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DEVELOPMENT OF SSR FINGERPRINTS OF INTERSPECIFIC POTATO SOMATIC HYBRIDS

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ABSTRACT : The objective of this study was to develop DNA fingerprints of potato progenies by SSR markers for genetic fidelity purpose. Interspecific potato somatic hybrid progenies, developed earlier by crossing somatic hybrids with Indian potato varieties, were used in this study. A total of 81 genotypes (progenies and parents) were analysed by two well-characterized potato SSR markers (STU6SNRN and STIIKA), and developed a data matrix for genetic fidelity purpose and other applications. High polymorphism was observed in STIIKA (PIC: 0.98) than STU6SNRN (PIC: 0.95), and also higher number of alleles was observed in STIIKA (22) than STU6SNRN (7). In STU6SNRN, alleles size 179, 182, 190 and 200 bp were predominant; whereas in STIKA, alleles size 195, 201, 221, 235, 242 and 245 were observed frequently in more than 50 per cent 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. Our study suggests that SSR fingerprints would be of practical utility to strengthen genetic fidelity of these somatic hybrids. Further, SSR fingerprints would also be valuable resources in resolving issues related to identification of true-to-type clone during field evaluation in multiple environments.

KEYWORDS Genetic fidelity, potato somatic hybrids, SSR markers

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third most essential food crop behind rice and wheat (Chakrabarti *et al*., 2017). The utilization of potato genetic resources is necessary to widen its genetic base through developing new varieties using breeding and biotechnological methods. Although, several wild species have been utilized in potato improvement, however they represent only a small fraction of the total genetic diversity (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. The difference in ploidy and endosperm balance number causes sexual incompatibility between wild species and cultivated potato species. To surmount this, development of genotypes is essential using diverse parents via protoplast fusion. Interspecific potato somatic hybrids were developed at this institute using protoplast fusion between cultivated and wild species viz., *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. etuberosum* for potato virus Y resistance (Tiwari *et al*., 2010). Further, advanced stage hybrid progenies have been developed by crossing these somatic hybrids with Indian cultivars to develop new potato varieties after multi-location trials (Luthra *et al*., 2016). Besides, several other potato somatic hybrids have been produced during the past four decades (review by Tiwari *et al*., 2018b).

Genetic fidelity of potato is crucial to ensure true-to-type clone in the varietal

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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 an easy-to-use, reproducible, locus-specific, co-dominance 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) so on. Hence, the aim of this study was DNA fingerprinting of potato somatic hybrids/ progenies using known SSR markers for their identification and genetic fidelity purpose.

MATERIALS AND METHODS

Plant materials

Eighty-one interspecific potato somatic hybrids (parents and progenies), developed earlier (Tiwari *et al*., 2018c), were used for DNA fingerprinting by SSR markers at ICAR (Indian Council of Agricultural Research)-Central Potato Research Institute, Shimla. Sample details are mentioned in Table 1. Phenotypic traits of selected

Table 1. Potato genotypes used in the study for SSR profiling.

Sr. No.	Genotype	Ploidy	Parents/Progenies	Total number of SSR alleles (STU and STIIKA)
1	MSH/14-4	4*x*	Kufri Garima × Bulk pollen	13
2	MSH/14-5	4*x*	Kufri Garima × Bulk pollen	10
3	MSH/14-7	4*x*	Kufri Garima × Bulk pollen	12
4	MSH/14-17	4*x*	Kufri Garima × Bulk pollen	13
5	MSH/14-18	4*x*	Kufri Garima × Bulk pollen	13
6	MSH/14-22	4*x*	Kufri Garima × Bulk pollen	11
7	MSH/14-23	4*x*	Kufri Garima × Bulk pollen	10
8	MSH/14-32	4*x*	Kufri Garima × Bulk pollen	12
9	MSH/14-57	4*x*	Kufri Jyoti × Bulk pollen	14
10	MSH/14-58	4*x*	Kufri Jyoti × Bulk pollen	12
11	MSH/14-60	4*x*	Kufri Jyoti × Bulk pollen	12
12	MSH/14-69	4*x*	Kufri Jyoti × Bulk pollen	10
13	MSH/14-81	4*x*	Kufri Sadabahar × Bulk pollen	10
14	MSH/14-85	4*x*	Kufri Sadabahar × Bulk pollen	9
15	MSH/14-87	4*x*	Kufri Sadabahar × Bulk pollen	8
16	MSH/14-88	4*x*	Kufri Sadabahar × Bulk pollen	8
17	MSH/14-89	4*x*	Kufri Sadabahar × Bulk pollen	9
18	MSH/14-90	4*x*	Kufri Sadabahar × Bulk pollen	11
19	MSH/14-91	4*x*	Kufri Sadabahar × Bulk pollen	2
20	MSH/14-92	4*x*	Kufri Sadabahar × Bulk pollen	8
21	MSH/14-96	4*x*	Kufri Sadabahar × Bulk pollen	8
22	MSH/14-97	4*x*	Kufri Sadabahar × Bulk pollen	11
23	MSH/14-98	4*x*	Kufri Sadabahar × Bulk pollen	13
24	MSH/14-100	4*x*	Kufri Sadabahar × Bulk pollen	10

Sr. No.	Genotype	Ploidy	Parents/Progenies	Total number of SSR alleles (STU and STIIKA)
25	MSH/14-103	4*x*	Kufri Sadabahar × Bulk pollen	8
26	MSH/14-104	4*x*	Kufri Sadabahar × Bulk pollen	9
27	MSH/14-105	4*x*	Kufri Sadabahar × Bulk pollen	9
28	MSH/14-109	4*x*	Kufri Sadabahar × Bulk pollen	12
29	MSH/14-112	4*x*	P8 × Kufri Jyoti	18
30	MSH/14-113	4*x*	P8 × Kufri Jyoti	18
31	MSH/14-114	4*x*	P8 × Kufri Jyoti	19
32	MSH/14-115	4*x*	P8 × Kufri Jyoti	18
33	MSH/14-116	4*x*	P8 × Kufri Jyoti	22
34	MSH/14-122	4*x*	P8 × Kufri Jyoti	18
35	MSH/14-123	4*x*	P8 × Kufri Jyoti	16
36	MSH/14-126	4*x*	P8 × Kufri Sadabahar	12
37	MSH/14-128	4*x*	Kufri Gaurav × P2	11
38	MSH/14-129	4*x*	Kufri Gaurav × P2	10
39	MSH/14-131	4*x*	Kufri Gaurav × P2	12
40	MSH/14-135	4*x*	Kufri Gaurav × P2	12
41	MSH/14-137	4*x*	Kufri Gaurav × P2	12
42	MSH/14-140	4*x*	Kufri Gaurav × P2	9
43	MSH/14-141	4*x*	Kufri Gaurav × P2	4
44	MSH/14-142	4*x*	Kufri Gaurav × P2	12
45	MSH/14-143	4*x*	Kufri Gaurav × P2	12
46	MSH/14-144	4*x*	Kufri Gaurav × P2	14
47	MSH/14-145	4*x*	Kufri Gaurav × P2	8
48	MSH/14-148	4*x*	Kufri Gaurav × P2	11
49	MSH/14-151	4*x*	Kufri Gaurav × P2	9
50	MSH/14-152	4*x*	Kufri Gaurav × P2	11
51	MSH/14-153	4*x*	Kufri Gaurav × P3	13
52	MSH/14-159	4*x*	Kufri Gaurav × P3	11
53	MSH/14-167	4*x*	Kufri Gaurav × P3	11
54	MSH/14-170	4*x*	Kufri Gaurav × P3	8
55	MSH/14-172	4*x*	Kufri Gaurav × P7	14
56	MSH/14-176	4*x*	Kufri Gaurav × P8	11
57	MSH/14-181	4*x*	Kufri Gaurav × P8	14
58	MSH/17-016	4*x*	Kufri Garima × Crd10	10
59	MSH/17-025	4*x*	Kufri Garima × P10	17
60	C-13	2*x*	*S. tuberosum* dihaploid	11
61	CPH	2*x*	S. *cardiophyllum* (wild species)	16
62	CRD6	4*x*	C-13 + *S. cardiophyllum*	14
63	CRD10	4*x*	C-13 + *S. cardiophyllum*	14
64	CRD16	4*x*	C-13 + *S. cardiophyllum*	14

Sr. No.	Genotype	Ploidy	Parents/Progenies	Total number of SSR alleles (STU and STIIKA)
65	CRD23	4*x*	C-13 + *S. cardiophyllum*	14
66	Kufri Sadabahar	4*x*	Potato variety	9
67	Kufri Gaurav	4*x*	Potato variety	17
68	Kufri Jyoti	4*x*	Potato variety	14
69	Kufri Garima	4*x*	Potato variety	9
70	Kufri Pukhraj	4*x*	Potato variety	12
71	P1	4*x*	C-13 + *S. pinnatisectum*	17
72	P2	4*x*	C-13 + *S. pinnatisectum*	13
73	P3	4*x*	C-13 + *S. pinnatisectum*	13
74	P4	4*x*	C-13 + *S. pinnatisectum*	14
75	P5	4*x*	C-13 + *S. pinnatisectum*	13
76	P6	4*x*	C-13 + *S. pinnatisectum*	14
77	P7	4*x*	C-13 + *S. pinnatisectum*	14
78	P8	4*x*	C-13 + *S. pinnatisectum*	15
79	P9	4*x*	C-13 + *S. pinnatisectum*	14
80	P10	4*x*	C-13 + *S. pinnatisectum*	15
81	P12	4*x*	C-13 + *S. pinnatisectum*	16
Total				986

Note: Bulk pollens are mixed pollens of somatic hybrids (*S. tuberosum* dihaploid C-13 + *S. pinnatisectum*)

advanced stage hybrids are mentioned in Table 1 and depicted in Fig. 1. Interspecific potato somatic hybrids were investigated for tuber and other traits by Tiwari *et al*. (2018c). Leaf samples were collected from field-grown plants at ICAR-CPRI, Regional Station, Kufri, Shimla, and ICAR-CPRI, Regional Station, Modipuram, Meerut, Uttar Pradesh, India.

SSR analysis

DNA was isolated from the leaf tissues of 81 genotypes using DNeasy Plant Mini kit (Qiagen, the Netherlands) and quality was checked on agarose gel (1%) and NanoDrop (Thermo Fisher Scientific, USA). FAM labelled two well-characterized SSR markers (STU6SNRN and STIIKA) in potato (Tiwari *et al*., 2018a; 2019) were used for DNA

Fig. 1. *Tubers phenotype of advanced stage clones of interspecific potato somatic hybrids. i) MSH/17-16: Yellow ovoid tubers, shallow eyes and yellow flesh; ii) MSH/14-7: Yellow ovoid tubers, shallow eyes and yellow flesh; iii) MSH/14-112: White ovoid tubers, shallow eyes and white flesh.*

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 two times and distinct SSR peaks were scored for 81 genotypes and analysed as described by Tiwari *et al*. (2019). Briefly, a data-sheet 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 – ý (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 81 genotypes (progenies and parents) of interspecific somatic hybrids of potato were fingerprinted using SSR markers STU6SNRN and STIIKA. DNA fingerprints were generated using high-throughput and high resolution equipment "3500 Genetic Analyzer" (Applied Biosystems). SSR polymorphism is presented in Table 2. Marker STU6SNRN showed 7 alleles (171, 174, 179, 182, 190, 200 and 206) with PIC value 0.95, whereas STIIKA amplified 22 alleles (121, 133, 137, 152, 154, 157, 186, 191, 195, 198, 201, 210, 219, 221, 223, 225, 231, 235, 242, 245, 254 and 256) with higher PIC value (0.98). In the somatic hybrid progenies SSR alleles ranged from 2 (MSH/14-91) to 22 (MSH/14-116). Total alleles count of both markers in the genotypes was 986. Tuber phenotypes are depicted in Fig. 1 and its SSR allelic profiles are shown Figs. 2 and 3. A data matrix of SSR alleles for all the progenies and parents (Supplementary Table S1, and summarised total alleles are presented in Table 1) showed distinctness among the genotypes specially progenies (Fig. 4). In the interspecific potato somatic hybrids the dissimilarity coefficient minimum value was 0.052 and the maximum value was 0.92 indicating level of genetic diversity in the genotypes.

Interspecific potato somatic hybrids progenies were obtained from hybridization

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 (5), 174 (34), 179 (67), 182 (80), 190 (81), 200 (80), 206 (14)	0.95
STIIKA	$(T)_{12}$ $(A)_9$ ATTCTTGTT $(TA)_2$ CA $(TA)_7$	F: TTCGTTGCTTACCTACTA R: CCCAAGATTACCACATTC	23	121 (7), 133 (7), 137 (5), 152 (8), 154 (7), 157 (3), 186 (20), 191 (32), 195 (66), 198 (36), 201 (42), 210 (11), 219 (27), 221 (74), 223 (33), 225 (23), 231 (51), 235 (41), 242 (39), 245 (45), 254 (3), 256 (23)	0.98

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

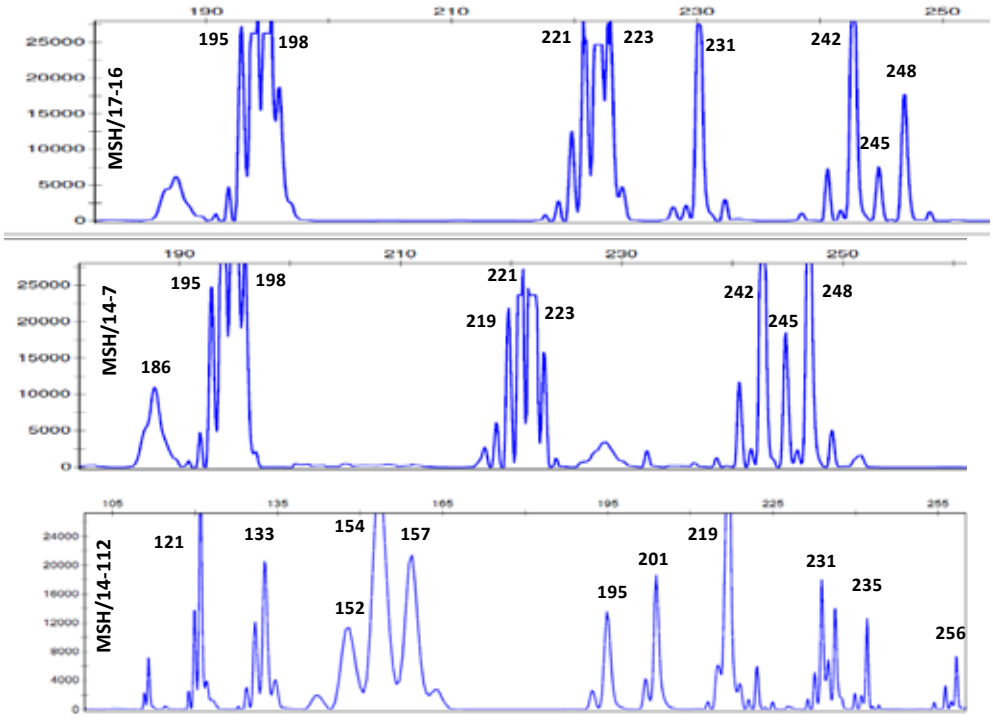

Fig. 2. SSR (STIIKA marker) allelic profile of advanced stage clones of interspecific potato somatic hybrids (MSH/17-16, MSH/14-7 & MSH/14-112). Value on peaks indicates SSR allele size (bp).

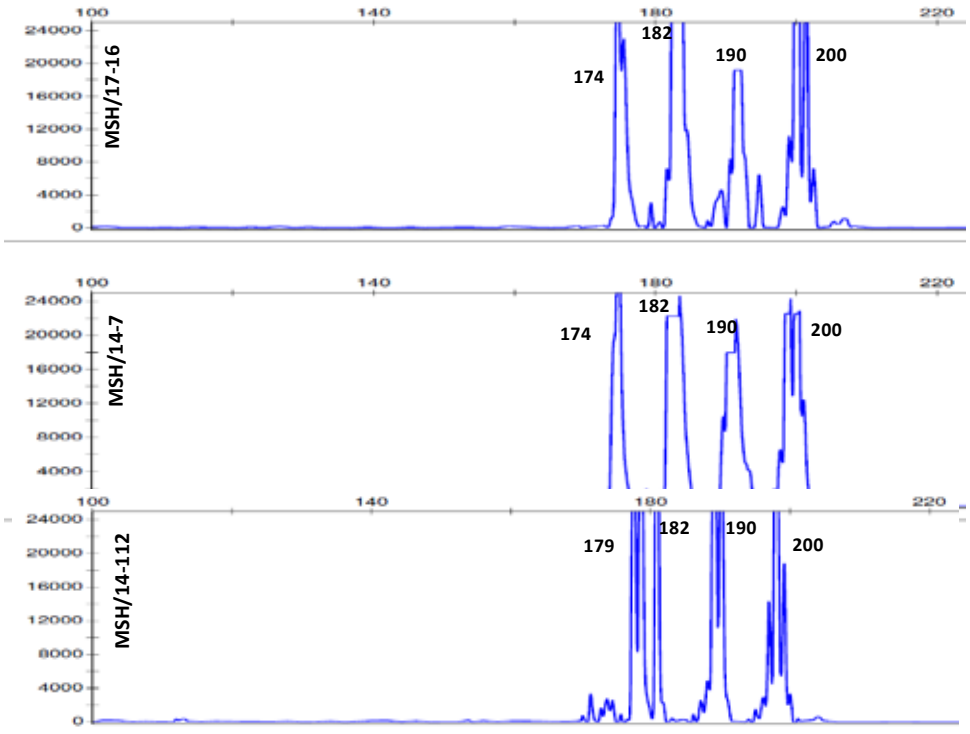

Fig. 3. *SSR (STU6SNRN marker) allelic profile of advanced stage clones of interspecific potato somatic hybrids (MSH/17-16, MSH/14-7 & MSH/14-112). Value on peaks indicates SSR allele size (bp).*

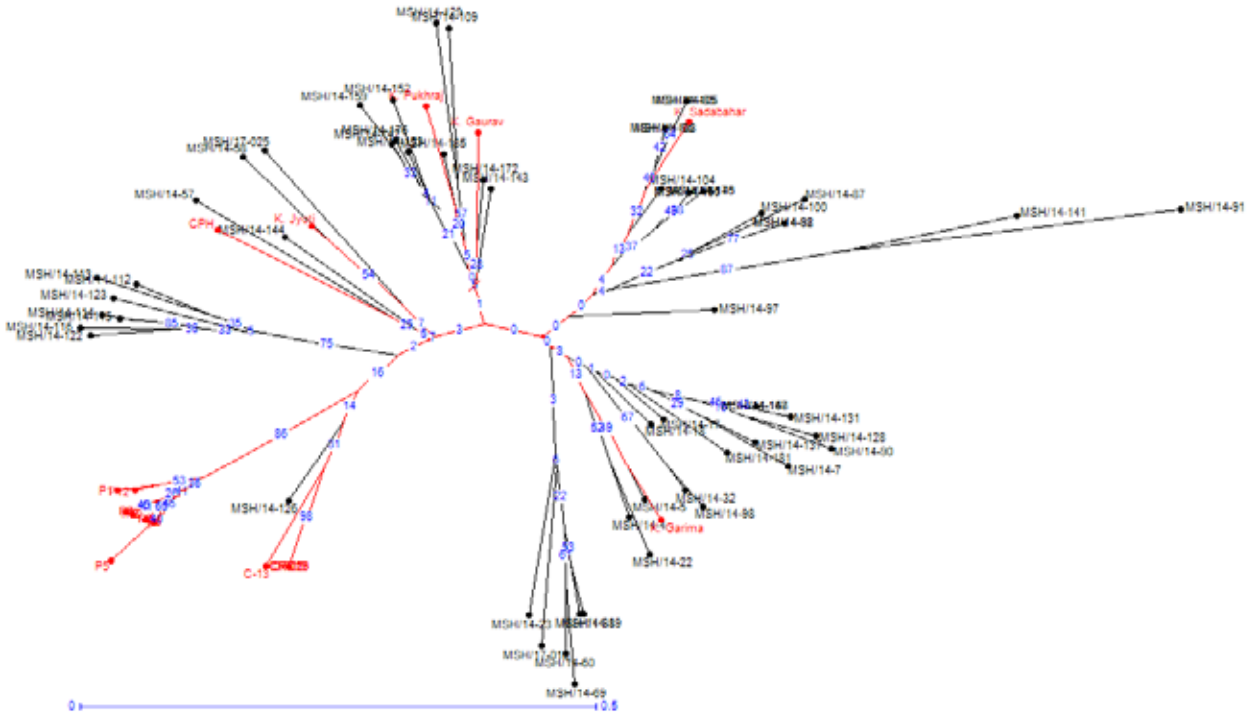

Fig. 4. *Genetic diversity analysis shows distinctness in interspecific potato somatic hybrids based on the Jaccard dissimilarity index by weighted Neighbor-Joining tree construction method using DARwin software. Bootstrap value was 100. Red colour shows the parents used to develop the progenies.*

between somatic hybrids (generated via protoplast fusion between cultivated and wild species) and common potato varieties (Tiwari *et al*., 2018c). These interspecific somatic hybrids (*S. pinnatisectum* and *S. cardiophyllum* derived) have a wider genetic base and possess high resistance to late blight (Tiwari *et al*., 2018c). Our results support findings of SSR analysis in potato by various previous workers. Previously, we characterized these markers in allelic profiling of potato varieties (Tiwari *et al*., 2018a) and wild species (Tiwari *et al*., 2019), and also for genetic fidelity testing of in vitro propagated plants (Tiwari *et al*., 2013a). 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 (Tiwari *et al*., 2013b), 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.

Solanum species is one of the riches 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 mentioned in Table 1). 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 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 (MSH/17-16, MSH/14-7, and MSH/14-112) could be released as new potato varieties in future after multi-location testing. Although worldwide several researchers have reported field evaluation of potato somatic hybrids (Cardi *et al*., 2002; 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, very limited 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 these hybrids at the time of release as varieties. In future, these fingerprints would be helpful in identification of trait-specific alleles in the hybrids.

CONCLUSION

Taken together, we have developed SSR fingerprints of advanced stage potato somatic hybrids. These 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 in identification of true-to-type clones of these hybrids in varietal development program in the country.

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LITERATURE CITED

Bradshaw JE, Bryan GJ and Ramsay G (2006) Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. *Potato Res* **49**: 49-65

Cardi T, Mazzei M and Frusciante L (2002) Field variation in a tetraploid progeny derived by selfing a *Solanum commersonii* (+) *S. tuberosum* somatic hybrid: a multivariate analysis. *Euphytica* **124**: 111–119

Caruso I, Castaldi L, Caruso G, Frusciante L and Carputo D (2008) Breeding potential of *Solanum tuberosum–S. commersonii* pentaploid hybrids: fertility studies and tuber evaluation. *Euphytica* **164**: 357-363

Chakrabarti SK, Conghua X and Tiwari JK (2017) The potato genome. Springer, Switzerland

Chandel P, Tiwari JK, Ali N, Devi S, Sharma SH, Sharma SA, Luthra SK and Singh BP (2015) Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. *Plant Cell Tiss Organ Cult* **123**: 579-589

Ghislain M, Nuñez J, Herrera MR, Pignataro J, Guzmán N, Bonierbale M and Spooner DM (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. *Mol Breed* **23**: 377-388

Luthra SK, Tiwari JK, Lal M, Chandel P and Kumar V (2016) Breeding potential of potato somatic hybrids: Evaluations for adaptability, tuber traits, late blight resistance, keeping quality and backcross (BC_1) progenies. *Potato Res* **59**: 375-391

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

Sarkar D, Tiwari JK, Sharma S, Poonam, Sharma S, Gopal J, Singh BP, Luthra SK, Pandey SK and Pattanayak D (2011) Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. *Plant Cell Tiss Organ Cult* **107**: 427-440

Smyda-Dajmund P, Śliwka J, Wasilewicz-Flis I, Jakuczun H and Zimnoch-Guzowska E (2017) BC_1 and F_1 progeny from *Solanum* × *michoacanum* (+) *S. tuberosum* somatic hybrids, autofused 4× *S. michoacanum* and cultivated potato. *Am J Potato Res* **94**: 323-333

Thieme R, Rakosy-Tican E, Gavrilenko T, Antonova O, Schubert J, Nachtigall M, Heimbach U and Thieme T (2008) Novel somatic hybrids (*Solanum tuberosum* L. + *Solanum tarnii*) and their fertile BC_1 progenies express extreme resistance to potato virus Y and late blight. *Theor Appl Genet* **116**: 691-700

Tiwari JK, Ali N, Devi S, Kumar V, Zinta R and Chakrabarti SK (2018a) Development of microsatellite markers set for identification of Indian potato varieties. *Scientia Hort* **231**: 22-30

Tiwari JK, Ali N, Devi S, Zinta R, Kumar V and Chakrabarti SK (2019) Analysis of allelic variation in wild potato (*Solanum*) species by simple sequence repeat (SSR) markers. *3 Biotech* **9**: 262

Tiwari JK, Chandel P, Gupta S, Gopal J, Singh BP and Bhardwaj V (2013a) Analysis of genetic stability of in vitro propagated potato micro tubers using DNA markers. *Physiol Mol Biol Plants* **19**: 587-595

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S and Chakrabarti SK (2018b) Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tiss Organ Cult* **132**: 225-238

Tiwari JK, Luthra SK, Devi S, Kumar V, Ali N, Zinta R and Chakrabarti SK (2018c) Development of advanced back-cross progenies of potato somatic hybrids and linked ISSR markers for late blight resistance with diverse genetic base- first ever produced in Indian potato breeding. *Potato J* **45**: 17-27

Tiwari JK, Poonam, Sarkar D, Pandey SK, Gopal J and Kumar SR (2010) Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. and *S. etuberosum* Lindl. *Plant Cell Tiss Organ Cult* **103**: 175-187

Tiwari JK, Singh BP, Gopal J, Poonam and Patil VU (2013b) Molecular characterization of the Indian Andigena potato core collection using microsatellite markers. *African J Biotechnol* **12**: 1025-1033