+
MISC033	+	+	+	-	-

+ = Amplification; - = No amplification

microsatellites similar to that in the present study. These differences can be attributed to the narrow genetic base of the genotypes used by Ashkenazi *et al*. (2001) and in the present study. Spooner *et al*. (2005) reported relatively poor allele numbers per microsatellite locus in Indian potato cultivars compared to other groups included in their study, suggesting a relatively narrow genetic base of Indian cultivars. It is believed that modern potato cultivars represent lower genetic diversity compared to landraces (Spooner *et al*., 2005). The ability of microsatellites to distinguish different genotypes in a sample set characterized by a narrow genetic base advocates the utility of the newly discovered microsatellites. Occurrence of a number of null alleles (Fig. 2) indicates that not only the microsatellite sequence but their flanking sequences are also quite variable in different species. Owing to the high level of polymorphism in *S. tuberosum* gene pool detected with microsatellites, the markers described here are highly suitable for the variety and hybrid identification and mapping of agronomically important genes or QTLs within *S. tuberosum.*

Estimation of heterozygosity and genetic distances

Genetic distances were estimated among the available potato germplasm using different microsatellite markers developed as above. Data was analyzed using cluster analysis software NTSysPC 2.1 (Rohlf, 1992). The distance matrix was used to construct the neighbor-joining tree that successfully distinguished all the cultivars and accessions used in this study (Fig. 3). All the wild species were placed in a cluster in one corner of the tree. However, the two hexaploid accessions (*S. demissum* 1837-1 and *S. demissum* 1846-4) were placed close to diploid accessions *S. sparsipillum* 1724-6 and *S. albicans* 1763-5, respectively. Similarly, the tetraploid accessions *S. tuberosum* ssp. *andigenum* (2040-10 and 2045) were placed farther from each other and also from modern cultivars currently under cultivation in India. Similarly, the advanced breeding line JEX/A 1270 of *andigenum* origin was significantly displaced on the tree. The modern cultivars K. Khasigaro and K. Bahar were accommodated nearest to the wild representatives.

Despite numerous phylogenetic studies based on microsatellites, no consensus could be achieved among biologists regarding the appropriateness of an evolutionary model for microsatellites. Microsatellite

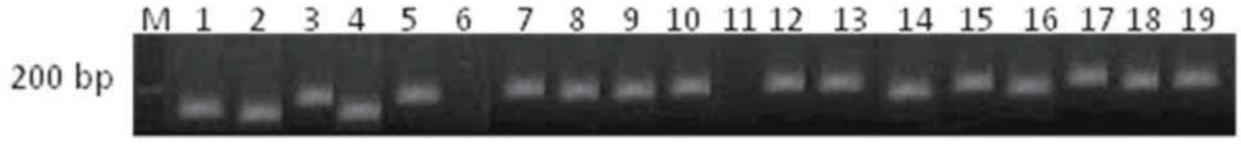

Fig. 2. Representative photograph displaying cross-amplification using primer pair LSS009 across representative potato germplasm available in India
M: 100 bp DNA ladder (Bangalore Genei, India); 1-17: advanced cultivars- J/95-221, J/95-227, J/95-229, J/95-242, MP/97-583, MP/97-625, MP/97-644, MP/97-921, MP/98-71, MS/92-1090, MS/93-1344, MS/94-889, MS/94-1118, MS/95-117, MS/95-1309, MS/97-621, MS/97-1606, SM/86-185; 18: *Solanum sparsipillum* accession 1724-6; 19: *Solanum albicans* accession 1763-5

evolution is probably more taxon specific rather than that assumed earlier. As these models have been designed for diploids, they do not best describe the dynamism displayed by a gene pool with different ploidy levels as in the present study. Consequently, the genetic distances and the similarity coefficients estimated in this study may not also appropriately explain the evolutionary events in potato. Although the data generated in this study had clustered the Indian cultivars together on the N-J tree (Fig. 3) and successfully distinguished all the 79 genotypes, it was not possible to decipher their phylogenetic relationships in a convincing way. Spooner *et al*. (2005) used a subset of cultivars used in this study, and similarly found those cultivars clustered together using genomic and chloroplast DNA microsatellites. However, the phylogenetic relationships on the N-J tree reported by Spooner *et al*. (2005) and in the present study do not fall in agreement with each other, as some of the close relatives in both the studies have been placed quite

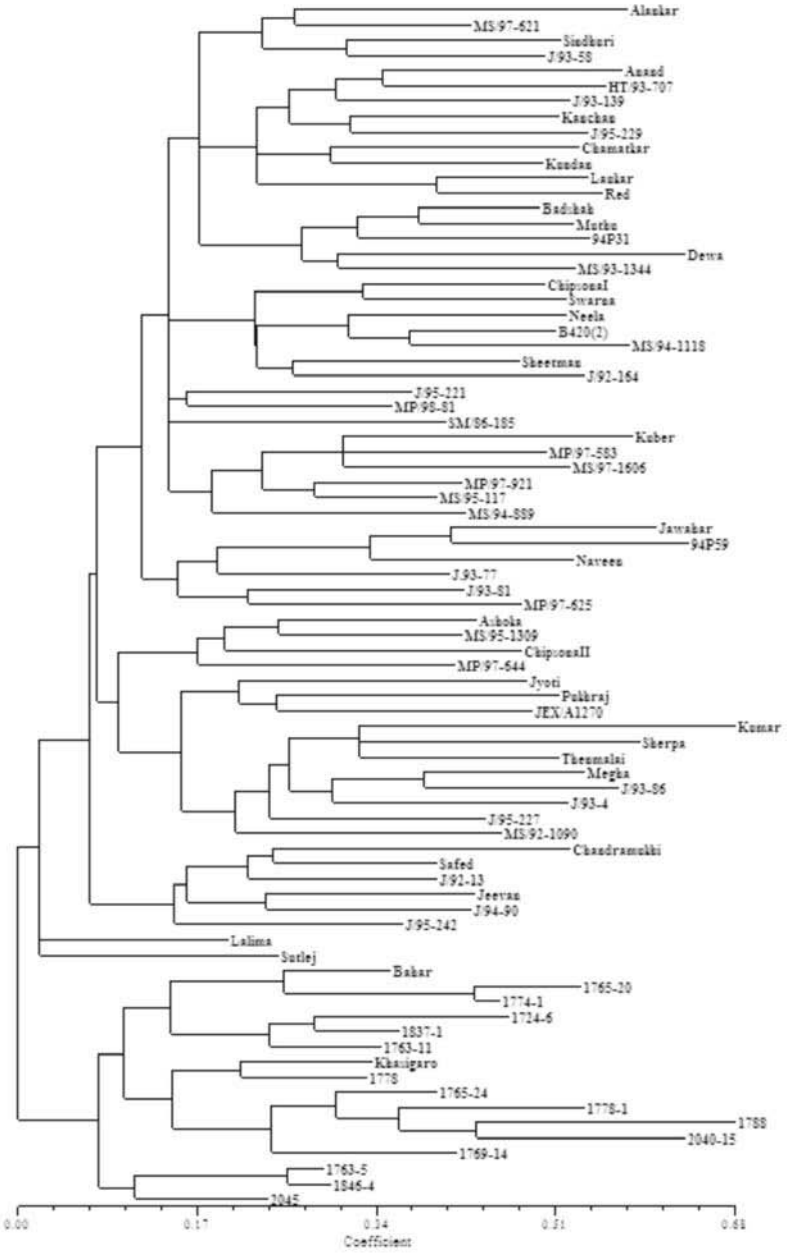

Fig 3. Neighbor-joining tree constructed from microsatellite marker analysis using Nei's (1972) similarity coefficient.

distantly on the N-J tree. For example, K. Chamatkar and K. Kundan, both desired from Ekishirazu (Spooner *et al*., 2005) were placed together on the N-J tree drawn by us, but have been placed on separate branches by Spooner *et al*. (2005). K. Jyoti is the common parent to K. Badshah, K. Swarna and K. Jawahar was shown by Spooner *et al*. (2005) but, the same relationship could not be traced in the present study. On the other hand, K. Muthu though unrelated to K. Jyoti, has been placed closer by Spooner *et al*. (2005) but not so in our N-J tree. However, K. Muthu has been found to be closer to K. Badshah in our study (Fig. 3), which is derived from a cross involving K. Jyoti (Spooner *et al*., 2005). This observation though unusual, points to the complex nature of microsatellite evolutionary dynamics, where unrelated genotypes may sometimes carry the same allele, and thus demands the adoption of two phase model (DiRienzo *et al*., 1994) to describe microsatellite mutations in place of any of the stepwise mutation model (Ohta and Kimura 1973) or infinite allele model (Kimura and Crow, 1964). The observation that 13 of the total 16 microsatellite markers extracted from the genomic library gave amplification in atleast one of the wild species is supported the fact that cultivated potatoes are probably polyphyletic in origin (Grun, 1990; Huaman and Spooner, 2002) and have an ability to cross with wild potatoes. It is also inferred from our results that ssp. *tuberosum* is distinct from ssp. *andigenum* and other wild accessions.

Genomes of closely related species exhibit a number of homologies. Transferability of markers between such species is thus obvious. This has been well exhibited between tomato and potato (Gebhardt *et al*., 1991; Tanksley *et al*., 1992; Ashkenazi *et al*., 2001). Ashkenazi *et al*. (2001) successfully amplified 60 % of the tomato microsatellite markers in potato. Likewise, we could amplify 50 % of potato microsatellite markers in tomato and eggplant. The amplification of potato microsatellites in other solanaceous species indicates the utility of the microsatellite markers and the homology between these species. The amplified region indicates the homology with regards to the primer pairs, but does not ensure the presence of microsatellites within them. Our experience in handling the rice genome (Grover *et al*., 2007) indicates that while flanking sequences may be well conserved, the microsatellite harbored between them may be missing. Present results suggest a lower level of homology between tomato and potato as compared to previous workers (Bonierbale *et al*., 1988; Gebhardt *et al*., 1991; Tanksley *et al*., 1992). In order to obtain the PCR product, as required in microsatellite analysis, more homology between the species under study is required as compared to hybridization based techniques like RFLP. Thus, the previous estimates (Bonierbale *et al*., 1988; Tanksley *et al*., 1992) of homology between tomato and potato were higher than those of Ashkenazi *et al*. (2001) and the present study. Further, RFLP probe hybridization is biased towards transcribed regions of the genomes which usually show higher homology than the rest of the genomes. Likewise, Gupta and Varshney (2000) reported low frequency of homologous microsatellites between wheat, barley and rice as compared to that for RFLPs.

The same size alleles in wild germplasm may represent different electromorphs and thus might not be indicative of low levels of microsatellite mutation activities in potato. This is also possible due to the generation of new alleles and under some evolutionary pressures reverting back to previously occurring alleles. Thus, the overall mechanism could be directionless in the sense that it could increase or decrease the locus length within defined limits. Our *in silico* studies in members of solanaceae prove conservation of microsatellites between different species and within the defined size range (unpublished results). The same has also been proved by amplification of nearly 50 % of microsatellites in related genera. Thus, despite the directionlessness of microsatellite evolution and occurrence of only a few alleles in ecologically diverse materials, high levels of heterozygosity in the microsatellite regions suggest that microsatellites may be useful in detecting genetic differences between closely related taxa.

Segregation analysis in backcross population

STMS markers are expected to follow Mendelian inheritance (tetrasomic inheritance in case of tetraploid species). A given marker that is polymorphic between two parents can be genotyped in F_2 or backcross populations to establish its mode of inheritance. We have performed similar investigation using diploid backcross population consisting of 67 plants with complex phylogeny (Gebhardt *et al*., 1989). Out of the total fifty one primer pairs tested, twenty nine produced polymorphic bands between parents P9 and P16. Eight primer pairs did not amplify any locus in the parents, while fifteen pairs exhibited no polymorphism between the parental genotypes. Out of the 29 markers that showed polymorphism among parents, 13 segregated in the backcross population. Being a backcross population, F_1 i.e., BC916/2 was expected to be heterozygous for the microsatellite markers that displayed polymorphism

between parents. For five out of these thirteen primer pairs, parent P9 displayed heterozygous alleles, while P16 displayed homozygous single allele, and the F_1 (BC916/2) inherited the heterozygous allele or *vice versa*. Linkage analysis could be performed on 11 of the segregating loci that segregated in the expected ratios of 3:1 or 1:1. In these cases, where microsatellites showed polymorphism among parents, a heterozygous marker in P9 would be present in 50 % of the cases in BC916/2, creating a 3:1 ratio, if P16 is homozygous, else producing 1:1 ratio. A homozygous marker in P9 will always be present as heterozygous in BC916/2 and will segregate in 1:1 ratio, except when P16 is homozygous for the same marker. Similarly, a heterozygous marker in P16 would be present in 50 % of the cases in BC916/2 and would always segregate either in 1:1 or 3:1 (presence versus absence of the markers in progeny).

Two of the loci polymorphic in parents, did not follow the Mendelian ratio. That can be attributed to the alloploid nature of potato or the complex phylogeny of the parental lines.

Scope of microsatellite research in potato

Feingold *et al*. (2005) discussed the various aspects of microsatellite discovery using experimental approaches despite the availability of atleast two online resources (Solanaceae Genomics Network and potato SSR-containing ESTs) for molecular marker development in potato. As one of these (Solanaceae Genomcis Network) has indicated the map location of some of the microsatellites without actually testing these loci for polymorphism, and SSR-containing ESTs suffers with higher levels of redundancies, the scope for mining of microsatellites both from genomic as well as cDNA sequences is still promising. The existing genomic sequence resources for potato and other closely related species are useful resources for future microsatellite marker development efforts. The development of new microsatellite markers in potato during the present study has proved valuable for genome characterization and can also be effectively exploited in mapping and gene tagging studies in potato and related species.

ACKNOWLEDGEMENTS

Authors are thankful to Indian Council of Agricultural Research (ICAR) for providing financial assistance in the form of a research project. Authors wish to extend thanks to Dr. C. Gebhardt, Max-Planck-Institut fur Zuchtungsforschung, Koln, Germany for providing the backcross population, Prof. Akhilesh Tyagi (now Director, National Institute of Plant Genome Research, New Delhi), Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi, Dr. N K Singh, NRC on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi and Dr. P Ananda Kumar, NRC on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi for extending their help towards the completion of the study.

REFERENCES

Achenbach U, Paulo J, Ilarionova E, Lubeck J, Strahwald J, Tacke E, Hofferbert H-R and Gebhardt C (2009) Using SNP markers to dissect linkage disequilibrium at a major quantitative trait locus for resistance to the potato cyst nematode *Globodera pallida* on potato chromosome V. *Theor. Appl. Genet.,* 118 : 619-629

Ashkenazi V, Chani E, Lavi U, Levy D, Hillel J, and Veilleux RE (2001) Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. *Genome*, 44 : 50-62.

Bell CJ and Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics*, 19 : 137-144.

Bonierbale M, Plaisted RL and Tanksley SD (1988) RFLP maps of potato and tomato based on a common set of clones reveal modes of chromosomal evolution. *Genetics*, 120 : 1095-1103.

Bormann CA, Rickert AM, Ruiz RAC, Paal J, Lubeck J, Strahwald J, Buhr K and Gebhardt C (2004) Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. *Mol. Plant Microbe Interact.,* 17 : 1126-1138

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

D'hoop BB, Paulo MJ, Mank RA, van Eck HJ and van Eeuwijk FA (2008) Association mapping of quality traits in potato (*Solanum tuberosum* L.). *Euphytica*, 161 : 47-60.

Di Rienzo A, Paterson AC, Garza JC, Valdes AM, Slatkin M and Freimer NB (1994) Mutational processes of simple sequence repeat loci in human populations. *Proc. Natl. Acad. Sci. USA*, 91 : 3166-3170

Dognalar S, Frary A, Daunay MC, Lester RN, and Tanksley SD (2002a) A comparative linkage map of eggplant (*Solanum melanogena*) and its implications for genome evolution in the Solanaceae. *Genetics*, 161 : 1697-1711.

Dognalar S, Frary A, Daunay MC, Lester RN and Tanksley SD (2002b) Conservation of gene function in the solanaceae as revealed by comparative mapping of domestication traits in eggplant. *Genetics*, 161 : 1713-1726.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus,* 12 : 13-15.

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.,* 5 : 435-445.

Feingold S, Lloyd J, Norero N, Bonierbale M and Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). *Theor. Appl. Genet.*, 111 : 456-466.

Frary A, Dognalar S, Daunay MC and Tanksley SD (2003) QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of solanaceous species. *Theor. Appl. Genet.*, 107 : 359-370.

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Uhrig H and Salamani F (1989) RFLP analysis and linkage analysis in *Solanum tuberosum* L. *Theor. Appl. Genet.*, 78 : 65-75.

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachstschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD, and Salamani F (1991) RFLP maps of potato and their alignment with the homologous tomato genome. *Theor. Appl. Genet.*, 83 : 49-57.

Ghislain M, Spooner DM, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R and Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theor. Appl. Genet.*, 108 : 881-890.

Ghislain M, Nunez J, del Rosarion Herrera M, Pignataro J, Guzman F, Bonierbale M and Spooner DM (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. *Mol. Breeding,* 23 : 377-388.

Grover A and Sharma PC (2004) Occurrence of simple sequence repeats in potato ESTs is not random: An *in silico* study on distribution and length of simple sequence repeats. *Potato J.*, 31 : 95-102.

Grover A and Sharma PC (2007) Microsatellite motifs with moderate GC content are clustered around genes on *Arabidopsis thaliana* chromosome 2. *In Silico Biol.,* 7 : 201-213.

Grover A, Aishwarya V and Sharma PC (2007) Biased distribution of microsatellite motifs in the rice genome. *Mol. Genet. Genomics*, 277 : 469-480.

Grun P (1990) The evolution of cultivated potatoes. *Econ. Bot.,* 44 : 39-55.

Gupta PK and Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica,* 113 : 163-185.

Hawkes JG (1990) The Potato: Evolution, Biodiversity and Genetic Resources. Smithsonian Institution Press, Washington, D.C. pp 259.

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

Huaman Z and Spooner DM (2002) Reclassification of landrace populations of cultivated potatoes (*Solanum* Sect. *Petota*). *Am. J. Potato Res.*, 89 : 947-965.

Jacobs JME, van Eck HJ, Arens P, Verkerk-Bakker B, te Lintel Hekkert B, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. *Theor. Appl. Genet.*, 91 : 289-300.

Katti MV, Ranjekar PK and Gupta VS (2001) Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Mol. Biol. Evol.*, 18 : 1161–1167.

Kimura M and Crow J (1964) The number of alleles that can be maintained in a finite population. *Genetics,* 49 : 725-738.

Knapp S, Bohs L, Nee M and Spooner DM (2004) Solanaceae- a model for linking genomics with biodiversity. *Comp. Funct. Genom.,* 5 : 285-291.

Liu Z-W, Biyashev BM and Saghai-Maroof MA (1996) Development of simple sequence DNA markers and their integration into a barley linkage map. *Theor. Appl. Genet.*, 93 : 869-876.

Livingstone KD, Lackney VK, Blauth JR, Wijk RV and Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. *Genetics,* 152 : 1183-1202.

Mayton H, Griffiths H, Simko I, Cheng S, Lorenzen J, De Jong W and Fry WE (2009) Foliar and tuber late blight resistance in a *Solanum tuberosum* breeding population. *Plant Breed.*, doi:10.1111/j.1439-0523.2009.01671.x.

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C and Waugh R (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. *Mol. Gen. Genet.*, 259 : 233-245.

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

Nei M (1972) Genetic distance between populations. *Amer. Naturalist,* 106 : 283-292.

Ohta T and Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genet. Res.,* 22 : 201-204.

Peloquin SJ, Boiteux LS and Carputo D (1999) Meiotic mutants in potato: Valuable variants. *Genetics,* 153 : 1493-1499.

Powell W, Morgante M, Doyle JJ, McNicol JW, Tingey SV and Rafalski AV (1996) Genepool variation in genus *Glycine* subgenus *Soja* revealed by polymorphic nuclear and chloroplast microsatellites. *Genetics*, 144 : 793-803.

Provan J, Powell W and Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theor. Appl. Genet.*, 92 : 1078-1084.

Raker C and Spooner DM (2002) The Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: microsatellite data. *Crop Sci.*, 42 : 1451-1458.

Reid A, Hof L, Esselink D and Vosman B (2009) Potato cultivar genome analysis. *Methods Mol. Biol.*, 508 : 295-308.

Röder MS, Plaschke J, Koing SU, Borner A, Sorrells ME, Tanksley SD, and Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet*., 246 : 327-333.

Rohlf FJ (1992) Numerical taxonomy and multivariate analysis system version 2.0. State University of New York (Stony Brook, New York).

Saitou N and Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., 4 : 406-425.

Scott KD (2001) Microsatellties derived from ESTs, and their comparison with those derived by other methods. In: Henry RJ (ed) *Plant genotyping: the DNA fingerprinting of plants*. CAB International. Wallingford.

Schneider K and Douches DS (1997) Assessment of PCR-based simple sequence repeats to fingerprint North American potato cultivars. *Am. Potato J*., 74 : 149-160.

Selkoe KA and Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.,* 9 : 615-629.

Seyfert AL, Cristescu MEA, Frisse L, Schaack S, Thomas WK and Lynch M (2008) The rate and spectrum of microsatellite mutation in *Caenorhabditis elegans* and *Daphnia pulex*. *Genetics,* 178 : 2113-2121.

Sharma PC, Grover A and Kahl G (2007) Mining microsatellites in eukaryotic genomes. *Trends Biotechnol*., 25 : 490-498.

Spooner DM, Nunez J, Rodriguez F, Naik PS and Ghislain M (2005) Nuclear and chloroplast DNA reassessment of the origin of Indian potato varieties and its implications for the origin of early European potato. *Theor. Appl. Genet.,* 110 : 1020-1026.

Spooner DM, Nunez J, Trujillo G, Herrera MR, Guzman F and Ghislain M (2007) Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. *Proc. Natl. Acad. Sci. USA,* 104 : 19398-19403.

Tang J, Baldwin SJ, Jacobs JME, van der Linden CG, Voorrips RE, Leunissen JAM, van Eck H and Vosman B (2008) Large-scale identification of polymorphic microsatellites using an *in silico* approach. *BMC Bioinformatics*, 9 : 374 doi:10.1186/1471-2105-9-374.

Tanksley SD, Ganal MW, Prince JP, de-Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W and Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. *Genetics*, 132 : 1141-1160.

van Eck HJ, Jacobs JME, van Dijk J, Stiekema WJ and Jacobsen E (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis. *Theor. Appl. Genet.,* 86 : 295-300.

van Eck HJ, Jacobs JME, Stam P, Ton J, Stiekema WJ and Jacobsen E (1994a) Multiple alleles for tuber shape in diploid potato detected by qualitative and quantitative genetic analysis using RFLPs. *Genetics*, 137: 303-309.

van Eck HJ, Jacobs JME, Van den Berg PMMM, Stiekema WJ and Jacobsen E (1994b) The inheritance of anthocyanin pigmentation in potato (*S. tuberosum* L.) and mapping of tuber skin colour loci using RFLPs. *Heredity*, 73: 410-421.

Van Os H, Andrzejeweski S, Bakker E, Barrena I, Bryan GJ, Caromel B, Ghareeb B, Isidore E, de Jong W, van Koert P, Lefebvre V, Milbourne D, Ritter E, Rouppe van der Voort JNAM, Rousselle-Bourgeois F, van Vliet J, Waugh R, Visser RGF, Bakker J and van Eck HJ (2006) Construction of a 10,000-marker ultradense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genomewide physical map. *Genetics*, 173 : 1075-1087.

Varshney RK, Graner A and Sorrells ME (2005). Genic microsatellite markers in plants: features and applications. *Trends Biotechnol*., 23: 48-54.

Veilleux RE, Shen LY and Paz MM (1995) Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. *Genome*, 38 : 1153-1162.

Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC, Sahi S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ and Kahl G (1999) Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mol. Gen. Genet*., 262 : 90-101.