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Molecular profiling of multi-coloured flesh potato (*Solanum tuberosum*) hybrids and interspecific somatic hybrids using SSR markers

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ABSTRACT

The objective of this study was to develop SSR profiles of potato (*Solanum tuberosum* L.) hybrids for genetic fidelity purpose. The multi-coloured flesh potato hybrids and interspecific somatic hybrids-derived progenies were for the study conducted at ICAR-CPRI, Shimla during 2019–20. A total of 165 potato genotypes were analysed using two well-known potato SSR markers (STU6SNRN and STIIKA). High polymorphism was observed in STIIKA (PIC: 0.93) than STU6SNRN (PIC: 0.82), and higher number of alleles were observed in STIIKA (23) than STU6SNRN (7). In STU6SNRN, alleles size 174, 179, 182, 190 and 200 bp were predominant whereas in STIIKA, alleles size 191, 195, 198, 201, 221, 223, 231, 242, 245 and 256 were observed frequently in more than 50% of the genotypes. Diversity analysis showed a clear distinction among the genotypes based on the Jaccard dissimilarity coefficient by the Neighbour-joining tree method using the DARwin software. SSR fingerprints would be valuable resources to strengthen genetic fidelity of these hybrids and identification of true-to-type clones.

Keywords: Genetic fidelity, Multi-coloured potato, Somatic hybrids, SSR markers.

Potato (*Solanum tuberosum* L.) is the third most essential food crop after rice and wheat. Potato yields high edible energy, protein and dry matter per unit area and time due to its high protein-calorie ratio and short crop duration. Potato is a rich source of carbohydrates, dietary fibres, ascorbic acid, thiamine, niacin, pantothenic acid and riboflavin, and is low in fat. It also contains a variety of beneficial compounds like phytonutrients, antioxidants, anthocyanins, carotenoids, flavonoids, caffeic acid, and tuber storage protein patatin which functions against free radicals (Brown 2005). Studies demonstrate that multi-coloured potatoes with red/purple/yellow flesh colours have various health benefits on account of richness of antioxidants and micronutrients like Fe and Zn (Dalamu *et al.* 2014, Luthra *et al.* 2018). With the realization of several health advantages of coloured potatoes, now focus has been reoriented to develop multi-colour potato varieties. The genus *Solanum* is a reservoir of genetic resources and provides a vast opportunity for potato improvement. The utilization of potato genetic resources is necessary to widen its genetic base by developing new varieties through breeding and biotechnological methods (Bradshaw *et al.* 2006). Of the available technologies, protoplast fusion is one such technique to use non-crossable wild species to harness the tertiary gene pool in genetic enhancement of the cultivated potato. Somatic hybrids, to widen the narrow genetic base of cultivated potato, have been developed at the institute (Tiwari *et al.* 2018b) such as *Solanum tuberosum* dihaploid 'C-13' (+) *S. pinnatisectum* (Sarkar *et al.* 2011), and 'C-13' (+) *S. cardiophyllum* (Chandel *et al.* 2015) for late blight resistance, and 'C-13' (+) *S. tuberosum* for potato virus Y resistance (Tiwari *et al.* 2010).

Genetic fidelity of potato is crucial to ensure true-to-type clone in the varietal improvement programme. A wide range of molecular markers have been used in potato for molecular characterization and genetic fidelity testing. Among them, simple sequence repeat (SSR) is easy-to-use, reproducible, locus-specific, co-dominant and polymorphic (Provan *et al.* 1996). Numerous researchers have used SSR in characterization of potato species (Ghislain *et al.* 2009), somatic hybrids (Chandel *et al.* 2015), varietal identification (Tiwari *et al.* 2018a), wild species (Tiwari *et al.* 2019) and genetic diversity (Provan *et al.* 1996). Hence, the aim of this study was to carry out DNA fingerprinting of hybrid progenies of different potatoes (multi-coloured flesh hybrids and interspecific somatic hybrids) by SSR markers for clonal identity and genetic fidelity purpose.

MATERIALS AND METHODS

Plant material: A total of 165 genotypes (84 multi-colour flesh potato hybrids and 81 interspecific potato

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somatic hybrids) including parents and progenies were used for SSR analysis at ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh during 2019–20. Sample details are mentioned in Table 1. Multi-coloured flesh potato hybrids were adapted from tuber traits like yield, tuber number, tuber skin colour, flesh colour, eye depth, general impression, tuber dry matter as described by Luthra *et al.* (2018). Interspecific potato somatic hybrids were investigated for tuber and other traits by Tiwari *et al.* (2018c). Leaf samples were collected from aeroponic and field-grown plants at ICAR-CPRI, Shimla, and some were from ICAR-CPRI, Regional Stations, Kufri and Modipuram.

SSR analysis: DNA was isolated from the leaf tissues of 165 genotypes using DNeasy Plant Mini kit (Qiagen, the Netherlands) and quality was checked on agarose gel (1%) and NanoDrop (Thermo Fisher Scientific, USA). FAM

Table 1 Genetic material used in the study

Genotype	Total number	Parents
Multi-coloured flesh tuber potato hybrid progenies		
MSP/15-1 to MSP/15-64	64	Bareilly Red × CP3770
Bareilly Red	1	A local collection (Bareilly, UP)
CP-3770	1	CIP 393280.64 (Lima, Peru)
MSP/16-4	1	CP4242 x CP2340
MSP/16-150, 216	2	K. Chipsona 1 × CP4242
MSP/16-272	1	K. Himsona × K Chipsona-3
MSP/16-300, 307, 375	3	MS/8-1148 × CP4242
MSP/17-007	1	CP4042 × K. Chipsona-3
MSP/17-089	1	K. Lauvkar × CP4242
MSP/17-147, 212	2	MP/04-816 × CP4242
MSP/17-341, 345	2	K Frysona × CP4242
MSP/17-532, 537	2	K Himsona × CP4242
JEX/A-122	1	*S. tuberosum* Gp. Andigenum
Kala Aaloo	1	A local collection (Lahul Spiti, HP)
CP 4242	1	Bora Valley (USA)
Sub-Total (A)	84	
Interspecific potato somatic hybrid progenies		
MSH/14-4, 5, 7, 17, 18, 22, 23, 32	8	Kufri Garima × Bulk pollen
MSH/14-57, 58, 60, 69	4	Kufri Jyoti × Bulk pollen
MSH/14-81, 85, 87, 88, 89, 90, 91, 92, 96, 97, 98, 100, 103, 104, 105, 109	16	Kufri Sadabahar × Bulk pollen
MSH/14-112, 113, 114, 115, 116, 122, 123, 126	8	P8 × Kufri Jyoti
MSH/14-128, 129, 131, 135, 137, 140, 141, 142, 143, 144, 145, 148, 151, 152	14	Kufri Gaurav × Interspecific somatic hybrid 'P2'
MSH/14-153, 159, 167, 170	4	Kufri Gaurav × Interspecific somatic hybrid 'P3'
MSH/14-172	1	Kufri Gaurav × Interspecific somatic hybrid 'P7'
MSH/14-176 and 181	1	Kufri Gaurav × Interspecific somatic hybrid 'P8'
MSH/17-16 and 25	2	Kufri Garima × Interspecific somatic hybrid 'Crd10'
C-13	1	Androgenic dihaploid of *Solanum tuberosum* cv. Kufri Chipsona-2
CPH	1	Diploid wild potato species (*S. cardiophyllum*)
Crd6, 10, 16, 23	4	Interspecific potato somatic hybrid (Dihaploid C-13 + *S. cardiophyllum*)
P1-12	12	Interspecific potato somatic hybrids (Dihaploid C-13 + *S. pinnatisectum*)
Kufri Sadabahar, Kufri Gaurav, Kufri Jyoti, Kufri Garima, Kufri Pukhraj	5	Common potato varieties
Sub-total (B)	81	
Total (A+B)	165	

Table 2 SSR markers polymorphism used in fingerprinting of potato progenies

SSR marker	SSR motifs	Sequence (5'→ 3')	Alleles	Alleles size (bp) (allele frequency)	PIC
STU6SNRN	$(TGG)_5$	F: GAAGTTTTATCAGAATCC R: ATCACCTCATCAGCAATC	7	171 (26), 174 (105), 179 (145), 182 (164), 190 (165), 200 (164), 206 (24)	0.82
STIIKA	$(T)_{12}(A)_9ATTCTTGTT(TA)_2$ $CA(TA)_7$	F: TTCGTTGCTTACCTACTA R: CCCAAGATTACCACATTC	23	121 (7), 133 (7), 137 (5), 152 (8), 154 (7), 157 (3), 186 (54), 191 (84), 195 (144), 198 (102), 201 (93), 219 (47), 221 (107), 223 (108), 225 (57), 231 (118), 235 (68), 242 (92), 245 (107), 254 (25), 256 (96)	0.93

PIC: Polymorphic Information Content; Allele frequency value in parenthesis indicates number of samples in which allele was observed.

labelled two well-characterized SSR markers (STU6SNRN and STIIKA) in potato (Tiwari *et al.* 2018a, 2019) were used for DNA fingerprinting. Polymerase chain reaction (PCR) included a total of 10 µl volume having 100 ng DNA, 1 µl (10 pM) each primer (forward and reverse), 1 U Taq polymerase, PCR buffer with 2.5 mM $MgCl_2$ and 200 µM dNTP and Milli-Q water (Qiagen). The PCR cycles included denaturation at 95°C/5 min; 35 cycles of 94°C/45 sec, annealing at 55°C /45 sec, and 72°C for 1 min; and extension at 72°C/7 min in GeneAmp PCR System (Applied Biosystems, CA, USA). The amplified PCR products were analysed with a 500-bp 'GS 500 ROX' standard using '3500 Genetic Analyzer' with GeneMapper® Software Version 4.1 (Applied Biosystems, CA, USA).

Scoring and data analysis: All reactions were repeated twice and distinct SSR peaks were scored for 165 genotypes and analysed as described by Tiwari *et al.* (2019). Briefly, a datasheet was prepared in the form of presence (1) and absence (0) of SSR alleles. SSR polymorphism in terms of the number of alleles, allele size and polymorphic information content (PIC) was determined using the formulae given by Nei (1973).

$$PIC = 1 - \Sigma (P^2_i)$$

where, P_i is the frequency of the i^{th} allele of a marker.

Cluster analysis was performed based on the Jaccard coefficient with the Neighbour-Joining method using the DARwin software (bootstrap value = 100).

RESULTS AND DISCUSSION

A total of 165 genotypes along with progenies and parents of multi-coloured flesh tubers (84) and interspecific somatic hybrids (81) of potato were analyzed using SSR markers STU6SNRN and STIIKA (Table 1). DNA fingerprints were generated using high-throughput and high resolution equipment "3500 Genetic Analyzer" (Applied Biosystems Instruments). SSR polymorphism is presented in Table 2. Marker STU6SNRN showed 7 alleles (171, 174, 179, 182, 190, 200 and 206) with PIC value 0.82, whereas STIIKA amplified 23 alleles (121, 133, 137, 152, 154, 157, 186, 191, 195, 198, 201, 219, 221, 223, 225, 231, 235, 242, 245, 254 and 256) with higher PIC value (0.93). In the multi-coloured flesh tuber potato progenies, SSR alleles ranged between 10 (MSP/15-6) to 18 (MSP/15-4, MSP/15-18 andMSP/15-34), whereas in the somatic hybrid progenies it ranged from 2 (MSH/14-91) to 22 (MSH/14-116). Total allele count of both markers in the genotypes was 2166. SSR allelic profiles of these selected hybrids are also shown Fig 1. A data matrix of SSR alleles for all the progenies and parents was prepared for further analysis. Genetic diversity analysis of all the hybrids based on the Jaccard dissimilarity index by weighted Neighbor-Joining tree construction method using DARwin software showed distinctness among the genotypes specially progenies (Supplementary Fig 1). In multi-coloured flesh potato hybrids, minimum dissimilarity value was 0.055 while maximum value was 0.75. Whereas, in the case of interspecific potato somatic hybrids minimum dissimilarity value was 0.052 and the maximum value was 0.92.

In this study, two types of potato progenies were used for SSR fingerprinting. First, multi-coloured flesh tuber potato hybrid progenies that are rich in antioxidants like anthocyanin and carotenoid, and micronutrients like Fe and Zn (Dalamu *et al.* 2014, Luthra *et al.* 2018). Second, interspecific potato somatic hybrid progenies that are the product of hybridization between somatic hybrids (generated via protoplast fusion between cultivated and wild species) and common potato varieties. These interspecific somatic hybrids have a wider genetic base and possess high resistance to late blight. These results support findings of SSR analysis in potato by various workers. Previously, we have well-characterized these markers in allelic profiling of potato varieties and wild species, and also for genetic fidelity testing of *in vitro* propagated plants (Tiwari *et al.* 2018a, 2019). Many SSR markers have been applied in characterization of potato germplasm (Ghislain *et al.* 2009, Provan *et al.* 1996), Indian potato varieties (Tiwari *et al.* 2018a), wild potato species (Tiwari *et al.* 2019), Andigena core collection, somatic hybrids (Chandel *et al.* 2015). Previous SSR work shows that SSR is one of the best markers for characterization of potatoes of cultivated and wild species (Provan *et al.* 1996). Minor variation in allele size could be possible due to software used to score SSR alleles.

In the recent years, unlike white-fleshed tuber, multi-coloured flesh potatoes have been given attention because they are beneficial for human health and rich in antioxidants like anthocyanin, carotenoids and other pigments (Brown *et*

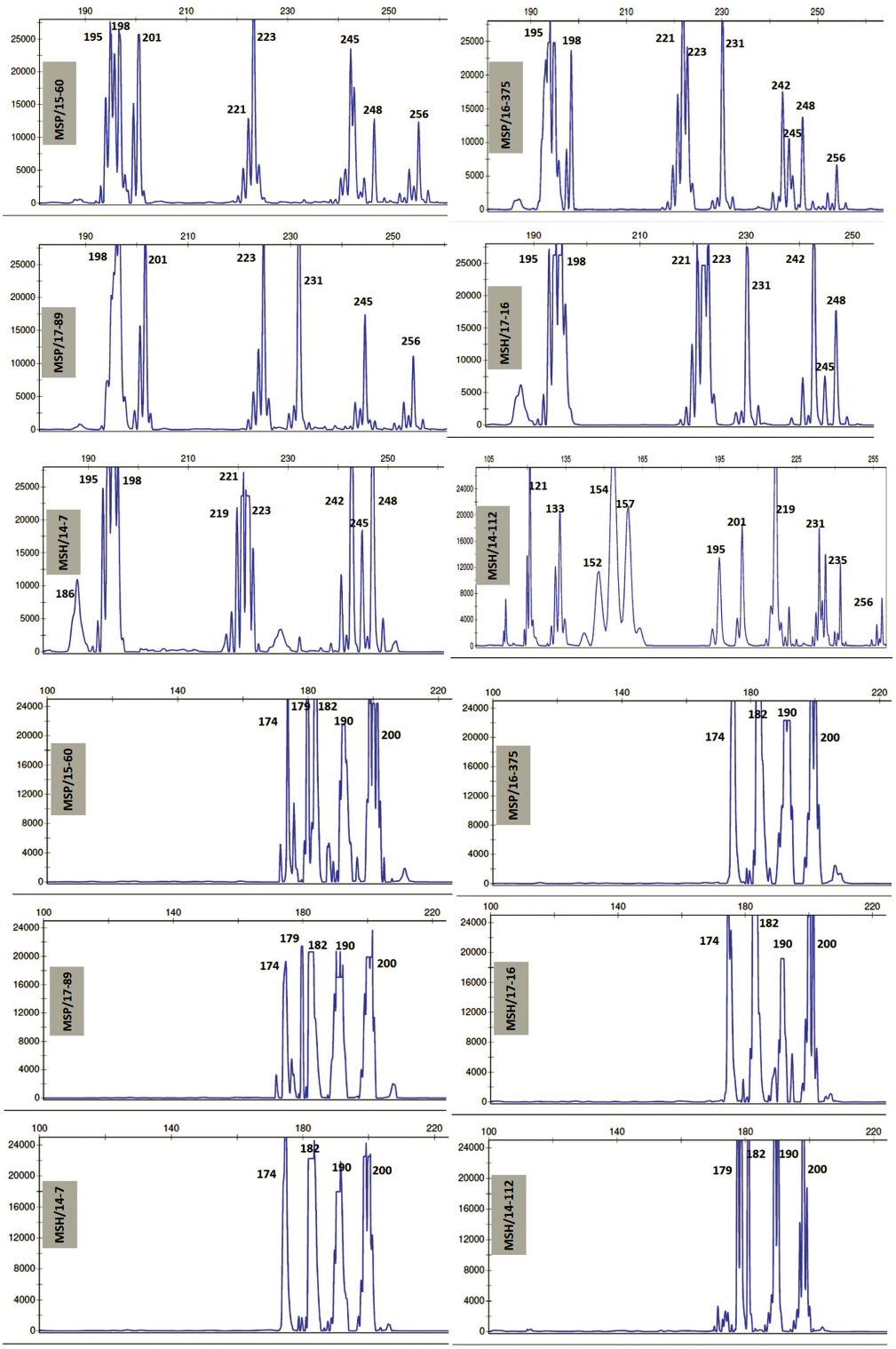

Fig 1 (a) SSR (STIIKA) profile of advance hybrids, viz. MSP/15-60, MSP/16-375, MSP/17-89, MSH/17-16, MSH/14-7 and MSH/14-112. (b) SSR (STU6SNRN) profile of hybrids, viz. MSP/15-60, MSP/16-375, MSP/17-89, MSH/17-16, MSH/14-7 and MSH/14-112. Value on peaks indicates SSR allele size (bp).

al. 2008). Also, such types of potatoes are being preferred by processing industries. Earlier, a set of progenies of multi-coloured flesh tuber hybrids (progenies: MSP/15-1 to 64; parents: Bareilly Red and CP3770) were evaluated for nutritional components (anthocyanin, carotenoids, ascorbic acid, iron and zinc), yield traits and molecular markers (ISSR and SSR) (Luthra *et al.* 2018). They concluded that these potato hybrids are rich in these health beneficial compounds. These nutritionally superior hybrids would be fulfilling nutritional requirements of people who are under-nourished. Further, they identified diagnostic SSR marker (STM2005), which can distinguish the parents Bareilly Red and CP3770 and their progenies (Luthra *et al.* 2018). In addition, here SSR fingerprints of all the progenies using very robust and polymorphic SSR markers (STU6SNRN and STIIKA) were developed for the use in genetic fidelity. For example, a few selected hybrids are shown in Fig 1, which could be released as new potato varieties in future after assessing their performance in multi-location trails. Besides, these hybrids have the potential to be used as parental lines in breeding to develop further new hybrids. These SSR fingerprints would be required to resolve genetic fidelity related issues during multi-location testing across the country. The SSR fingerprints of these hybrids would be helpful in characterizing the advance hybrids for identification of clone specific SSR alleles linked to particular traits.

Solanum species has one of the richest genetic resources in plants and has immense potential to widen the genetic base of cultivated potato by using non-crossable wild species. Earlier, we developed interspecific potato somatic hybrids by protoplast fusion between *Solanum tuberosum*

dihaploid 'C-13' and wild *S. pinnatisectum* for very high resistance to late blight and broad genetic base (Sarkar *et al.* 2011). The diploid wild species are not crossable with cultivated tetraploid potato due to the difference in ploidy and endosperm balance number. Further, we hybridized these interspecific somatic hybrids with Indian potato varieties to develop new potato genotypes with resistance to late blight and broad genetic base. As a result, we obtained several segregating progenies with desired tuber traits, and some hybrids were molecular characterized by ISSR markers (Tiwari *et al.* 2018c). Currently, we have developed advanced stage of these interspecific potato somatic hybrids with desirable tuber traits, late blight resistance and a wider genetic base. Based on the field potential, we have further selected promising somatic hybrids clones with high yield, resistance to late blight, dry matter and tuber traits, which have produced elite progenies (Luthra *et al.* 2016). A few advanced stage hybrids could be released as new potato varieties in future after multi-location testing. Though worldwide several researchers have reported field evaluation of potato somatic hybrids (Caruso *et al.* 2008), yield, quality, late blight and potato virus Y resistance traits (Thieme *et al.* 2008), and late blight resistance (Smyda-Dajmund *et al.* 2017). However, few have reached to advanced stage of clonal selection, as we have achieved. In this study, we have developed detailed SSR fingerprints of these somatic hybrids progenies, which would be useful to address the issues related to genetic fidelity of the hybrids at the time of release as varieties. In future, these fingerprints would be helpful in identification of trait-specific alleles in the hybrids.

SSR fingerprints of advanced stage potato hybrids (multi-coloured flesh tuber hybrids, and interspecific somatic hybrids) have been developed. Both segments of hybrids have particular advantages like rich in nutritional component and health benefits in the former one, while the latter interspecific somatic hybrids have high resistance to late blight and a broader genetic base. The SSR fingerprints developed in this study would strengthen their genetic fidelity during their release as new potato varieties. DNA fingerprinting of these hybrids would also implicate identification of true-to-type clones of these hybrids in varietal development program in the country.

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