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Development of simple sequence repeats (SSRs) markers for identification of wild species and somatic hybrids of potato

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ABSTRACT

SSR markers were analysed to reveal allelic variations in wild species and interspecific somatic hybrids of potato. SSR allelic profiles showed high polymorphism and distinctness in the samples. A total of 131 alleles of 13 polymorphic SSR loci were scored in 48 samples of wild species and somatic hybrids of potato. Alleles per locus ranged from 6 (STM5127/ STM5114/ STM1053/ STI0030) to 20 (STM1052) whereas PIC value varied between 0.81 (STM5127/ STM5114) to 0.94 (STM1052) in the samples. This study suggests that SSR-based genotyping and development SSR markers dataset would strengthen their utilization in identification and molecular characterization of wild species and somatic hybrids of potato.

Key words: *Solanum tuberosum, Solanum* species, wild, molecular characterization.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world in terms of human consumption after rice and wheat. To ensure the global food and nutritional security in the current scenario of climate change, increasing population pressure and food demands, increasing potato production would be one of the plausible options. To achieve this, there is need to widen the narrow genetic base of the cultivated potato and also develop new varieties using wild and cultivated species (Chakrabarti *et al*., 3).

Potato belongs to a diverse gene pool of more than 200 *Solanum* species, which is a reservoir of various traits such as biotic and abiotic stress like late blight resistance, heat and drought tolerance (Bradshaw *et al.*, 1). A few species has been utilized in breeding and somatic fusion to develop interspecific somatic hybrids (Tiwari *et al.*, 12). Moreover, many useful genes derived from wild sources cannot be transferred through conventional technique because of sexual incompatibilities are primarily due to difference in ploidy and endosperm balance number (EBN). Hence, somatic hybridization provides means of bypassing sexual incompatibility between *Solanum* species and leads to fertile plants that can be used directly in breeding programs. We have demonstrated the development of interspecific somatic hybrids between *Solanum tuberosum* dihaploid 'C-13' (+) *S. pinnatisectum* (Sarkar *et al.*, 9) and 'C-13' (+) *S. cardiophyllum* for late blight resistance (Chandel *et al.*, 4), and 'C-13' (+) *S. etuberosum* for potato virus Y resistance (Tiwari *et al.*, 10). Hence, before the utilization of wild species in potato improvement, it is necessary to characterize them through molecular markers and then deploy them in breeding and biotechnology programme. Among the various markers used in crops, SSR is an excellent marker system to analyze closely related species due to its co-dominance, locus specific, reproducibility and capability of high-throughput genotyping nature (Provan *et al.*, 7). Earlier many researchers have used SSR markers to investigate diversity as well as other similar studies in potato (Tiwari *et al.*, 11, 13), development of potato genetic identity (PGI) kit of 24 SSR markers (Ghislain *et al.* 5).

The present study was undertaken to investigate allelic variations in wild species and somatic hybrids by SSR markers, and to develop markers for their identification.

MATERIALS AND METHODS

Wild species and interspecific potato somatic hybrids were available at ICAR-Central Potato Research Institute (CPRI), Shimla. The 48 samples used in this study were *viz.*, dihaploid C-13, *S. pinnatisectum*, *S. cardiophyllum*, *S. etuberosum*, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11, P12, P13, P14, E1-1, E1-2, E1-3, E1-4, E1-5, E2-1, E2-2, E2-3, E2-4, E2-6, E2-7, E4-1, E4-2, E6-1, E6-2, E6-3, E8, E10, E11, E12, E14-1, E15, E17-1, E17-2, Crd6, Crd7, Crd8, Crd10, Crd16 and Crd23 (Tiwari *et al.*, 10; Sarkar *et al.*, 9; Chandel *et al.*, 4). Plants were grown in the earthen pots (in triplicates) at the institute. Leaf tissues of these samples were used for DNA isolation using the DNeasy® Plant Mini Kit (Qiagen, Limburg, Netherlands). Fourteen SSR markers were used for polymerase chain reaction (PCR) amplification. The PCR reaction was performed in 20 µL volume

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with DNA template (100 ng) in 1× PCR buffer (2.5 mM/L $MgCl_2$ and 200 µM/L dNTP), each primer (0.5 µM/L), and *Taq* polymerase (1U) (Qiagen) following denaturation at 94 °C for 5 min; followed by 35 cycles of 94 °C for 45 sec, 51-60 °C for 45 sec, and 72 °C for 1 min; and final extension at 72 °C for 8 min in a Veriti Thermal Cycler (Life Technologies, CA, USA). The amplified SSR fragments were analysed with a 500-bp 'GS 500 ROX' standard on '3500 Genetic Analyzer' using GeneMapper® Software Version 4.1 (Applied Biosystems, CA, USA).

PCR reactions were repeated at least twice, and only reproducible, distinct, and scorable peaks (SSR fragments) across the run were considered for analysis. A data matrix of the samples was prepared on the basis of presence (1) or absence (0) of the fragments. Number of alleles, allele size, absolute frequencies and polymorphic information content (PIC) of markers were calculated. The PIC value of SSR markers was calculated according to the formula: $PIC = 1 - \sum (Pi^2)$, where *Pi* is the frequency of the *i*th allele of a marker detected in accessions (Nei, 6). A similarity matrix of SSR profiles was estimated based on the Jaccard's similarity coefficient. Cluster analysis was performed with unweighted pair-group method (UPGMA) using the software NTSYS-PC 2.21 (Rohlf, 8). Cluster tree (circular) was prepared using DARwin software using bootstrap value 100.

RESULTS AND DISCUSSION

Allelic profiles of 48 samples of wild species and interspecific potato somatic hybrids showed a high polymorphism by 13 SSR markers and one marker STI0012 did not show any amplification. A summary of SSR alleles and PIC values of the markers are presented in Table 1. SSR analysis showed a total of 131 alleles of 13 polymorphic SSR loci distributed in the samples studied. SSR alleles per locus ranged from 6 (STM5127/ STM5114/ STM1053/ STI0030) to 20 (STM1052) whereas PIC value varied between 0.81 (STM5127/ STM5114) to 0.94 (STM1052) in wild species and somatic hybrids. To illustrate, SSR allelic profiles is shown in Fig. 1 by STU6SNRN marker using the 3500 Genetic Analyzer (Applied Biosystems). A dataset of allelic profiles of the SSR markers is summarised in supplementary file. Total alleles counts of 13 SSR markers in all 48 samples were 2973. Sample P9 scored highest allele counts (77), whereas minimum count was observed in P14 (42). In particular, allele counts were as follow: dihaploid C-13 (67), *S. pinnatisectum* (49), *S. cardiophyllum* (56), *S. etuberosum* (44), P1 (70), P2 (64), P3 (62), P4 (56), P5 (45), P6 (73), P7 (61), P8 (60), P9 (77), P10 (68), P11 (46), P12 (67), P13 (59), P14 (42), E1-1 (65), E1-2 (72), E1-3 (64), E1-4 (65), E1-5 (64), E2-1 (64), E2-2 (62), E2-3 (67), E2-4 (66), E2-6 (71), E2-7 (64), E4-1 (48), E4-2 (63), E6-1 (76), E6-2 (61), E6-3 (60), E8 (76), E10 (61), E11 (72), E12 (64), E14-1 (57), E15 (56), E17-1 (70), E17-2 (68), Crd6 (66), Crd7 (49), Crd8 (54), Crd10 (56), Crd16 (72) and Crd23 (54). Cluster analysis of wild species and somatic hybrids distinguished all the samples using 13 SSR markers (Fig. 2).

Table 1. Summary of SSR alleles and PIC values analysed in wild species and somatic hybrids of potato.

SN	SSR marker	Chr.	SSR alleles #	SSR allele size (bp)	PIC
1.	STM5127	1	6	231, 236, 241, 247, 251, 254	0.81
2.	STM5114	2	6	279, 287, 293, 296, 300, 303	0.82
3.	STM1053	3	6	161, 165, 167, 170, 172, 176	0.81
4.	STPoAc58	5	13	140, 144, 147, 150, 157, 163, 186, 192, 195, 219, 228, 235, 240	0.91
5.	STM0019	6	11	98, 113, 135, 140, 152, 171, 189, 192, 202, 205, 209	0.85
6.	STM0031	7	10	73, 81, 92, 123, 139, 149, 155, 167, 176, 184	0.84
7.	STM1104	8	8	146, 152, 159, 162, 165, 168, 172, 175	0.85
8.	STM1052	9	20	109, 119, 126, 141, 148, 153, 164,w 179, 184, 189, 195, 201, 207, 220, 226, 242, 246, 248, 250, 256	0.94
9.	STM1106	10	11	96, 103, 111, 131, 135, 139, 146, 150, 153, 156, 159	0.90
10.	STM0037	11	8	49, 64, 68, 73, 75, 82, 85, 87	0.85
11.	STI0030	12	6	64, 74, 85, 92, 101, 105	0.82
12.	STIKA	-	16	175, 183, 185, 192, 195, 199, 202, 210, 221, 224, 226, 229, 232, 235, 237, 248	0.91
13.	STU6SNRN	-	10	170, 175, 179, 182, 187, 190, 195, 199, 202, 209	0.88
	Total	-	131		-

PIC: Polymorphic information content; No amplification was observed in SSR marker STI0012.
[a] SSR repeat motifs are described in Ghislain *et al*., 5.

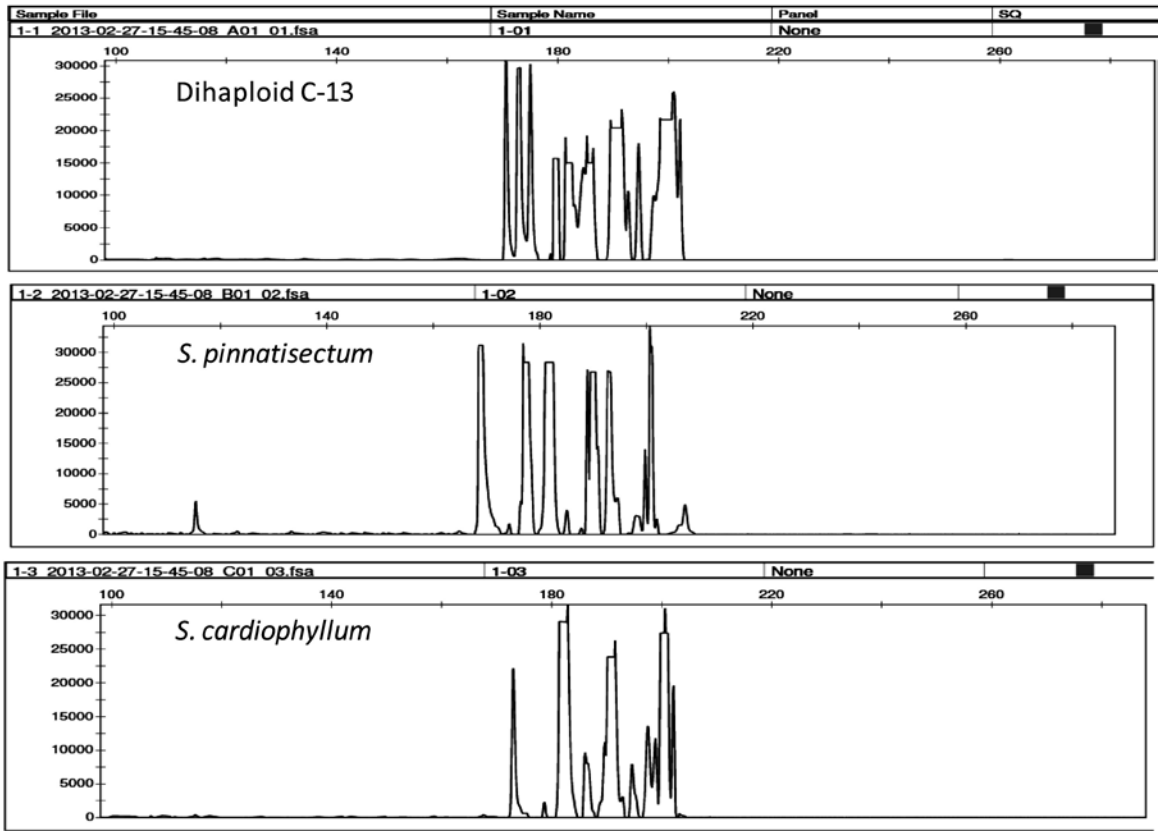

Fig. 1. SSR allelic profiles of wild species and dihaploid C-13 by STU6SNRN marker on '3500 Genetic Analyzer' (Applied Biosystems).

The SSR markers showed high polymorphism based on the PIC (0.81-0.94) distributed across the 13 SSR loci in wild species and interspecific somatic hybrids of potato. Our findings are congruent with the earlier studies using SSR in potato genetic variation analysis (Ghislain *et al.*, 5; Provan *et al.*, 7), validation of Andigena core collection (Tiwari *et al.*, 13) and so on. The SSR studies have shown good discrimination power and polymorphism in closely related potato genotypes for varietal identification (Tiwari *et al.*, 11), allelic diversity analysis in potato landraces (Carputo *et al.*, 2). Further, researchers have suggested that SSR markers are a useful tool to investigate genetic variation in closely related taxa of potato (*Solanum tuberosum* subsp. *tuberosum* and subsp. *andigenum*) due to allelic polymorphism and high degree of heterozygosity in microsatellite regions (Provan *et al.*, 12). Allelic profiles in terms of size, number and absolute frequencies observed in our study had little deviations in comparison with earlier findings analysis (Ghislain *et al.*, 5; Provan *et al.*, 7). Probably this could be due to the equipment and software technologies used to analyse SSR fragments. We employed a high-throughput machine having high precision i.e. '3500 Genetic Analyzer', whereas earlier workers might have used semi-automated gel-based machines to score the SSR fragments. Moreover, we have already used the '3500 Genetic Analyzer' system for molecular characterizations of our interspecific somatic hybrids (Sarkar *et al.*, 9; Chandel *et al.*, 4). Previously, we have observed high level of allelic diversity (4 to 35 alleles per SSR locus) with a range of PIC value (0.53 to 0.92) by SSR markers in Indian potato varieties (Tiwari *et al.*, 11). Cluster analysis in this study using 13 SSR markers reflected genetic distinctness in wild species and somatic hybrids. Thus, above studies show the versatility of the SSR system for molecular characterization of potatoes.

To conclude, allelic profiling of wild potato species by 13 polymorphic SSR markers developed in this study would be an important resource for potato improvement. Further, these wild species and interspecific somatic hybrids are one of the key genetic resources of potato for identification of resistant genes for biotic and abiotic stresses. Further, study should be focused on identification SSR alleles linked to desirable trait and their real deployment in potato improvement applying breeding and biotechnological tools.

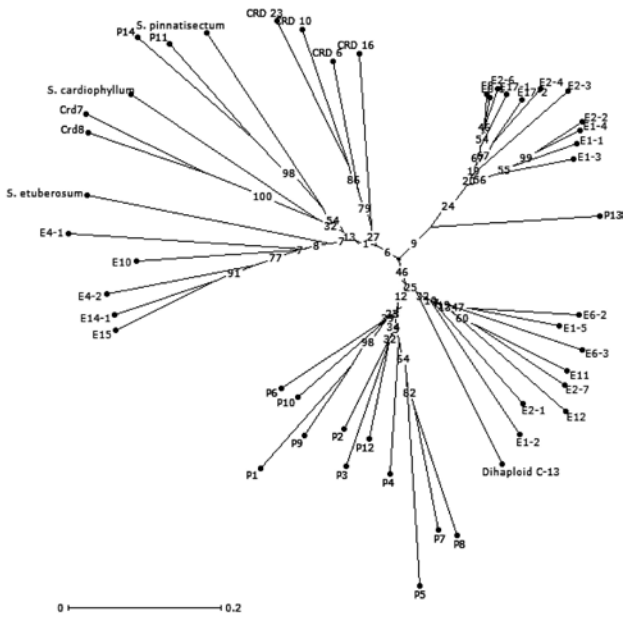

Fig. 2. Cluster analysis of wild species and somatic hybrids samples based on the Jaccard's coefficient by unweighted Neighbor-Joining method using 13 SSR markers. Bootstrap values are shown on the nodes.

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