Analysis of allelic variation in wild potato (*Solanum*) species by simple sequence repeat (SSR) markers

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Abstract

Allelic variation in wild potato (*Solanum*) species was analysed using 14 simple sequence repeat (SSR) markers. SSR allelic profiles showed high polymorphism and distinctness among the wild species. A total of 109 alleles of 14 polymorphic SSR markers were scored in 82 accessions belonging to 22 wild potato species. Allele size ranged from a minimum of 104 bp (STI0030) to a maximum of 304 bp (STM5114). Number of SSR alleles per marker ranged from 4 (STM5127/STM1053) to 13 (STM0019), whereas PIC value varied between 0.66 (STM1053) and 0.91 (STM0019). Cluster analysis using SSR allelic profiles of 82 accessions grouped showed 5 major clusters (I–V) based on the Dice similarity coefficient using neighbour-joining clustering method. Distinct allelic variations were observed among the accessions irrespective of the origin country, series and species. Our study suggests that SSR-based molecular characterization of wild potato species is accession specific and development of an allelic dataset for all the accessions would strengthen their utilization in potato research in future.

Keywords Alleles · Diversity · Potato · SSR marker · *Solanum* species

Introduction

Potato (*Solanum tuberosum* L.) plays a key role in the global food and nutritional security after rice, wheat and maize (Chakrabarti et al. 2017). With increasing population and more food demand compounded with disease pests' pressure and climate change, enhancing potato production is necessary through development of new varieties using diverse genetic resources like wild/semi and cultivated *Solanum* species in potato. Potato belongs to a diverse gene pool which includes more than 200 *Solanum* species possessing various desirable traits and biotic/abiotic stresses (Machida-Hirano and Niino 2017). A few potato species have been utilized in breeding (Bradshaw et al. 2006), pre-breeding and biotechnology like somatic fusion (Tiwari et al. 2018b) and still many more species need to be applied.

The potato (*Solanum*) species consist of diploids (73%), triploids (4%), tetraploids (15%), pentaploids (2%) and hexaploids (6%) (Gopal et al. 2003). The cultivated potato is *Solanum tuberosum* ssp. *tuberosum* (long-days adapted) and is found naturally in southern Chile, whereas *Solanum tuberosum* ssp. *andigena* (short-days adapted) is widely distributed in the Andean regions of Venezuela and northern Argentina (Gopal et al. 2003). Besides, primitive cultivated species are confined to the Andes of South America, which are *S. phureja* ($2n=2x=24$), *S. chaucha* ($2n=3x=36$), *S. stenotomum* ($2n=2x=24$), *S. goniocalyx* ($2n=2x=24$), *S. curtilobum* ($2n=5x=60$), *S. juzepczukii* ($2n=3x=36$) and *S. ajanhuiri* ($2n=2x=24$) (Machida-Hirano and Niino 2017). Wild species are adapted to stress environments such as frost (e.g. *S. acaule*), dry semi-desert (e.g. *S. berthaultii*), cool temperate (e.g. *S. colombianum* Dun.), and coastal plains (*S. commersonii* and *S. chacoense*). Wild species also possess resistance to various pests and diseases, for example, resistance to late blight—the most devastating disease of potato—has been introgressed into the cultivated types such as *S. tuberosum* subsp. *andigena*, and wild *S. demissum*, *S. stoloniferum*, *S. phureja*, *S. chacoense*, *S. bulbocastanum* as well as *S. acaule* in Europe and America (Bradshaw et al. 2006). In India, late blight resistance in the most popular potato cv. Kufri Jyoti was derived from wild *S. demissum*

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and now this variety is highly susceptible. Thus, there are several wild potato species which might be important for potato improvement in the future (Tiwari et al. 2017).

It is essential to characterize wild potato species before their deployment in improvement programmes using molecular markers for their diversity pattern, germplasm management and other similar studies. DNA markers have been used for a long time in potato characterization. Among them, simple sequence repeat (SSR) is an excellent marker system to study closely related genotypes due to co-dominance, locus specific, reproducibility and being capable of high-throughput genotyping (Provan et al. 1996; Powell et al. 1996). Earlier, several researchers have used SSR markers to investigate diversity in potato (Liao and Guo 2014; Tiwari et al. 2013, 2018a), development of potato genetic identity (PGI) kit of 24 SSR markers (Ghislain et al. 2009) and many others. Our aim in this study was to investigate allelic variations in wild potato (*Solanum*) species by SSR markers, analyse the molecular diversity and develop an SSR allelic dataset for their future application.

Materials and methods

Plant material

Eighty-two accessions belonging to 22 wild potato (*Solanum*) species were used in this study. Wild accessions were grown in the earthen pots (in triplicates) at Indian Council of Agricultural Research (ICAR)-Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh, India. In vitro plants were raised from true potato seeds (TPS) and multiplied through tissue culture as described by Sarkar et al. (2011). A single mother plant per accession was clonally regenerated. TPS were obtained from the international gene banks namely Potato Introduction Station, NRSP-6, Sturgeon Bay, Wisconsin (USA); Centre for Genetic Resources, Wageningen University and Research, the Netherlands (CGN); and International Potato Centre, Lima, Peru. A brief description of the wild species is summarised in Table 1 and images are shown in Fig. 1.

DNA analysis

Leaf tissues of 82 accessions were collected and total genomic DNA was isolated using the DNeasy® Plant Mini Kit (Qiagen, Venlo, Limburg, Netherlands) following manufacturer's instructions. DNA quantification was performed with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and quality was checked on 1% (w/v) agarose gel for SSR analysis. Fourteen SSR markers (12 markers, one per chromosome from the PGI kit, Ghislain et al. 2009; and two polymorphic markers, STIKA

and STU6SNRN, Provan et al. 1996) were used for polymerase chain reaction (PCR) amplification (Table 1). The PCR reaction was performed in 25 µL volume with DNA template (100 ng) in 1 × PCR buffer (2.5 mM/L MgCl₂ and 200 µM/L dNTP), each primer (0.5 µM/L), and Taq polymerase (1U) (Qiagen, Venlo, Limburg, Netherlands) following initial denaturation at 94 °C/5 min; followed by 35 cycles of 94 °C/45 s, 51–60 °C/45 s, and 72 °C/1 min; and final extension at 72 °C/8 min in a Veriti Thermal Cycler (Life Technologies, Carlsbad, California, 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, California, USA).

Data scoring and analysis

PCR reactions were repeated at least twice, and only reproducible, distinct, and scorable SSR alleles across the run were considered for analysis. A data matrix of 82 accessions was prepared on the basis of presence (1) or absence (0) of the alleles. Number of alleles, allele size, absolute frequencies and polymorphic information content (PIC) of markers were calculated for 82 samples. The PIC value of SSR markers was calculated according to the formula: $PIC = 1 - \sum (P_i^2)$, where P_i is the frequency of the i th allele of a marker detected in accessions (Nei 1973). A similarity matrix of SSR profiles was estimated based on the Dice similarity coefficient using the neighbour-joining clustering method using DARwin software with bootstrap value 100 (Perrier and Jacquemoud-Collet 2006). The principal component analysis (PCA) was analysed to assess the genetic association among the wild species using the software NTSYSpc (Rohlf 2006).

Results

SSR polymorphism

Allelic profiling of 82 accessions belonging to 22 wild potato species showed polymorphism using 14 SSR markers (Table 1). A summary of distribution of SSR alleles' size, number of alleles, and PIC value is presented in Table 2. SSR analysis showed 109 alleles of 14 polymorphic SSR loci in wild species. The allele size ranged from a minimum of 104 bp (STI0030) to a maximum of 304 bp (STM5114). The number of alleles per marker ranged from 4 (STM5127/STM1053) to 13 (STM0019), whereas the PIC value varied between 0.66 (STM1053) and 0.91 (STM0019) (Table 3). The most informative SSR allele was 208 bp of STM1052, which amplified to maximum in 80 accessions, whereas 121 bp of STI0030 marker was found to be minimum in

Table 1 Details of 82 accessions belonging to 22 wild potato (*Solanum*) species used in SSR analysis. Scientific name of wild species along with ploidy/EBN/Chr. no. details are mentioned from the search US Gene bank search: <https://npgsweb.ars-grin.gov>; or CGN Gene bank search: <https://cgngenis.wur.nl>; or adapted from Hijmans and Spooner (2001). Nomenclature is based on Hawkes (1990)

S. no.	<i>Solanum</i> species	Origin country	Genbank ID ^a	Chr. no./ Ploidy/ EBN	Series	Lab code ^b	# Alleles ^c	Cluster
1.	<i>Solanum acaule</i> Bitter	Bolivia	PI 210029	48/4x/2	<i>Acaulia</i>	ACL29	35	I
2.	<i>Solanum acaule</i> Bitter subsp. <i>aemulans</i>	Argentina	CGN17938	48/4x/2	<i>Acaulia</i>	ACL38	30	I
3.	<i>Solanum berthaultii</i> Hawkes	Bolivia	PI 310925	24/2x/2	<i>Tuberosa</i>	BER25	39	II
4.	<i>Solanum berthaultii</i> Hawkes	Bolivia	PI 265857	24/2x/2	<i>Tuberosa</i>	BER57	30	III
5.	<i>Solanum berthaultii</i> Hawkes	Bolivia	PI 265858	24/2x/2	<i>Tuberosa</i>	BER58	36	II
6.	<i>Solanum berthaultii</i> Hawkes	Bolivia	PI 498096	24/2x/2	<i>Tuberosa</i>	BER96	38	II
7.	<i>Solanum cardiophyllum</i> Lindl. subsp. <i>cardiophyllum</i>	Mexico	CGN18325	24/2x/1	<i>Pinnatisecta</i>	CPH25	37	I
8.	<i>Solanum cardiophyllum</i> Lindl. subsp. <i>cardiophyllum</i>	Mexico	CGN18326	24/2x/1	<i>Pinnatisecta</i>	CPH26	32	I
9.	<i>Solanum cardiophyllum</i> Lindl.	Mexico	PI 283062	24/2x/1	<i>Pinnatisecta</i>	CPH62	32	I
10.	<i>Solanum cardiophyllum</i> Lindl.	Mexico	PI 283063	24/2x/1	<i>Pinnatisecta</i>	CPH63	37	I
11.	<i>Solanum cardiophyllum</i> Lindl.	Mexico	PI 595465	24/2x/1	<i>Pinnatisecta</i>	CPH65	38	I
12.	<i>Solanum cardiophyllum</i> Lindl. subsp. <i>cardiophyllum</i>	Mexico	CGN22387	24/2x/1	<i>Pinnatisecta</i>	CPH87	33	I
13.	<i>Solanum chacoense</i> Bitter	Argentina	PI 197760	24/2x/2	<i>Yungasensa</i>	CHC60	38	I
14.	<i>Solanum chomatophilum</i> Bitter	Peru	PI 310990	24/2x/2	<i>Conicibaccata</i>	CHM90	35	I
15.	<i>Solanum hannemani</i> ^d	Argentina	CGN18001	24/2x/2	<i>Tuberosa</i>	HAN01	31	I
16.	<i>Solanum hjertingii</i> Hawkes	Mexico	PI 283103	48/4x/2	<i>Longipedicellata</i>	HJT03	28	I
17.	<i>Solanum hougasii</i> Correll	Mexico	PI 161727	72/6x/4	<i>Demissa</i>	HOU27	38	II
18.	<i>Solanum huancabambense</i> Ochoa	Peru	CGN18306	24/2x/2	<i>Yungasensa</i>	HCB06	27	I
19.	<i>Solanum huancabambense</i> Ochoa	Peru	CGN17719	24/2x/2	<i>Yungasensa</i>	HCB19	35	I
20.	<i>Solanum iopetalum</i> (Bitter) Hawkes	Mexico	PI 239402	72/6x/4	<i>Demissa</i>	IOP02	46	II
21.	<i>Solanum iopetalum</i> (Bitter) Hawkes	Mexico	PI 230459	72/6x/4	<i>Demissa</i>	IOP59	35	II
22.	<i>Solanum iopetalum</i> (Bitter) Hawkes	Mexico	PI 275180	72/6x/4	<i>Demissa</i>	IOP80	44	II
23.	<i>Solanum jamesii</i> Torr.	United States of America	PI 498407	24/2x/1	<i>Pinnatisecta</i>	JAM07	33	III
24.	<i>Solanum jamesii</i> Torr.	United States of America	CGN18346	24/2x/1	<i>Pinnatisecta</i>	JAM46	36	I
25.	<i>Solanum jamesii</i> Torr.	United States of America	CGN18349	24/2x/1	<i>Pinnatisecta</i>	JAM49	39	III
26.	<i>Solanum jamesii</i> Torr.	United States of America	CIP762777	24/2x/1	<i>Pinnatisecta</i>	JAM77	47	III
27.	<i>Solanum jamesii</i> Torr.	United States of America	PI 558089	24/2x/1	<i>Pinnatisecta</i>	JAM89	42	III
28.	<i>Solanum lesteri</i> Hawkes & Hjert.	Mexico	CGN24429	24/2x/1	<i>Polyadenia</i>	LES29	32	V
29.	<i>Solanum lesteri</i> Hawkes & Hjert.	Mexico	PI 558434	24/2x/1	<i>Polyadenia</i>	LES34	35	V
30.	<i>Solanum lesteri</i> Hawkes & Hjert.	Mexico	CGN18337	24/2x/1	<i>Polyadenia</i>	LES37	44	V
31.	<i>Solanum lesteri</i> Hawkes & Hjert.	Mexico	CGN23988	24/2x/1	<i>Polyadenia</i>	LES88	32	V
32.	<i>Solanum medians</i> Bitter	Peru	PI 283081	24/2x/2	<i>Tuberosa</i>	MED81	39	I
33.	<i>Solanum microdontum</i> Bitter	Argentina	PI 218224	24/2x/2	<i>Tuberosa</i>	MCD24	27	I
34.	<i>Solanum microdontum</i> Bitter	Argentina	PI 473171	24/2x/2	<i>Tuberosa</i>	MCD71	30	I
35.	<i>Solanum microdontum</i> Bitter	Argentina	PI 195185	24/2x/2	<i>Tuberosa</i>	MCD85	42	I

Table 1 (continued)

S. no.	<i>Solanum</i> species	Origin country	Genbank ID ^a	Chr. no./ Ploidy/ EBN	Series	Lab code ^b	# Alleles ^c	Cluster
36.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN23011	24/2x/1	<i>Pinnatisecta</i>	PNT11	46	III
37.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN23012	24/2x/1	<i>Pinnatisecta</i>	PNT12	40	III
38.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN18331	24/2x/1	<i>Pinnatisecta</i>	PNT31	48	III
39.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN17740	24/2x/1	<i>Pinnatisecta</i>	PNT40	47	III
40.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN17741	24/2x/1	<i>Pinnatisecta</i>	PNT41	49	III
41.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN17442	24/2x/1	<i>Pinnatisecta</i>	PNT42	33	I
42.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN17443	24/2x/1	<i>Pinnatisecta</i>	PNT43	35	I
43.	<i>Solanum pinnatisectum</i> Dunal	Mexico	CGN17744	24/2x/1	<i>Pinnatisecta</i>	PNT44	48	III
44.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN23013	24/2x/1	<i>Polyadenia</i>	PLD13	31	V
45.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN23014	24/2x/1	<i>Polyadenia</i>	PLD14	31	V
46.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 275237	24/2x/1	<i>Polyadenia</i>	PLD37	28	V
47.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 275238	24/2x/1	<i>Polyadenia</i>	PLD38	29	V
48.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 320342	24/2x/1	<i>Polyadenia</i>	PLD42	34	V
49.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 558443	24/2x/1	<i>Polyadenia</i>	PLD43	39	V
50.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 558445	24/2x/1	<i>Polyadenia</i>	PLD45	35	V
51.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN17746	24/2x/1	<i>Polyadenia</i>	PLD46	31	V
52.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN17747	24/2x/1	<i>Polyadenia</i>	PLD47	34	V
53.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN17748	24/2x/1	<i>Polyadenia</i>	PLD48	32	V
54.	<i>Solanum polyadenium</i> Greenm.	Mexico	CGN17749	24/2x/1	<i>Polyadenia</i>	PLD49	30	V
55.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 310963	24/2x/1	<i>Polyadenia</i>	PLD63	33	V
56.	<i>Solanum polyadenium</i> Greenm.	Mexico	PI 230480	24/2x/1	<i>Polyadenia</i>	PLD80	36	V
57.	<i>Solanum polyadenium</i> Greenm.	Mexico	CIP761014	24/2x/1	<i>Polyadenia</i>	PLDCIP14	26	V
58.	<i>Solanum polyadenium</i> Greenm.	Mexico	CIP760724	24/2x/1	<i>Polyadenia</i>	PLDCIP24	29	V
59.	<i>Solanum polytrichon</i> Rydb.	Mexico	CGN18318	24/2x/2	<i>Longipedicellata</i>	PLT18	35	II
60.	<i>Solanum polytrichon</i> Rydb.	Mexico	CGN17750	24/2x/2	<i>Longipedicellata</i>	PLT50	39	II
61.	<i>Solanum polytrichon</i> Rydb.	Mexico	CGN17751	24/2x/2	<i>Longipedicellata</i>	PLT51	41	II
62.	<i>Solanum polytrichon</i> Rydb.	Mexico	CGN22361	24/2x/2	<i>Longipedicellata</i>	PLT61	32	II
63.	<i>Solanum polytrichon</i> Rydb.	Mexico	CGN22362	24/2x/2	<i>Longipedicellata</i>	PLT62	36	I
64.	<i>Solanum stenophyllidium</i> Bitter	Mexico	CGN17603	24/2x/1	<i>Pinnatisecta</i>	SPH03	36	I
65.	<i>Solanum stoloniferum</i> Schltdl.	Mexico	PI 275240	48/4x/2	<i>Longipedicellata</i>	STO40	38	II
66.	<i>Solanum stoloniferum</i> Schltdl.	Mexico	PI 225661	48/4x/2	<i>Longipedicellata</i>	STO61	33	I
67.	<i>Solanum trifidum</i> Correll	Mexico	PI 283104	24/2x/1	<i>Pinnatisecta</i>	TRF04	37	IV
68.	<i>Solanum trifidum</i> Correll	Mexico	CGN22722	24/2x/1	<i>Pinnatisecta</i>	TRF22	28	I
69.	<i>Solanum trifidum</i> Correll	Mexico	CGN18335	24/2x/1	<i>Pinnatisecta</i>	TRF35	41	IV
70.	<i>Solanum trifidum</i> Correll	Mexico	PI 255537	24/2x/1	<i>Pinnatisecta</i>	TRF37	32	IV

Table 1 (continued)

S. no.	<i>Solanum</i> species	Origin country	Genbank ID ^a	Chr. no./ Ploidy/ EBN	Series	Lab code ^b	# Alleles ^c	Cluster
71.	<i>Solanum trifidum</i> Correll	Mexico	PI 255539	24/2x/1	<i>Pinnatisecta</i>	TRF39	47	IV
72.	<i>Solanum trifidum</i> Correll	Mexico	PI 255541	24/2x/1	<i>Pinnatisecta</i>	TRF41	38	IV
73.	<i>Solanum trifidum</i> Correll	Mexico	PI 255542	24/2x/1	<i>Pinnatisecta</i>	TRF42	45	IV
74.	<i>Solanum trifidum</i> Correll	Mexico	PI 283064	24/2x/1	<i>Pinnatisecta</i>	TRF64	40	IV
75.	<i>Solanum trifidum</i> Correll	Mexico	PI 283065	24/2x/1	<i>Pinnatisecta</i>	TRF65	41	IV
76.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275255	24/2x/2	<i>Tuberosa</i>	VER55	34	II
77.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275256	24/2x/2	<i>Tuberosa</i>	VER56	39	II
78.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275257	24/2x/2	<i>Tuberosa</i>	VER57	30	II
79.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275258	24/2x/2	<i>Tuberosa</i>	VER58	35	II
80.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275259	24/2x/2	<i>Tuberosa</i>	VER59	30	II
81.	<i>Solanum verrucosum</i> Schltdl.	Mexico	PI 275260	24/2x/2	<i>Tuberosa</i>	VER60	25	II
82.	<i>Solanum vernei</i> Bitter & Wittm.	Argentina	PI 320330	24/2x/2	<i>Tuberosa</i>	VRN30	21	I

^aPotato gene bank IDs, starting with PI, CGN and CIP are obtained from Potato Introduction Station, NRSP-6, Sturgeon Bay, Wisconsin (USA); Centre for Genetic Resources, the Netherlands (CGN); and International Potato Centre, Lima, Peru, respectively

^b'Lab code' was used in this study to denote the accessions on the cluster tree and images, due to space limitations

^cTotal number of alleles of all 14 SSR markers scored in the accession

^dAuthority of *Solanum hannemanii* is not available

3 accessions. A total of 2929 SSR alleles were amplified in all 82 accessions by 14 markers, which varied from 21 (VRN30) to 49 (PNT41) in the accessions. A dataset of SSR alleles was developed for all the accessions (Table 1 and Suppl. File S1). Several markers had either nil or very minor or non-scorable fragments shown as 'Nil' (null alleles) in Suppl. File S1.

Cluster analysis

Cluster analysis of wild species distinguished all the accessions based on the Jaccard's similarity coefficient (0.19–0.80) by 14 SSR markers (Fig. 2). All accessions were classified into major five clusters (I–V) based on the allelic profiles. Most accessions were categorised into the respective species cluster except a few like VRN30. Allelic variation was observed within the species. For example, accessions (BER25, BER58 and BER96) of *S. berthaultii* species were grouped into cluster II except BER57 (cluster III); *S. jamesii* and *S. pinnatisectum* species accessions were grouped into cluster III except JAM46, PNT42 and PNT43 (cluster I); *S. trifidum* accessions were grouped into cluster IV except TRF22 (cluster I). Genetic association in the wild species was shown by principal component analysis (PCA). The PCA analysis showed a total variance of 45.15% by calculating the first three components as 33.60, 6.67 and 4.87% and PCA plot showing the first two principal components (Dim-1 and Dim-2) is depicted in Suppl. File S2.

Discussion

We observed allelic variations in 82 accessions of 22 wild potato (*Solanum*) species by 109 alleles of 14 polymorphic SSR markers. All markers showed polymorphism based on the PIC values (0.66–0.91) distributed across the SSR loci in the accessions. SSR studies have shown good discrimination power and polymorphism in closely related potato species such as varietal identification (Powell et al. 1996) and characterization of wild species (Carputo et al. 2013). SSR allows labelling of genotypes to reveal genetic variation in potato (Ghislain et al. 2009) and germplasm management of reference collection (Reid et al. 2011). Our findings are congruent with earlier SSR studies on allelic variation including observation on null alleles (Provan et al. 1996; Ghislain et al. 2009) and validation of Andigena core collection (Tiwari et al. 2013). Allelic profiles in terms of size, number and absolute frequencies observed in this study showed little deviation in comparison with earlier findings (Provan et al. 1996; Ghislain et al. 2009). Probably, this could be due to the equipment types and software technologies used to analyse SSR alleles. We used a high-throughput fragment analysis system, i.e., '3500 Genetic Analyzer' (Applied Biosystems) that has high precision, whilst earlier workers might have used a semi-automated gel-based method to score SSR alleles. Moreover, we used this system for molecular characterization of wild species and interspecific somatic hybrids (Sarkar et al. 2011; Chandel et al. 2015) and identification of potato varieties using SSR markers (Tiwari et al. 2018a).

Fig. 1 Twenty-two wild potato (*Solanum*) species were used for SSR analysis. Due to space limitation on the image panel, accessions are mentioned here: *Solanum acaule* (CGN17938), *S. berthaultii* (PI 265858), *S. cardiophyllum* (PI 283062), *S. chacoense* (PI 197760), *S. chomatophilum* (PI 310990), *S. hannemanii* (CGN18001), *S. hjertingii* (PI 283103), *S. hougasii* (PI 161727), *S. huancabambense*

(CGN18306), *S. iopetalum* (PI 275180), *S. jamesii* (CGN18346), *S. lesteri* (PI 558434), *S. medians* (PI 283081), *S. microdontum* (PI 195185), *S. pinnatisectum* (CGN17744), *S. polyadenium* (PI 230480), *S. polytrichon* (CGN17750), *S. stenophyllidium* (CGN17603), *S. stoloniferum* (PI 225661), *S. trifidum* (PI 255537), *S. verrucosum* (PI 275256), and *S. vernei* (PI 320330)

We observed null alleles in a few accessions with some SSR markers. Similar observations of null alleles have been reported earlier by Galarreta et al. (2011) on characterization of potato landraces and by Tiwari et al. (2013) on characterization of Andigena core collection.

Our earlier study showed high allelic diversity with a range of PIC values (0.53–0.92) using SSR markers in characterization of Indian potato varieties (Tiwari et al. 2018a). Similarly, high SSR allelic diversity has also been reported in potato landraces by Galarreta et al. (2011). Researchers have suggested that SSR markers are a useful tool to investigate genetic variations 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 (Raker and Spooner 2002). Cluster and PCA analysis in this study using 14 SSR markers reflected genetic distinctness among the accessions of the wild species. Cluster analysis showed the maximum value of Jaccard's similarity coefficients to

be 0.80 by 14 SSR to distinguish the accessions. An SSR database of potato varieties of the European Union common catalogue has been developed showing unique profiles (Reid et al. 2011). We developed a dataset of SSR markers for identification of potato varieties (Tiwari et al. 2018a). Thus, the above studies show the versatility of the SSR system for molecular characterization of potatoes.

South America is the centre of origin of potato and the *Solanum* species originated from the Andean regions of Peru, Bolivia and Argentina in South America and Mexico/Central America (Hawkes 1990). Distinct variations have been observed in this study by SSR markers within the species and between/among the accessions with respect to allelic distribution, which could be due to genomic changes during evolutionary process of the species (Provan et al. 1996). In potato, since each TPS is genetically different from another TPS, allelic variation could be expected among the accessions and within the species. Diversity based on the SSR alleles has been observed in the accessions irrespective

Table 2 Molecular profiling of wild potato species by SSR markers. SSR markers source: Ghislain et al. (2009) (#1–12) and Provan et al. (1996) (#13–14)

S. no.	SSR marker	Repeat motif	Sequence (5'→3')	Map location	Alleles size (bp)	Number of alleles	T_a (°C)	PIC
1	STM5127	(TCT) _n	F: TTCAAGAATAGGCAA AACCA R: CTTTTTCTGACTGAGTTG CCTC	I	237–247	4	55	0.74
2	STM5114	(ACC) _n	F: AATGGCTCTCTCTGTATG CT R: GCTGTCCCAACTATCTTT GA	II	275–304	6	60	0.80
3	STM1053	(TA) _n (ATC) _n	F: TCTCCCCATCTTAATGTT TC R: CAACACAGCATACAGATC ATC	III	165–180	4	53	0.66
4	STI0012	(ATT) _n	F: GAAGCGACTTCCAAAATC AGA R: AAAGGGAGGAATAGA AACCAAAA	IV	148–226	7	56	0.81
5	STPoAc58	(TA) _n	F: TTGATGAAAGGAATGCAG CTGTG R: ACGTTAAAGAAGTGAGAG TACGAC	V	218–251	7	57	0.77
6	STM0019 ^a	(AT) _n (GT) _n (AT) _n (GT) _n (GC) _n (GT) _n	F: AATAGGTGTACTGACTCT CAATG R: TTGAAGTAAAAGTCCTAG TATGTG	VI	108–247	13	55	0.91
7	STM0031	(AC) _n ... (AC) _n GCAC (AC) _n (GCAC) _n	F: CATACGCACGCACGT ACAC R: TTCAACCTATCATTTTGT GAGTCG	VII	137–272	8	53	0.83
8	STM1104	(TCT) _n	F: TGATTCTCTTGCTACTG TAATCG R: CAAAGTGGTGTGAAGCTG TGA	VIII	147–180	7	53	0.81
9	STM1052	(AT) _n GT (AT) _n (GT) _n	F: CAATTCGTTTTTTCATG TGACAC R: ATGGCGTAATTTGATTGA ATACGTAA	IX	141–300	10	55	0.77
10	STM1106	(ATT) _n	F: TCCAGCTGATTGGTTAGG TTG R: ATGCGAATCTACTCGTCA TGG	X	124–294	11	51	0.87
11	STM0037	(TC) _n (AC) _n AA (AC) _n (AT) _n	F: AATTAACTTAGAAGATT AGTCTC R: ATTTGGTTGGGTATGATA	XI	130–289	10	52	0.85
12	STI0030	(ATT) _n	F: TTGACCCTCCAATATAG ATTCTTC R: TGACAACTTTAAAGCATA TGTCAGC	XII	104–139	6	58	0.81
13	STU6SNRN	(TGG) ₅	F: GAAGTTTATCAGAATCC R: ATCACCTCATCAGCAATC	–	145–204	9	55	0.85
14	STIKA	(T) ₁₂ (A) ₉ ATTCTTGTT(TA) ₂ CA(TA) ₇	F: TTCGTTGCTTACCTACTA R: CCCAAGATTACCACATTC	–	175–233	7	55	0.82
Total						109		

PIC polymorphic information content, T_a annealing temperature

^aAll SSR amplified at single locus except, STM019 amplifies at two loci (Ghislain et al. 2009)

of the origin country, series and species. The 82 accessions used in this study originated mostly from Mexico (61) followed by Argentina (7), Bolivia (5), the United States of America (5) and Peru (4). Interestingly, the wild species that

originated from South American countries like Argentina (*S. acaule*, *S. chacoense*, *S. hannemanii*, *S. microdontum* and *S. vernei*) and Peru (*S. chomatophilum*, *S. huancabambense* and *S. medians*) have been grouped into cluster I. On the

Table 3 Distribution of SSR allele size (bp) and its absolute frequency in wild potato species

SSR primer	Allele size (absolute frequency)
STM5127	237 (43), 241 (40), 244 (25), 247 (37)
STM5114	275 (32), 281 (55), 284 (50), 287 (37), 290 (34), 304 (9)
STM1053	165 (65), 170 (78), 173 (28), 180 (11)
STI0012	148 (26), 161 (22), 167 (44), 194 (54), 199 (30), 205 (13), 226 (7)
STPoAc58	218 (47), 226 (59), 232 (23), 236 (21), 240 (13), 247 (9), 251 (5)
STM0019	108 (18), 118 (27), 126 (20), 145 (20), 156 (24), 162 (27), 166 (24), 172 (12), 180 (10), 189 (27), 195 (14), 206 (38), 247 (14)
STM0031	137 (35), 149 (40), 155 (12), 184 (9), 204 (14), 229 (37), 254 (12), 272 (18)
STM1104	147 (27), 151 (47), 154 (42), 160 (30), 164 (58), 171 (11), 180 (10)
STM1052	141 (24), 154 (5), 165 (4), 179 (10), 201 (38), 208 (80), 217 (8), 230 (6), 161 (13), 300 (12)
STM1106	124 (58), 127 (33), 130 (48), 139 (30), 146 (32), 155 (21), 169 (7), 182 (11), 235 (10), 268 (7), 294 (17)
STM0037	130 (17), 142 (10), 149 (10), 160 (19), 178 (47), 187 (18), 193 (15), 221 (7), 237 (13), 289 (7)
STI0030	104 (7), 109 (5), 115 (5), 121 (3), 135 (8), 139 (10)
STU6SNRN	145 (16), 153 (31), 169 (22), 177 (36), 181 (69), 190 (78), 194 (41), 199 (66), 204 (20)
STIKA	175 (21), 194 (64), 200 (54), 208 (62), 213 (33), 218 (17), 233 (29)

Absolute frequency is the number of accessions in which the SSR amplified, out of total of 82 accessions used in the study. (e.g., absolute frequency will be a maximum of 82, if SSR amplified in all 82 accessions; and 0 if not amplified in any accession)

Fig. 2 Diversity analysis of 82 accessions belonging to 22 wild species using 14 SSR markers based on the Dice coefficient using the weighted neighbour-joining tree construction method. Bootstrap values are shown on the nodes. Different colours are shown to represent the species

contrary, Mexico-originated species (*S. cardiophyllum*, *S. hjertingii*, *S. hougasii*, *S. iopetalum*, *S. lesteri*, *S. pinnatisectum*, *S. polyadenium*, *S. polytrichon*, *S. stenophyllidum*, *S. stoloniferum*, *S. trifidum*, and *S. verrucosum*) have been grouped differently into all five clusters, whereas Bolivia (*S. acaule* and *S. berthaultii*) and the United States of America (*S. jamesii*) species have been grouped into various clusters.

To conclude, allelic profiling of wild potato species by SSR markers, development of a dataset of alleles and diversity among the accessions of the species are important information for future application of these species. These wild species are an important resource of resistance genes for various biotic and abiotic stresses and could be characterized for more traits like late blight resistance (Tiwari et al. 2015). Future study should be focused on the identification of SSR alleles linked to target traits and their deployment in future improvement programmes applying breeding and biotechnological approaches.

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Author contributions JKT designed the experiment. JKT, NL, SD and RZ performed the SSR analysis. VK provided the samples. JKT analysed the data and wrote the manuscript, and SKC edited it. All authors read and confirmed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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