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Identification of simple sequence repeat (SSR) markers linked to interspecific potato somatic hybrids

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Potato belongs to the genus *Solanum*, which is one of the richest genetic resources in plants, of which only small fraction has been utilized by breeding and biotechnological methods (Chakrabarti *et al.* 2017). Of them, somatic hybridization is one of the potential method to utilize non-crossable wild species to harness the tertiary genepool into cultivated potato (Tiwari *et al.* 2018b). The sexual incompatibility between wild species and cultivated species is caused by the difference in endosperm balance number and ploidy number. Hence, somatic hybridization has been proven in potato improvement. We have demonstrated successful development of interspecific somatic hybrids between *Solanum tuberosum* dihaploid C-13 and *S. pinnatisectum* (Sarkar *et al.* 2011); C-13 and *S. cardiophyllum* for late blight resistance (Chandel *et al.* 2015); and C-13and *S. etuberosum* for potato virus Y resistance (Tiwari *et al.* 2010). This has enabled us to widen the genetic base of cultivated potato by utilizing these somatic hybrids in breeding as parental lines to develop new varieties. Further, somatic hybrids have been evaluated in the field for various traits and promising clones were selected for improvement through breeding (Luthra *et al.* 2016), and advance stage hybrids have been developed using these somatic hybrids (Tiwari *et al.* 2018c). Besides, several other potato somatic hybrids have been produced during the past four decades (Tiwari *et al.* 2018b).

Although, conventional breeding method has played a pivotal role in potato improvement, inclusion of molecular markers for identification and genetic fidelity testing is essential. Among several molecular markers, SSR is an easy-to-use, reproducible, locus-specific, co-dominance and highly polymorphic in nature (Provan *et al.* 1996). SSR markers have been used in potato for various applications; like molecular characterization of cultivated/semi-cultivated/ wild potato species (Ghislain *et al.* 2009), wild species (Tiwari *et al.* 2019), somatic hybrids (Chandel *et al.* 2015), varietal identification (Tiwari *et al.* 2018a), and genetic diversity (Provan *et al.* 1996). Hence, the aim of this study was to develop diagnostic SSR markers for identification of advance stage potato hybrids/progenies developed using interspecific somatic hybrids and common potato varieties.

A total of 50 potato genotypes were used in the present investigation (2018–19) for SSR marker analysis at ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh. The genotypes were CPH, Kufri Bahar, Kufri Garima, Kufri Jyoti, Kufri Pukhraj, Kufri Sadabahar, Crd6, Crd10, Crd16, P1, P6, P8, P10, MSH/17-1 and MSH/17-2 (Crd 6 × Crd6), MSH/17-4 to MSH/17-6 (Crd 10 × P1), MSH/17-7 to MSH/17-13 (Kufri Bahar × P8), MSH/17-14 to MSH/17-15 (Kufri Garima × Crd6), MSH/17-16 (Kufri Garima × Crd10), MSH/17-17 to MSH/17-21 (Kufri Garima × P8), MSH/17-22 to MSH/17-25 (Kufri Garima × P10), MSH/17-26 to MSH/17-27 (Kufri Jyoti × Crd 16), MSH/17-28 to MSH/17-29 (Kufri Jyoti × P8), MSH/17-31 to MSH/17-33 (Kufri Jyoti × P6), and MSH/14-112 to MSH/14-116, MSH/14-112 and MSH/14-123 (P8 × Kufri Jyoti). Leaf samples were collected from the field grown plants from ICAR-CPRI, Regional Station, Kufri, Shimla. Leaf samples of 50 parents/progenies were analysed using 59 simple sequence repeat (SSR) markers. The annealing temperature was 55°C for the SSR markers (STG0016, STM0001, STM0003, STM0004, STM0007, STM0010, STM0011, STM0013, STM0015, STM0017, STM0019, STM0023, STM0031, STM0032, STM0037, STM0046, STM1001, STM1003, STM1007, STM1024, STM1036, STM1041, STM1043, STM1046, STM1052, STM1057, STM1058, STM1064, STM1069, STM1097, STM2002, STM2003, STM2004, STM2005, STM2020, STM2021, STM2023, STM2026, STM3003, STM3012, STM3018, STM51217), except some STG0025 (56°C), STI0001 (60°C), STI0003 (60°C), STI0004 (60°C), STI0012 (56°C), STI0014 (54°C), STI0030 (58°C), STI0032 (61°C),

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Table 1 Allelic profile of diagnostic SSR markers for identification of progenies of potato somatic hybrids

SSR marker	SSR motif	Sequence (5'→3')	Ta (°C)	Allele size (bp)	PIC
STM0003	(AC)9 (AT)9	F:GGAGAATCATAACAACCAG R:AATTGTAACTCTGTCTGTGTG	55	103, 132, 144	0.55
STI0001	(AAT)n	F: CAGCAAAATCAGAACCCGAT R: GGATCATCAAATTCACCGCT	60	169, 172, 175, 178, 184, 188	0.83

Samples for STM0003: P8, Kufri Jyoti, MSH-14/112 to MSH-14/116, MSH-14/122 and MSH-14/123. Samples for STI0001: Kufri Jyoti Kufri Garima, Card-10, Card-16, P 10, MSH/17-16, MSH/17-25, and MSH/17-27.

STI0033 (61°C), STG0001 (58°C), STG0010 (60°C), STM1053 (53°C), STM1104 (53°C), STM1106 (51°C), STM5114 (60°C), STM5121 (50°C) and STPoAC58 (57°C) (Provan *et al.* 1996, Ghislain *et al.* 2009). DNA isolation was performed using DNeasy Plant Mini kit (Qiagen, the Netherlands) and quality was checked on agarose gel (1%) and NanoDrop (Thermo Fisher Scientific, USA). Polymerase chain reaction (PCR) included total of 10 µl 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 (except above) 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 resolved on high resolution agarose gel (3%). Of which only selected SSR markers were synthesized with FAM labelled and PCR amplified. The amplified PCR products 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 twice and only reproducible and distinct SSR markers were selected.

A total of 59 SSR markers were analyzed in total 50 parents and progenies developed by crossing interspecific potato somatic hybrids and common varieties. The PCR products were checked on high-resolution agarose gel (3%) to visualize polymorphic bands in the parents and progenies. However, only two SSR markers (STM0003 and STI0001) were identified as diagnostic SSR marker for the identification of the parents and progenies. Alleles of STM0003 were 103, 132 and 144 bp, whereas alleles of

Table 2 SSR markers allelic profile in advance stage hybrids of somatic hybrid progenies

Genotype	SSR allele size (bp)						Allele no.
	103	132	144				
a) STM0003							
1. P8	1	0	1		2		
2. Kufri Jyoti	0	1	1		2		
3. MSH-14/112	1	0	1		2		
4. MSH-14/113	1	0	1		2		
5. MSH-14/114	1	0	1		2		
6. MSH-14/115	1	0	1		2		
7. MSH-14/116	1	0	1		2		
8. MSH-14/122	1	0	1		2		
9. MSH-14/123	1	0	1		2		
Allele no.	8	1	9		18		
b) STI0001	169	172	175	178	184	188	
1. Kufri Jyoti	1	1	1	1	1	1	6
2. Kufri Garima	0	1	1	1	1	1	5
3. Crd10	1	1	1	1	1	0	5
4. Crd16	1	1	0	1	1	1	5
5. P10	1	1	1	0	1	1	5
6. MSH/17-16 (Kufri Garima × Crd10)	0	1	1	1	1	1	5
7. MSH/17-25 (Kufri Garima × P10)	1	1	1	1	1	1	6
8. MSH/17-27 (Kufri Jyoti × Crd 16)	0	0	0	1	1	1	3
Allele no.	5	7	6	7	8	7	40

(Presence: 1; Absence: 0)

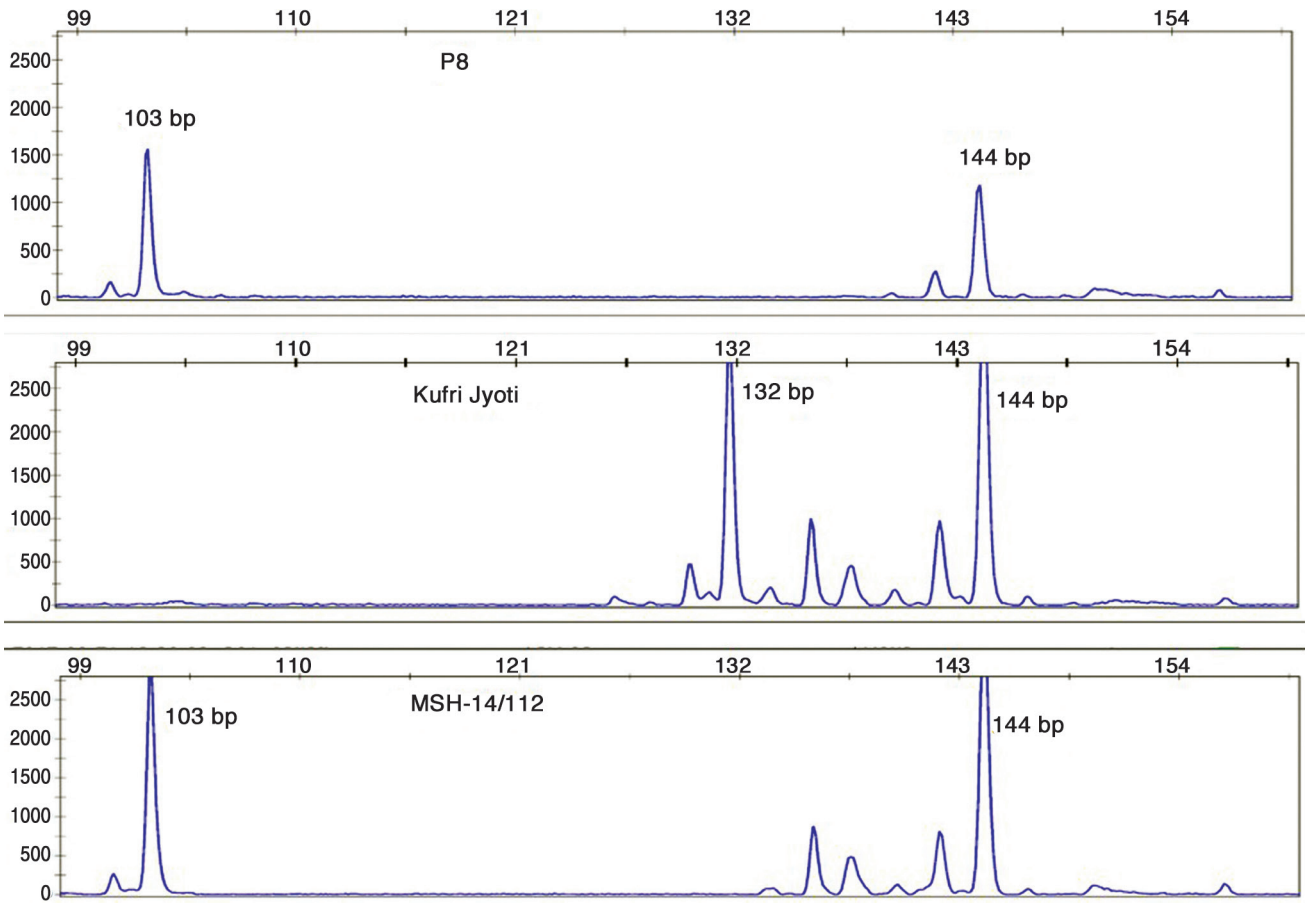

Fig 1 FAM labelled SSR marker (STM0003) profile showing distinct markers in parents (somatic hybrid: P8, and potato cv. Kufri Jyoti) and their progeny (MSH/14-112).

STI0001 marker were 169, 172, 175, 178, 184 and 188 bp (Table 1). PIC value of STM0003 and STI0001 markers was 0.55 and 0.83, respectively. The SSR profile of STM0003 showed distinct polymorphism in another set of parents (P8 and Kufri Jyoti) and progenies (MSH-14/112, MSH-14/113, MSH-14/114, MSH-14/115, MSH-14/116, MSH-14/122 and MSH-14/123) (Table 2). On the other hand, STI0001 showed distinct polymorphism in progenies (MSH/17-16, MSH/17-25, and MSH/17-27) and parents (Kufri Jyoti, Kufri Garima, Card-10, Card-16 and P10) (Table 2). To illustrate, SSR profiles of FAM dye-labelled STM0003 using high resolution machine '3500 Genetic Analyzer' showing distinct diagnostic marker is depicted in Fig 1. The variation in allele size in high resolution machine and simple agarose gel picture is obvious due to allele size determining softwares. Our results are in agreement with allelic profile of this marker. Interestingly, both STI0001 and STM0003 markers profiles can be used for identification of varieties developed from this progenies. Many of the advance stage potato hybrids are in pipeline to develop new varieties in future. Studies have shown that SSR has excellent discriminatory power for molecular characterization of potato species and land races characterization (Provan *et al.* 1996). SSR profiles in terms of allele size and number could vary due to scoring technology used in the study.

Taken together, in this study diagnostic SSR marker STM0003 (103, 132 and 144 bp) was identified for the parents (P8 and Kufri Jyoti), and their hybrids (MSH-14/112, MSH-14/113, MSH-14/114, MSH-14/115, MSH-14/116, MSH-14/122 and MSH-14/123). Similarly, another diagnostic SSR marker STI0001 (169, 172, 175, 178, 184 and 188 bp) was identified for the parents (Kufri Jyoti, Kufri Garima, Card-10, Card-16 and P10) and their hybrids (MSH/17-16, MSH/17-25, and MSH/17-27). Nevertheless, future study would be required to investigate genetic stability and association of linked markers with the traits in these hybrids. Further, determining genes, its functional validation and SSR markers would be future line of work.

SUMMARY

The aim of present study was to identify simple sequence repeat (SSR) markers linked to potato somatic hybrid progenies. A total of 50 breeding lines (parents and progenies) were analyzed using 59 simple sequence repeat (SSR) markers. Of which, SSR marker STM0003 clearly distinguished the parents i.e. somatic hybrid P8 (*Solanum tuberosum* dihaploid C-13 + wild species *S. pinnatisectum*) and potato cv. Kufri Jyoti, and their progenies (MSH-14/112, MSH-14/113, MSH-14/114, MSH-14/115, MSH-14/116, MSH-14/122 and MSH-14/123). STM0003 showed three

distinct alleles (103, 132 and 144 bp), where both P8 and progenies contained 103 and 144 bp, and Kufri Jyoti had 132 and 144 bp alleles. On the other hand, STI0001 distinguished progenies namely MSH/17-16 (Kufri Garima × Crd10), MSH/17-25 (Kufri Garima × P10) and MSH/17-27 (Kufri Jyoti × Crd 16) with respect to their parents, and STI0001 contained six alleles (169, 172, 175, 178, 184 and 188 bp). The study suggests that STM0003 and STI0001 are diagnostic markers to identify these somatic hybrid derived progenies and parents.

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