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Short Communication

Genetic diversity of different late blight responsive Indian potato cultivars as revealed by SSR markers

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Abstract

Forty seven resistant and susceptible to late blight Indian potato cultivars were used to assess genetic diversity present among them. A set of 24 microsatellite markers applied which generated 193 alleles with an average of 7.04 per loci and 4.10 per genotype. Eight alleles were specific to resistant cultivars, whereas 11 were specific to susceptible. Genetic diversity among the moderately resistant genotypes was found to be higher than the susceptible and resistant ones. The cluster analysis grouped the cultivars into 2 main and 4 sub clusters with late blight resistant genotypes distributed across the clusters. The diverisity among the genotypes was very high; however, diversity within groups *viz*., resistant, moderately resistant and susceptible was found to be very low (6%). The findings of the study would be of great help to the breeders in selecting genotypes for developing improved varieties of potato.

Key words: Microsatellite, gene pool, late blight, disease, linkage, similarity coefficient

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop of the world after rice and wheat. At present, India ranked second only to China in terms of annual production and both countries together contribute more than half of the global potato production (Patil et al. 2016). Last decade has witnessed an increase of 51.70% in its production in India and stands top among the vegetables (FAOSTAT 2018). Despite the rapid progress, potato production in India suffers from recurrent late blight *Phytophthora infestans* (Mont.) attack in epiphytotic form every year and causes tremendous losses (Sharma et al. 2016; Patil et al. 2017). To protect the crop from the blight, farmers spent a huge amount of money on fungicides. Incorporation and improving genetic resistance to *P. infestans* with careful selection of parents and better utilization of heterosis is an increasingly important aspect of potato breeding (Vanishree et al. 2016). Simple sequence repeats (SSRs, or microsatellites) have been used to great benefit in potato for studies on diversity, genetic structure, classification and even linking resistance trait (Tiwari et al. 2013). Few studies estimating the genetic diversity between and within late blight resistant and susceptible potato varieties have been done using morphological characters (Muhammed 2014), isozymes (Bisognin and Douches 2002) and RAPD markers (Pattanayak et al. 2002; El-Komy et al. 2012) but it is well known fact that microsatellite markers reveal greater clarity and depth of diversity than earlier markers used. Therefore, present study was carried out to estimate the genetic diversity among the late blight resistant and susceptible Indian potato cultivars based on 24 highly informative SSR markers.

Forty seven Indian potato varieties released by the ICAR-Central Potato Research Institute were grown under disease free tissue culture conditions. Total genomic DNA was extracted from 27 days old plants following modified CTAB procedure (Doyle and Doyle 1987) and its quantity as well as quality was determined with NanoDrop 2000 (Thermo Scientific) and 0.8% agarose gel respectively. Standard protocol of PCR

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reaction was carried out using highly informative microsatellite-based genetic identity kit for potato (Tiwari et al. 2013). The electrophorograms were used for the diversity analysis using NTSYS-pc (2.02j) (Rohlf 2001). DNA amplification profiles were scored in a binary fashion with ‘0’ indicating absence and ‘1’ indicating presence of a band. A similarity matrix on the basis of band sharing was calculated from the binary data using Dice coefficient (Nei and Li 1979) and were graphically expressed using the group average agglomerative clustering to generate dendrogram. The degree of polymorphism between late blight resistant and susceptible potato cultivars was calculated for each primer from the binary data matrix by using Shannon’s index of phenotypic diversity having equations (Paul et al. 1997): H_0 = “$SP_i lnP_i$, where, P_i is the frequency of phenotype i and H_0 is genetic diversity within late blight resistant (R), moderate resistant (MR) and susceptible (S) cultivars (groups) detected by a particular primer. $H_{0(P)} = 1/3[H_{0(R)} + H_{0(S)} + H_{0(MR)}]$, where, $H_{0(P)}$ is average diversity in the R, S and MR groups revealed by a particular primer. H_{Sp} = “S $P_i lnP_i$, where, H_{Sp} is diversity in all the 47 cultivars considered together for a particular primer. H_0, $H_{0(P)}$ and H_{Sp} were calculated for all the primers and the average estimate of 24 primers was calculated. Then proportion of diversity present within populations was calculated as $H_{0(P)}/H_{Sp}$ and compared with that between populations, $(H_{Sp} – H_{0(P)})/H_{Sp}$.

All the 47 Indian potato cultivars which consisted of 2 clonal selections, 2 full sibs, 20 half sibs involving different common parents, 2 grand half sibs (common maternal grandparent) and 11 with distinct parents (Table 1) were grouped into 13 R, 20 MR and 14 S cultivars based on their reaction to disease caused by *P. infestans* (Singh et al. 2018). Screening with 24 SSR markers revealed a total of 193 alleles with 100% polymorphism. The average number of alleles per locus was 7.04 (Table 2). Four markers STG0010, STI0004, SYM1052 and STPoAc58 had the highest number of alleles *i.e*., 11. Further, 8 alleles were found specific to R cultivars, whereas 11 were specific to S but none found specific to MR cultivars. They shared all the alleles either with R or S cultivars. El-Komey et al. (2012) reported one RAPD marker UBC_{720bp} generating specific fragment only by the late blight susceptible and moderately resistant cultivars while screening with 9 potato varieties using RAPD and SSR markers and also obtained 296 (57.4%) and 29 (93.1%) polymorphic alleles respectively. A total of 180 alleles were obtained with 13 R cultivars, of which 174 were found to be polymorphic alleles (96.67%) and a total of 184 alleles were obtained with 14 S cultivars of which 181 found to be polymorphic (98.34%). Whereas, of 187 total alleles obtained with 20 MR cultivars, 185 were polymorphic in nature (98.93%). Bisognin and Douches (2002) obtained 42 polymorphic alleles while assessing the genetic diversity among late blight resistant and susceptible potato germplasm (33 diploid and 27 tetraploid) with isozyme loci and SSR markers and also reported 23 allozymes unique to wild species. The diversity within the MR (2.21) cultivars was found to be greater than the S (2.21) and R (2.13) potato cultivars as estimated by allele frequencies detected by each marker. Overall diversity of all 47 Indian potato cultivars was estimated to be 2.34 which higher than diversity found among the individual groups. These results with SSR markers are in line with study by Pattanayak et al. (2002). The estimation of diversity specially among the disease resistant and susceptible cultivars in potato is not only restricted to late blight but also extend to bacterial wilt and Potato Virus Y (PVY) resistance and susceptibility (Carputo et al. 2013). Marker STI0001 alone showed high diversity among the S potato cultivars (3.45), whereas, STI0012, STI0032, STM1052 and STPoAc58 revealed greater diversity both in R and MR cultivars. The average proportion of diversity present within the Indian potato cultivars (R+MR+S) was found to be 94% whereas, the proportion of diversity between the cultivars of R, MR and S groups was found to be low (6%).

Cultivars were grouped based on Nei and Lie coefficient for genetic similarities and dendrogram obtained 2 major and 4 minor clusters. More divergence was found between Kufri Garima (R) and other varieties (0.42), whereas Kufri Kuber (R) and Kufri Jeevan (MR) were found to be genetically similar. The results are in line with many previous genetic diversity studies made on Indian potato cultivars (Sharma and Nandineni 2014; Vanishree et al. 2016). The low genetic diversity found in Indian potato cultivars is attributed to the sharing of common parents (2 full sibs, 20 half sibs and 2 grand half sibs). The study also showed no clear association among the potato cultivars for late blight resistance, moderately resistance and susceptibility. Previous studies assessing the diversity among late blight resistance and susceptible potato lines have also reported the absence of clear association between them (Pattanayak et al. 2002; Bisognin and Douches 2002; Carputo et al. 2013) and to our knowledge this is the first report of estimating the diversity within and between the late blight resistant and susceptible Indian potato cultivars using SSR markers.

Table 1. A list of Indian potato cultivars, their parentage, year of release and the magnitude of late blight resistance

S.No.	DUS Cultivar	Selection	Parentage	Year of release	Late blight resistance
1	Kufri (K) Red	-	Clonal selection from Darjeeling Red Round	1958	S
2	K. Safed	-	Clonal selection from Phulwa	1958	S
3	K. Kuber	ON 2236	(*S.curtilobum* x *S.tuberosum*) x *S.andigenum*	1958	S
4	K. Kumar	S 1758	Lumbri x Katahdin	1958	MR
5	K. Kundan	Hybrid 9	Ekishirazu x Katahdin	1958	MR
6	K. Neela	A 1528	Katahdin x Shamrock	1963	MR
7	K. Sindhuri	C 140	K. Red x K. Kundan	1967	S
8	K. Sheetman	C 3745	Craigs Defiance x Phulwa	1968	MR
9	K. Jeevan	SLB/E 427	M 109-3 x Seedling 698-D	1968	MR
10	K. Naveen	SLB/E-402	3070d(4) x Seedling 692-D	1968	MR
11	K. Jyoti	SLB/Z- 389(b)	3069d(4) x 2814a(1)	1968	S
12	K. Alankar	A 3649	Kennebec x ON 2090	1968	MR
13	K. Chamatkar	ON 1202	Ekishirazu x Phulwa	1968	S
14	K. Khasigaro	SLB/A-67	Taborky x Seedling 698-D	1968	MR
15	K. Muthu	SLB/Z-785	3046(1) x M109-3	1971	MR
16	K. Lauvkar	A 7416	Serkov x Adina	1972	S
17	K. Dewa	C 3804	Craigs Defiance x Phulwa	1973	S
18	K. Badshah	JF 4870	K. Jyoti x K. Alankar	1979	R
19	K. Bahar	E 3797	K. Red x Gineke	1980	S
20	K. Lalima	BS/C-1753	K. Red x AG 14 (Wis. x 37)	1982	S
21	K. Sherpa	F 5242	Ultimus x Adina	1983	R
22	K. Swarna	PCN/76- 110	K. Jyoti x $(VTn)^2$ 62.33.3	1985	R
23	K. Megha	SS/C-562	SLB/K-37 x SLB/Z-73	1989	R
24	K. Ashoka	PJ 376	EM/C -1020 x Allerfruheste Gelbe	1996	S
25	K. Sutlej	JI 5857	K. Bahar x K. Alankar	1996	MR
26	K. Jawahar	JH 222	K. Neelamani x K. Jyoti	1996	MR
27	K. Chandramukhi	A 2708	Seedling 4485 x K. Kuber	1968	S
28	K. Pukhraj	JEX/C-166	Craigs Defiance x Jex/B-687	1998	MR
29	K. Chipsona-I	MP/90-83	MEX.750826 x MS/78-79	1998	R
30	K. Chipsona-II	MP/91-G	F-6 x QB/B92-4	1998	R
31	K. Giriraj	SM/85-45	SLB/J-132 x EX/A 680-16	1998	MR
32	K. Anand	MS/82-717	K. Ashoka x PH/F-1430	1999	MR
33	K. Kanchan	SE/I-1307	SLB/Z-405(a) x Pimpernel	1999	MR
34	K. Arun	MS/92- 2105	K. Lalima x MS/82-797	2005	MR
35	K. Pushkar	JW 160	QB/A 9-120 x Spatz	2005	R
36	K. Shailja	SM/87-185	K. Jyoti x EX/A 680-16	2005	MR
37	K. Surya	HT/92-621	K. Lauvkar x LT-1	2006	S
38	K. Himalini	SM/91- 1515	I-1062 x Tollocan	2006	MR
39	K. Chipsona-III	MP/97-583	MP/91-86 x K. Chipsona-II	2006	R
40	K. Girdhari	SM/93-237	K. Megha x Bulk Pollen of 10 genotypes	2008	HR
41	K. Himsona	SM/91- 1515	MP/92-35 x K. Chipsona II	2008	MR
42	K. Khyati	J/93-86	MS/82-638 x K. Pukhraj	2008	R
43	K. Sadabahar	MS/93- 1344	MS/81-145 x PH/F-1545	2008	MR
44	K. Frysona	MP/98-71	MP/92-30 x MP/90-94	2009	R
45	K. Gaurav	JX576	JE 812 x K. Jyoti	2012	S
46	K. Chipsona-IV	MP/01-916	Atlantic x MP/92-35	2010	R
47	K. Garima	MS/99- 1871	PH/F 1045 x MS/82-638	2012	R

Source: CPRI, Technical Bulletin No. 12, 27, 51 and 78 (2011) and Singh et al. (2018); MR = Moderately resistant, R = Resistant, S = Susceptible and NT=Not tested

Table 2. The polymorphism and diversity found among the Resistant, Moderately resistant and Susceptible potato cultivars

S.No.	Marker	Resistant			Moderately resistant			Susceptible			All cultivars			$H_{0(P)}$	$H_{0(P)}/H_{Sp}$	$(H_S-H_{0(P)})/H_{Sp}$
		Total alleles	Poly. alleles	$H^*_{0(R)}$	Total alleles	Poly. alleles	$H^{\wedge}_{0(MR)}$	Total alleles	Poly. alleles	$H^{\#}_{0(S)}$	Total alleles	Poly. alleles	$H^{+}_{0(Sp)}$			
1	STG0001	9	8	2.37	9	9	2.43	9	9	2.45	9	9	2.49	2.42	0.97	0.03
2	STG0010	10	10	3.06	11	11	2.76	11	11	3.04	11	11	3.11	2.95	0.95	0.05
3	STG0016	9	8	2.52	9	8	2.78	9	9	2.72	9	9	2.81	2.67	0.95	0.05
4	STG0025	7	7	2.18	7	7	2.35	7	7	2.38	7	7	2.39	2.30	0.96	0.04
5	STI0001	10	10	2.95	11	11	2.95	11	11	3.45	11	11	3.15	3.00	0.95	0.05
6	STI0003	7	7	2.35	7	7	2.28	7	7	2.02	7	7	2.39	2.33	0.97	0.03
7	STI0004	6	5	1.03	6	6	1.69	6	5	1.58	6	6	1.60	1.43	0.89	0.11
8	STI0012	11	11	3.16	11	11	3.21	11	11	3.38	11	11	3.40	3.25	0.96	0.04
9	STI0014	7	7	2.23	9	9	2.80	9	9	2.44	9	9	2.78	2.49	0.90	0.10
10	STI0030	7	7	2.37	9	9	2.65	9	9	2.80	9	9	2.77	2.61	0.94	0.06
11	STI0032	10	10	3.05	10	10	3.13	10	10	2.89	10	10	3.13	3.02	0.96	0.04
12	STI0033	8	8	2.09	7	7	2.01	8	8	2.40	8	8	2.38	2.17	0.91	0.09
13	STM0019	8	7	2.23	8	8	2.60	8	8	2.60	8	8	2.60	2.48	0.95	0.05
14	STM0031	6	6	1.91	7	7	2.23	7	7	1.97	7	7	2.24	2.03	0.91	0.09
15	STM0037	4	4	1.29	4	4	1.09	4	4	1.24	4	4	1.22	1.21	0.99	0.01
16	STM1052	10	10	3.12	10	10	3.22	8	8	2.61	11	11	3.25	2.98	0.92	0.08
17	STM1053	3	3	0.72	3	3	0.87	3	3	0.69	3	3	0.79	0.76	0.96	0.04
18	STM1064	7	7	1.72	8	7	2.15	7	7	1.46	8	8	1.99	1.78	0.89	0.11
19	STM1104	8	7	1.86	8	8	2.16	8	7	2.27	9	9	2.26	2.10	0.93	0.07
20	STM1106	7	7	2.10	7	7	1.95	7	7	2.18	7	7	2.14	2.08	0.97	0.03
21	STM5114	6	5	1.02	5	5	1.24	7	7	1.64	7	7	1.46	1.30	0.89	0.11
22	STM5121	4	3	0.73	4	4	0.79	4	3	0.73	4	4	0.78	0.75	0.96	0.04
23	STM5127	7	7	2.10	6	6	1.86	5	5	1.39	7	7	1.92	1.78	0.93	0.07
24	STPoAc58	10	10	3.00	11	11	3.03	9	9	2.68	11	11	3.05	2.90	0.95	0.05
Total/Average		**181**	**174**	**2.13**	**187**	**185**	**2.26**	**184**	**181**	**2.21**	**193**	**193**	**2.34**	**2.20**	**0.94**	**0.06**

Poly. = Polymorphic; $H^*_{0(R)}$ = Genetic diversity among the resistant varieties, $H^{\wedge}_{0(MR)}$ = Genetic diversity among moderately resistant cultivars, $H^{\#}_{0(S)}$ = Genetic diversity among the susceptible cultivars, $H^{+}_{0(Sp)}$ = Genetic diversity among all the Indian potato cultivars, $H_{0(P)}$ = Genetic diversity detection capacity of particular marker, $H_{0(P)}/H_{sp}$ = Diversity present within the population and $(H_{sp}-H_{0(P)})/H_{sp}$ = Diversity present between the population

Quantification of genetic diversity present within the varieties by various molecular markers would be of immense help for the breeders to improve inheritable disease resistance in potato through selection of efficient and diverse parents. The present study demonstrates the successful utilization of SSR markers to assess the genetic variability within and between the late blight resistant and susceptible potato cultivars. The specific alleles identified for both resistant and susceptible potato cultivars may be further utilized to associate with disease and put to use in breeding programs.

Authors' contribution

Conceptualization of research (VUP, SKC, VB); Designing of the experiments (VUP, GV, VB); Contribution of experimental materials (VB, SKC, GV); Execution of field/lab experiments and data collection (VUP, GV, LS); Analysis of data and interpretation (VUP, VB, GB); Preparation of manuscript (VUP, VB, GB, SKC).

Declaration

The authors declare no conflict of interest.

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