Potato J (2018) 45 (1): 17-27

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

Jagesh Kumar Tiwari[1], SK Luthra[2], Sapna Devi[1], Vinod Kumar[1], Nilofer Ali[1], Rasna Zinta[1] and SK Chakrabarti[1]

ABSTRACT: Elite hybrid progenies of late blight resistant potato somatic hybrids were developed through hybridization with common potato varieties, and also linked ISSR markers were identified with resistant parent/progenies. Previously developed potato somatic hybrids (considered as F_1 being protoplast fusion product between *Solanum tuberosum* dihaploid 'C-13' + *S. pinnatisectum*) were back-crossed with potato varieties (*S. tuberosum*) and true potato seed (TPS) were produced. TPS-raised seedlings were advanced to back-cross progenies clones (BC_1-C_1, BC_1-C_2, BC_1-C_3 and BC_1-C_4) during the five years based on tuber traits in field trials and field resistance to late blight. The BC_1-C_2 progenies were profiled by ISSR markers and alleles linked to late blight resistant somatic hybrid parent P8 and their progenies (P8 × Kufri Jyoti) were identified. Eight promising advanced hybrids of BC_1-C_4 progenies (Kufri Garima × Bulk pollen: MSH/14-7; Kufri Gaurav × P2: MSH/14-129 and -131 for yield enhancement; P8 × Kufri Jyoti: MSH/14-112, -113, -115, -122 and -123 for late blight resistance and dry matter) were identified. This study concludes successful exploitation of interspecific potato somatic hybrids possessing high resistance to late blight and developed promising clones with linked ISSR alleles for genetic fidelity and breeding purposes. This is the first ever report in India towards widening the genetic base of potato by exploitation of interspecific somatic hybrids.

KEY WORDS: Back-cross progenies, ISSR, late blight resistance, potato, somatic hybrids

INTRODUCTION

The *Solanum* species is a wealth of genetic resources, of which only a few species have been exploited in potato breeding (Chakrabarti *et al.*, 2017). Owing to sexual incompatibilities between wild and cultivated species, somatic hybridization has been applied worldwide in potato to broaden the narrow genetic base of Indian varieties (Machida-Hirano and Niino 2017; Tiwari *et al.*, 2017). Several potato somatic hybrids have been produced during the past four decades (review by Tiwari *et al.*, 2018) and applied to study potato tuber biology (Tiwari *et al.*, 2015b).

Late blight is the most serious disease of potato caused by the oomycetes (*Phytophthora infestans* Mont. de Bary). Besides fungicides application, the use of resistant cultivars is one of the sustainable options to manage this disease (Chakrabarti *et al.*, 2014). Hence, developing late blight resistant genotype is important through utilization of diverse gene pool like somatic hybrids (Chandel *et al.*, 2015). In the past decades, many known potato resistance genes (*R1-R11*) were broken down by new strains of the pathogen (Tiwari *et al.*, 2015a). Although, conventional breeding method has played a pivotal role in potato improvement, inclusion of markers technology would fasten it through clonal selection at

[1]ICAR-Central Potato Research Institute, Shimla 171001, Himachal Pradesh, India
Email: jageshtiwari@gmail.com
[2]ICAR -Central Potato Research Institute Campus, Modipuram, Meerut, Uttar Pradesh 250 110, India

early stage with linked markers and ensure genetic fidelity of the genotype. Molecular markers have been used in potato for long time for example varietal identification (Tiwari *et al.*, 2018), and late blight and virus resistance (Tiwari *et al.*, 2012, 2013c).

Earlier we developed late blight resistant interspecific potato somatic hybrids between *Solanum tuberosum* dihaploid 'C-13' and wild *S. pinnatisectum* and characterized by various phenotypic and molecular approaches (Sarkar *et al.*, 2011). Somatic hybrids were evaluated in the field for various traits including late blight resistance and promising clones were selected for improvement through breeding (Luthra *et al.*, 2016). To our knowledge elite somatic hybrids-derived clones were maximum end up to registration. Objectives of this study were to develop elite clones/ genetic stock by hybridization between potato somatic hybrids and common varieties, and to develop linked markers with late blight resistant somatic parent for genetic fidelity and breeding purposes.

MATERIAL AND METHODS

Plant materials

Interspecific potato somatic hybrids were developed earlier by protoplast fusion between *i*) cultivated *Solanum tuberosum* dihaploid 'C-13' and wild species *S. pinnatisectum* (P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10) for high resistance to late blight (Sarkar *et al.*, 2011, Tiwari *et al.*, 2013b), and *ii*) 'C-13' and *S. etuberosum* E1-1, E1-2, E1-3, E1-7, E2-1, E2-7 and E11) for potato virus Y resistance (Tiwari *et al.*, 2010). These somatic hybrids and common potato varieties (Kufri Pukhraj, Kufri Jyoti, Kufri Sadabahar, Kufri Garima and Kufri Gaurav) were used in the study. Somatic hybrids were maintained under in vitro conditions at the Indian Council of Agricultural Research (ICAR)-Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh, India for research use. Healthy seed tubers of the potato varieties- multiplied routinely via tissue culture at the institute- were used in the study.

Hybridization and development of back-cross progenies

In first year, hybridizations were performed between potato somatic hybrids (considered as F_1 being protoplast fusion product) and varieties, and TPS were produced during the main crop season under natural flowering conditions at high hill of ICAR-CPRI, Regional Station (RS), Kufri, Shimla (31.1°N and 77.2°E; 2500 m above mean sea level). In second year, seedlings were raised from TPS and transplanted in field, and first progeny BC_1-C_1 were selected under sub-tropical plain at ICAR-CPRI, RS, Modipuram (29° N and 76° E; 222 m above mean sea level). Subsequently in third to fifth years, BC_1-C_1 progeny was advanced to BC_1-C_2, BC_1-C_3 progenies and BC_1-C_4 (advanced hybrids) after successive clonal selections in the field trials at Modipuram. A layout of hybridization and progenies development is shown in **Figure 1**.

Tubers were planted by 20-25th October of every year in multiple rows trials consisting of 120 tubers. Plants were dehaulmed at 90 days after planting (DAP) and harvested at 10 days after dehaulming. Advanced hybrids (BC_1-C_4 progeny) were selected based on phenotypes (plant emergence, plant vigour and foliage maturity) and tuber traits (tuber yield, tuber dry matter and tuber characters) as detailed described by Luthra *et al.* (2016). Observations were recorded on various characters such as *i*) plant emergence (emergence of > 95% plants at 45 DAP), *ii*) plant vigour (on 1-5 scale; 1- very poor and 5- very good at 60 DAP), *iii*) foliage maturity (on 1-5 scale; 1- very late and 5- very early at 90 DAP), *iv*) total tuber yield (t/ha), v) total

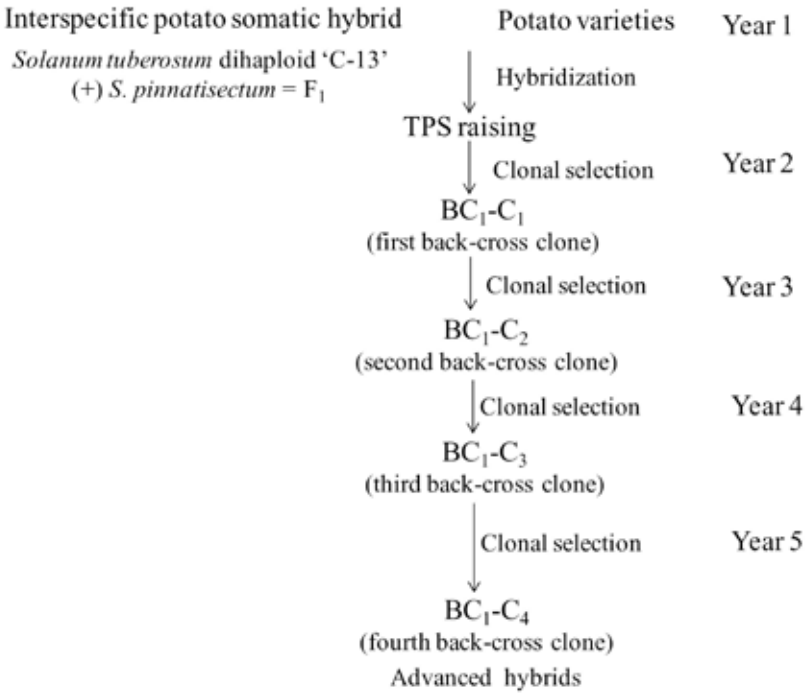

Fig. 1. A schematic presentation of development of back-cross progenies (BC_1-C_1, BC_1-C_2, BC_1-C_3 and BC_1-C_4) by hybridization between interspecific potato somatic hybrids (S. tuberosum + S. pinnatisectum) and Indian varieties.

dry matter (%) and v) tuber characteristics at 90 days crop duration in the order of skin colour, tuber shape, eye depth and flesh colour (Y- yellow, O- oval, Ob- oblong, S- shallow, M- medium, W- white, C- cream, WC- white cream, LY- light yellow). Total dry matter was estimated from tubers by oven drying of 50 g sample at 80°C for 72 h as described by Luthra *et al.* (2016). The quantitative data were statistically analyzed using the software Windostat 8.5 (Ameerpet, Hyderabad, India).

Molecular markers analysis

Total genomic DNA was isolated from leaf tissues of 58 samples (BC_1-C_2) using a DNeasy plant mini kit (Qiagen, Venlo, Limburg, Netherlands). DNA was quantified using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and checked quality on agarose gel 1% (w/v). DNA of 58 samples was used in analysis using 24 ISSR markers **(Table 1)**. The polymerase chain reaction (PCR) mixture (25 µL) included template DNA (100 ng), PCR buffer (1), $MgCl_2$ (2.5 mM), dNTP (200 µM), one primer (0.5 µM), and Taq polymerase (1 U) (Qiagen, Venlo, Limburg, Netherlands). PCR was performed in a thermo-cycler (Life Technologies, Carlsbad, California, USA) following temperature profiles: 94°C/4 min, followed by 35 cycles of 94°C/1 min, 55°C/1 min, and 72°C/1 min; with a final extension at 72°C/7 min. ISSR fragments were resolved on 1% agarose gel. The well resolved and scorable PCR products were scored and a binary data matrix of presence (1)/absence (0) of bands or fragments was prepared. Reactions were repeated twice to confirm the amplification. A tree was constructed based on the Jaccard similarity coefficient following unweighted pair-group method (UPGMA) clustering method using NTSYS-PC 2.21 software (Rohlf, 2006).

Table 1. Primer details used in molecular profiling of 58 BC_1-C_2 progenies of somatic hybrids crossed with Indian potato varieties.

SN	Primer name	Sequence (5′à3′)	Ta (°C)	PIC
1	ISSR1	HVH(TG)7T	55	0.94
2	ISSR2	HVH(TCC)5	55	0.93
3	ISSR5	BDB(TCC)5	55	0.93
4	ISSR10	(AG)8YG	55	0.88
5	ISSR16	(AC)8	55	NA
6	ISSR19	(GCC)5	55	NA
7	ISSR1425	BDV(CAG)5	55	NA
8	ISSR2102	HVH(CTT)5	55	NA
9	ISSR2103	HVH(GTC)5	55	NA
10	ISSR2104	BDB(GAT)5	55	0.93
11	ISSR2105	BDB(GAC)5	55	0.91
12	ISSRMC1	VHV(GT)7G	55	0.79
13	ISSRMC2	DHB(CGA)5	55	0.75
14	ISSRP92	BDB(CA)7	55	0.93
15	ISSRP93	BDB(CAC)5	55	NA
16	ISSRAm2	(CAG)5	55	NA
17	ISSR1424	BDB(CAC)5	55	NA
18	ISSR1428	DBD(AC)7	55	0.95
19	ISSR827	[AC] 8 G	55	0.92
20	ISSR834	[AG] 8 YT	55	NA
21	ISSR841	[GA] 8 YC	55	0.94
22	ISSR857	[AC] 8 YG	55	NA
23	ISSR1417	BDT (CA)7	55	0.95
24	ISSR1423	HVH (TGT)5	55	0.79

NA: not amplified or very faint/minor and non-scorable amplification
PIC: Polymorphic Information Content

Late blight resistance test

Eight advanced hybrids of the BC_1-C_4 progeny was tested for late blight resistance under highly congenial conditions at Kufri hill. Tubers were planted in field in replicated (three replications) trials for two years along with control varieties viz., Kufri Girdhari (highly resistant), Kufri Himalini (moderately resistant) and Kufri Bahar (highly susceptible). Disease reaction was recorded from the date of first lesion on leaf of susceptible cultivar and percentage infections were recorded at weekly intervals for five times till complete loss of the susceptible control. The area under the disease progress curve (AUDPC) was calculated (Shaner and Finney, 1977) and resistance/susceptible genotypes were categorised based on the AUDPC values as highly resistant (HR: < 50), resistant (R: 50-100), moderately resistant (MR: 101-300), and susceptible (S: > 300) (Luthra *et al.*, 2016).

RESULTS AND DISCUSSION

Development of back-cross progenies

Several crosses were made between somatic hybrids and indigenous potato varieties to develop advanced clones with desirable agronomic traits, and resistance derived from somatic hybrids. Total 4071 TPS were generated from 32 crosses, 887 seedlings were transplanted in the field and 181 BC_1-C_1, 58 BC_1-C_2 and 17 BC_1-C_3 progenies were selected. Finally, eight advanced hybrids (BC_1-C_4) were identified as potential clones for further use. The BC_1-C_4 progeny belonged to different crosses such as Kufri Garima Bulk pollen (MSH/14-7); P8 Kufri Jyoti (MSH/14-112, MSH/14-113, MSH/14-115, MSH/14-122, MSH/14-123); and Kufri Gaurav P2 (MSH/14-129 and MSH/14-131). Besides, TPS of various crosses were sown but clones could not be recovered either due to mortality of transplanted seedlings or highly non-desirable tuber traits. Details of hybridization, TPS generated, seedlings transplanted and clones selected are summarized in **Table 2**.

Molecular markers analysis

In search of polymorphic and diagnostic markers linked with late blight resistant somatic hybrids parent, 58 BC_1-C_2 progeny and their parents were characterized using 24 ISSR markers. Fourteen ISSR namely ISSR1 (PIC-0.94), ISSR2 (PIC-0.93), ISSR5 (PIC-0.93), ISSR10 (PIC-0.88), ISSR2104 (PIC-0.93),

Table 2. Summary of back-cross progenies (BC_1C_1 to BC_1C_4) developed by hybridization between interspecific somatic hybrids (*Solanum tuberosum* dihaploid 'C-13' + *S. pinnectisectum*) and Indian potato varieties.

	Year 1		Year 2		Year 3			Year 4			Year 5		
SN	Hybridization (Parents /crosses)	TPS sown	Seedling transplanted	BC_1C_1 selected	BC_1C_1 planted	BC_1C_2 selected	Accession (MSH/14-)	BC_1C_2 planted	BC_1C_3 selected	Accession (MSH/14-)	BC_1C_3 planted	BC_1C_4 selected (Advanced hybrids)	Accession (MSH/14)
1	K. Garima × Bulk pollen	1540	165	37	37	8	4, 5, 7, 17, 18, 22, 23, 32	8	1	7	1	1	7
2	K. Jyoti × Bulk pollen	390	115	35	35	4	57, 58, 60, 69	4	-	-	-	-	-
3	K. Sadabahar × Bulk pollen	290	106	36	36	17	74-109	17	3	87, 92, 103	-	-	-
4	P8 × K. Jyoti	475	40	14	14	7	112, 113, 114, 115, 116, 122, 123	7	7	112, 113, 114, 115, 116, 122, 123	7	5	112, 113, 115, 122, 123
5	P8 × K. Sadabahar	2	1	1	1	1	126	1	-	-	-	-	-
6	K. Gaurav × P2	440	127	26	26	14	128, 129, 131, 135, 137, 140, 141, 142, 143, 144, 145, 148, 151, 152	14	6	129, 131, 137, 142, 143, 144	6	2	129, 131
7	K. Gaurav × P3	490	197	19	19	4	153, 159, 167, 170	-	-	-	-	-	-
8	K. Gaurav × P7	90	34	3	3	1	172	-	-	-	-	-	-
9	K. Gaurav × P8	230	84	7	7	2	176, 181	-	-	-	-	-	-
10	P3 × K. Pukhraj	1	1	1	1	0	-	-	-	-	-	-	-
11	P10 × K. Jyoti	11	1	1	1	0	-	-	-	-	-	-	-
12	E1-2 × K. Pukhraj	3	1	1	1	0	-	-	-	-	-	-	-
13	E1-1 × K. Jyoti	1	-	-	-	-	-	-	-	-	-	-	-
14	E1-2 × K. Jyoti	4	-	-	-	-	-	-	-	-	-	-	-
15	E1-3 × K. Jyoti	2	-	-	-	-	-	-	-	-	-	-	-
16	E1-7 × K. Pukhraj	3	-	-	-	-	-	-	-	-	-	-	-
17	E2-1 × K. Pukhraj	3	-	-	-	-	-	-	-	-	-	-	-
18	E2-7 × K. Jyoti	2	-	-	-	-	-	-	-	-	-	-	-
19	E2-7 × K. Pukhraj	1	-	-	-	-	-	-	-	-	-	-	-
20	E11 × K. Jyoti	4	-	-	-	-	-	-	-	-	-	-	-
21	K. Bahar × Bulk pollen	35	-	-	-	-	-	-	-	-	-	-	-
22	P10 × Kufri Pukhraj	3	2	-	-	-	-	-	-	-	-	-	-
23	P2 × Kufri Jyoti	8	2	-	-	-	-	-	-	-	-	-	-
24	P3 × Kufri Jyoti	10	2	-	-	-	-	-	-	-	-	-	-
25	P3 × Kufri Sadabahar	5	1	-	-	-	-	-	-	-	-	-	-

SN	Year 1 Hybridization (Parents /crosses)	Year 2 TPS sown	Year 2 Seedling transplanted	Year 2 BC_1-C_1 selected	Year 3 BC_1-C_1 planted	Year 3 BC_1-C_2 selected	Year 3 Accession (MSH/14-)	Year 4 BC_1-C_2 planted	Year 4 BC_1-C_3 selected	Year 4 Accession (MSH/14-)	Year 5 BC_1-C_3 planted	Year 5 BC_1-C_4 selected (Advanced hybrids)	Year 5 Accession (MSH/14)
26	P5 × Kufri Jyoti	4	2	-	-	-	-	-	-	-	-	-	-
27	P6 × Kufri Jyoti	2	1	-	-	-	-	-	-	-	-	-	-
28	P6 × Kufri Pukhraj	8	3	-	-	-	-	-	-	-	-	-	-
29	P7 × Kufri Jyoti	2	-	-	-	-	-	-	-	-	-	-	-
30	P2 × Kufri Sadabahar	1	-	-	-	-	-	-	-	-	-	-	-
31	P9 × Kufri Jyoti	10	2	-	-	-	-	-	-	-	-	-	-
32	P9 × Kufri Pukhraj	1	-	-	-	-	-	-	-	-	-	-	-
Total		4071	887	181	181	58	-	58	17	-	14	8	

Genotypes P1-P10 are somatic hybrids of *Solanum tuberosum* dihaploid 'C-13' (+) *S. pinnatisetcum*. Bulk pollen of somatic hybrids P2, P3, P5, P6, P7, P8, P9 and P10; BC_1-C_1, BC_1-C_2, BC_1-C_3 and BC_1-C_4 represent back-cross progenies clonal generations 1, 2, 3 and 4, respectively. Somatic hybrid E1-2 (*S. tuberosum* + *S. etuberosum*); Interspecific somatic hybrids are considered as F_1, as they are fusion product between two parents.

ISSR2105 (PIC-0.91), ISSRMC1 (PIC-0.79), ISSRMC2 (PIC-0.75), ISSRP92 (PIC-0.93), ISSR1428 (PIC-0.95), ISSR827 (PIC-0.92), ISSR841 (PIC-0.94), ISSR1417 (PIC-0.95) and ISSR1423 (PIC-0.79) were polymorphic and others did not amplify in the samples **(Tables 1 and 3)**.

Selected amplification sin P8 Kufri Jyoti-derived all progenies (MSH/14-112, MSH/14-113, MSH/14-114, MSH/14-115, MSH/14-116, MSH/14-122 and MSH/14-123) are shown in **Figure 2**. Well-resolved fragments were observed in ISSR2 (345, 424 and 506 bp) and ISSR841 (467, 707 and 1317 bp). Among them, somatic hybrids-linked diagnostic fragments (ISSR2-424 and ISSR841-1317 bp) were developed for identification of late blight resistant somatic parent P8 and their progenies clones **(Figure 2)**. A cluster tree showing divergence of the progenies was constructed using amplification profiles of ISSR markers **(Figure 3)**.

Field evaluation and late blight test of advanced hybrids

Eight advanced hybrids (BC_1-C_4) were selected based on tuber traits and phenotypic performance in field and data are summarised in **Table 4** and depicted in **Figure 4**. At harvest three advanced hybrids MSH/14-7, -129 and -131 produced 25, 32 and 4%, respectively higher total tuber yield over the control Kufri Bahar- the most popular variety of the region. These hybrids were identified as promising hybrids for future evaluations based on yield performance and may be released as varieties after multi-locations testing. Other five advanced hybrids namely MSH/14-112, -113, -115, -122 and -123 from the cross P8 Kufri Jyoti produced moderate tuber yield, but possessed medium foliage maturity, good plant vigour and high tuber dry matter (19-22%) over the controls. These hybrids were identified as elite genetic

Table 3. Molecular profiling of 58 BC_1C_2 progenies SSR and ISSR markers.

Parents/cross	Accession (MSH/14-)	ISSR fragments amplified (total/polymorphic)
		ISSR2 (3/0), ISSR5 (5/3), ISSR841 (6/5) and ISSR1423 (5/3)
K. Jyoti × Bulk pollen	57, 58, 60, 69	ISSR1 (6/5), ISSR2 (3/2), ISSR5 (3/1), ISSR2104 (4/2), ISSR2105 (6/6), ISSRP92 (6/5), ISSR1428 (8/7), ISSR827 (8/8), ISSR841 (6/3) and ISSR1417 (7/6)
K. Sadabahar × Bulk pollen	74-109	No amplification
P8 × K. Jyoti	112, 113, 114, 115, 116, 122, 123	ISSR1 (11/11), ISSR2 (7/7), ISSR5 (7/7), ISSR10 (10/8), ISSR2104 (4/2), ISSR2105 (8/8), ISSRP92 (9/9), ISSR1428 (6/5), ISSR827 (4/3), ISSR841 (3/2) and ISSR1417 (6/4)
P8 × K. Sadabahar	126	No amplification
K. Gaurav × P2	128, 129, 131, 135, 137, 140, 141, 142, 143, 144, 145, 148, 151, 152	ISSR1423 (2/0), ISSR1 (5/4), ISSR2 (4/2), ISSR5 (3/2), ISSR2104 (4/2), ISSR2105 (4/3), ISSR1428 (4/1), ISSR827 (6/5) and ISSR841 (5/3)
K. Gaurav × P3	153, 159, 167, 170	ISSR1 (6/5), ISSR2 (3/1), ISSR5 (3/1), ISSR2104 (4/3), ISSR2105 (3/3), ISSR1428 (6/5), ISSR841 (6/1), ISSR1417 (6/3) and ISSR1423 (5/2)
K. Gaurav × P7	172	ISSR1 (6/5), ISSR2 (3/3), ISSR5 (3/1), ISSR2104 (5/5), ISSRP92 (8/8), ISSR1428 (7/5), ISSR827 (7/7) and ISSR841 (6/4)
K. Gaurav × P8	176, 181	ISSR1 (5/5), ISSR2 (3/3), ISSR5 (3/3), ISSR10 (4/3), ISSR2104 (4/2), ISSR2105 (4/1), ISSRMC1 (6/2), ISSRMC2 (5/4), ISSR1428 (7/6), ISSR841 (4/2) and ISSR1417 (6/3)

Note: ISSR products were amplified on 1% agarose gel.

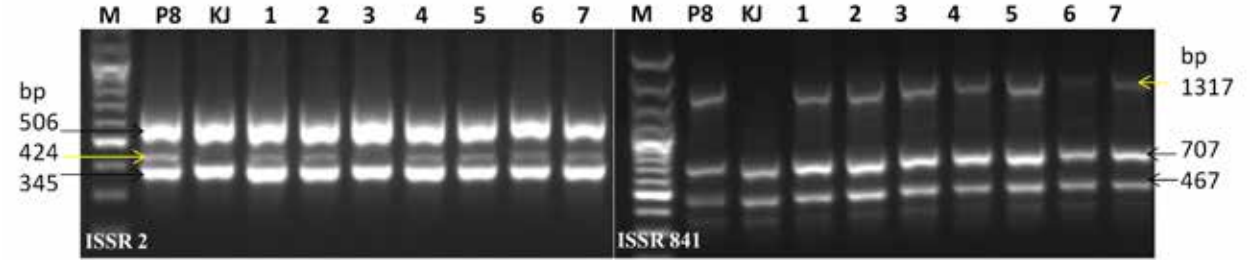

Fig. 2. Selected polymorphic ISSR markers (ISSR2 and ISSR841) showing banding profiles of advanced hybrids developed by crossing between somatic hybrid (S. tuberosum + S. pinnatisectum) P8 and cv. Kufri Jyoti (KJ). Progenies (SN 1-7): MSH/14-112, MSH/14-113, MSH/14-114, MSH/14-115, MSH/14-116, MSH/14-122, MSH/14-123. Somatic hybrid-specific diagnostic bands possessing late blight resistance are indicated with yellow arrows.

material for registration purpose and to be used in breeding programs.

All eight advanced hybrids (MSH/14-7, 112, -113, -115, -122, -123, -129 and -131) were evaluated for late blight resistance for two years in Kufri hill under natural congenial conditions **(Table 4)**. The hybrids MSH/14-112, -113, -115, -122 and -123 were found high resistance to late blight over the years (AUPDC: 5-45), where as hybrids MSH/14-7 (AUPDC: 252-303), MSH/14-131 (AUPDC: 210-230) and MSH/14-129 (AUPDC: 116-137) was moderately resistant.

Potato somatic hybrids (*Solanum tuberosum* dihaploid 'C-13' + wild *S. pinnatisectum*) used in this study were developed earlier via protoplast fusion and possess high resistance to late blight. Following a series of back-crossing with common varieties, a number of hybrid clones (BC_1-C_1, BC_1-C_2, BC_1-C_3 and BC_1-C_4) were developed and advanced hybrids (BC_1-C_4) were selected based on yield performance, elite tuber traits and phenotypes having resistance to late

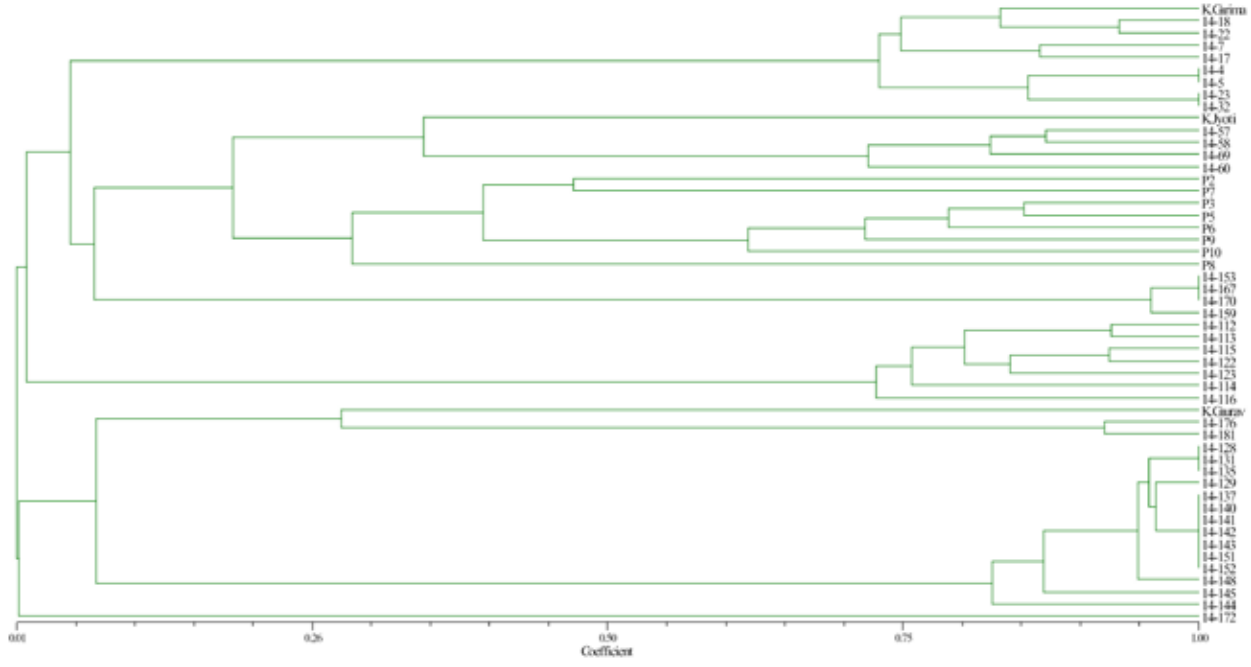

Fig. 3. A cluster tree of somatic hybrids progenies and hybridization parents showing distinctness using UPGMA method based on the Jaccard similarity coefficient of ISSR markers data.

Fig. 4. Tuber phenotypes of eight advanced hybrids (BC_1-C_4) progenies.

blight. Eight advanced hybrids (MSH/14-7, 112, -113, -115, -122, -123, -129 and -131) were selected for registration as elite genetic stock and multi-locations testing for release as varieties. These somatic hybrids demonstrate a successful introgression of desirable traits from *S. pinnatisectum* into cultivated potato (*S. tuberosum*). A few researchers have developed advanced progenies using somatic hybrids as a parent in breeding (Cardi *et al.*, 2002; Caruso *et al.*, 2008). Interestingly, we attempted reciprocal crosses to see behaviour of somatic hybrids during the crossing. Somatic hybrids as female parents transferred late blight resistance trait, while as male parent increased yield with moderate resistance to late blight under highly congenial natural condition. It shows that cytoplasmic transfer played an important role in transfer of trait from the somatic hybrids into cultivated varieties. Further, the BC_1-C_1 progenies were characterized using ISSR markers, and identified ISSR alleles (ISSR2-424 and ISSR841-1317 bp) linked with late blight resistant parent P8 and their progenies. ISSR is a dominant marker system and it would augment identification of P8 derived progenies/clones at early stage selection using simple agarose gel system. Many molecular markers have been used in potato breeding for late blight resistance (Tiwari *et al.*, 2013),

Table 4. Field evaluation and late blight test of eight advanced hybrids (BC_1-C_4) of interspecific potato somatic hybrids (*S. tuberosum* dihaploid 'C-13' + *S. pinnatisetcum*) crossed with Indian varieties.

SN	Genotype/ parents	Plant emergence (%)	Plant vigour	Foliage maturity	Total tuber yield (t/ha)	Total dry matter (%)	Tuber characters	Late blight reaction (AUDPC)		
								Year I	Year II	Class
	MSH/14-7 (K. Garima × Bulk pollen)	89.17	5.00	3.00	42.18	16.81	Y, O, S, Y	283.3	252.0	MR
	MSH/14-112 (P8 × K. Jyoti)	95.00	4.50	3.50	23.58	21.09	W, O, S, W	45.3	25.7	HR
	MSH/14-113 (P8 × K. Jyoti)	80.00	5.00	1.00	29.10	20.29	W, O, S, W	38.3	10.5	HR
	MSH/14-115 (P8 × K. Jyoti)	89.17	3.00	3.50	25.82	21.94	W, Ob, S, C	5.0	11.65	HR
	MSH/14-122 (P8 × K. Jyoti)	94.74	4.00	3.50	24.14	22.35	WC, O, S, C	9.3	36.75	HR
	MSH/14-123 (P8 × K. Jyoti)	86.67	4.50	3.00	33.42	18.70	WC, O, S, C	95.8	71.15	R
	MSH/14-129 (K. Gaurav × Bulk pollen)	65.83	5.00	3.00	44.34	15.69	Y, O, S, Y	237.3	116.1	MR
	MSH/14-131 (K. Gaurav × Bulk pollen)	86.67	4.50	3.00	35.06	15.55	Y, O, S, C	230.8	210.0	MR
	K. Bahar	87.50	4.50	3.50	33.61	20.47	W, O, M, W	nd	nd	nd
	K. Garima	93.33	5.00	3.00	47.06	18.49	LY, O, S, LY	nd	nd	nd
	K. Mohan	81.67	5.00	3.50	55.27	15.81	WC, O, S, C	nd	nd	nd
	K. Pukhraj	85.00	5.00	4.00	43.81	16.39	L, O, M, Y	nd	nd	nd
	K. Himalini	nd	nd	nd	nd	nd	nd	119.3	133.0	MR
	K. Gardhari	nd	nd	nd	nd	nd	nd	0	0	HR
	K. Jyoti	nd	nd	nd	nd	nd	nd	332.2	360.0	S
	Mean	86.22	4.58	3.12	36.44	18.63	-	126.96	111.53	-
	CD (at 5%)	2.17	0.42	0.12	1.84	0.61	-	2.06	1.91	-

K.: Kufri; Bulk pollen: mixed pollens of somatic hybrids P2, P3, P5, P6, P7, P8, P9 and P10; Plant emergence (%), Plant vigour at 60 days based on 1 to 5 scale (1: Very week to 5: Highly vigorous); Foliage maturity at 90 days based on 1 to 5 scale (1: Very late i.e. green to 5: Very early i.e. dry); Tuber traits at 90 days crop duration in the order of skin colour, tuber shape, eye depth and flesh colour (Y- yellow, O- oval, Ob- oblong, S- shallow, M- medium, W- white, C- cream, WC- white cream, LY- light yellow); Late blight resistance class is based on the area under disease progressive curve (AUDPC): highly resistant (HR: < 50), resistant (R: 50-100), moderately resistant (MR: 101-300), and susceptible (S: >300) (Luthra *et al.* 2016); nd: not determined.

and particularly SSR markers in potato characterization (Tiwari *et al.*, 2013, 2018).

Earlier studies show successful development of advanced self and back-cross progenies of somatic hybrids by crossing with cultivated potato. Improvement for traits such as yield, tuber traits (number and weight) and morphology were observed in progenies over parents (Möllers *et al.*, 1994; Carrasco *et al.*, 2000). The exploitation of wild species-derived somatic hybrids resulted in transfer of multiple traits into cultivated potato such as higher tuber yield (Pavek and Corsini, 2001), and useful traits in F_2 progeny (*S. commersonii*: Cardi *et al.*, 2002); higher tuber yield, better quality, and late blight and PVY resistance in BC_1 (*S. tarnii*: Thieme *et al.*, 2008); and late blight resistance (*S. michoacanum*: Smyda-Dajmund *et al.*, 2017). Recently, based on the field studies we identified potential somatic hybrids (C-13 + *S. pinnatisectum*) for breeding applications for the sub-tropical plain conditions- where most potato is grown in India, yield, high tuber dry matter, better storage quality and late blight resistance are important traits for breeding (Luthra *et al.*, 2016).

To conclude, somatic fusion technique has proven that is efficient in developing cultivated potato genotypes with elite traits. The advanced hybrid clones (BC_1-C_4) were developed with good agronomic traits (yield, tuber traits and dry matter), late blight resistance, and ISSR alleles linked with resistant parent P8 for genetic fidelity and breeding purposes. These advanced clones have potential for registration and release as new potato varieties. In future, characterization using genomics tool like more molecular markers, fragment sequencing and chromosomal location would be attempted to understand behaviour of somatic hybrids-derived progenies.

ACKNOWLEDGEMENTS

The authors are grateful to the Competent Authority, ICAR-Central Potato Research Institute, Shimla for providing necessary supports under the Biotechnology program (HORTCPRICIL201500300131).

LITERATURE CITED

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, Tiwari JK (Eds.) (2017) The Potato Genome. Springer International Publishing AG 2017. DOI https://doi.org/10.1007/978-3-319-66135-3.

Chakrabarti SK, Singh BP, Thakur G, Tiwari JK, Kaushik SK, Sharma S and Bhardwaj V (2014) QTL mapping underlying resistance to late blight in a diploid potato population of *Solanum spegazzinii S. chacoense*. *Potato Res* **57**: 1–11.

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.

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.

Machida-Hirano R and Niino T (2017) Potato Genetic Resources In: Chakrabarti SK, Conghua X, Tiwari JK (eds) The Potato Genome. Compendium of Plant Genomes. Springer, Cham. DOI https://doi.org/10.1007/978-3-319-66135-3_2

Moller C, Frei U and Wenzel G (1994) Field evaluation of tetraploid somatic potato hybrids. *Theor Appl Genet* **88**, 147-52.

Pavek JJ and Corsini DL (2001) Utilization of potato genetic resources in variety development. *Am J Potato Res* **78**:433–441.

Rohlf F (2006) NTSYSpc: Numerical taxonomy system (ver. 2.2). Exeter Publishing, Ltd., Setauket, NY, USA

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.

Shaner G and Finney RF (1977) The effect of nitrogen fertilization on the expression of slow mild-mildewing resistance in knox wheat. *Phytopathology* **67**, 1051-1056.

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* DOI 10.1007/s12230-017-9568-2

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

Tiwari JK, Kumar V, Devi S, Luthra SK, Chakrabarti SK, Rawat S, Nagesh M (2017) Genomics in management and genetic enhancement of potato germplasm. In: Chakrabarti SK, Conghua X, Tiwari JK (eds) The Potato Genome. Compendium of Plant Genomes. Springer, Cham. DOI https://doi.org/10.1007/978-3-319-66135-3_8

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

Tiwari JK, Devi S, Sharma S, Chandel P, Rawat S and Singh BP (2015a) Allele mining in *Solanum* germplasm: cloning and characterization of RB-homologous gene fragments from late blight resistant wild potato species. *Plant Mol Biol Rep* **33**: 1584–1598.

Tiwari JK, Devi S, Sundaresha S, Chandel P, Ali N, Singh B, Bhardwaj V and Singh BP (2015b) Microarray analysis of gene expression patterns in the leaf during potato tuberization in the potato somatic hybrid *Solanum tuberosum* and *Solanum etuberosum*. *Genome* **58**, 305–313.

Tiwari JK, Gopal J and Singh BP (2012) Marker-assisted selection for virus resistance in potato: options and challenges. *Potato J* 39: 101-117.

Tiwari JK, Poonam, Kumar V, Singh BP, Sharma S, Luthra SK and Bhardwaj V (2013b) Evaluation of potato somatic hybrids of dihaploid *S. tuberosum* (+) *S. pinnatisectum* for late blight resistance. *Potato J* **40**: 176-179.

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, Sundaresha S, Singh BP, Kaushik SK, Chakrabarti SK, Bhardwaj V and Chandel P (2013c) Molecular markers for late blight resistance breeding of potato: an update. *Plant Breed* **132**: 237–245.

MS received: 12 March 2018; Accepted: 28 June 2018