Potato J (2020) 47 (1): 46-53

GENETIC DIVERSITY OF DIFFERENT CAROTENOID CONTAINING INDIAN POTATO CULTIVARS AS REVEALED BY SSR MARKERS

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ABSTRACT: Specialty potatoes, particularly rich in nutrients have gained importance in recent times which has lead formulation of separate breeding programs. Characterizing the genetic base of essential cultivars in any crop is very important for the breeders to utilize and improve it through these breeding programs. Forty one Indian potato cultivars characterized for their total carotenoid content were grouped into two, 24 with low carotenoid (≤ 100 µg/100g f.w.) content and 17 with medium (100 - 350 µg/100g f.w.), based on their total carotenoid content. Twenty four nuclear microsatellite markers were used to assess the genetic diversity present within and between the different groups which generated a total of 177 alleles with an average of 7.37 per loci and 4.01 per genotype. Four alleles were found specific to potato cultivars with low carotenoids content, whereas, none were found specific to medium carotenoids containing cultivars. Genetic diversity among the low carotenoid varieties was found to be higher (2.31) than the medium containing (2.26). The cluster analysis grouped all the 41 varieties into 2 main clusters and 4 sub clusters with no clear relation between diversity and total carotenoid content. The average proportion of diversity present within the Indian potato cultivars (low + medium) was found to be 98%, whereas, the proportion of diversity between the cultivars of low and medium carotenoid containing was found to be very low (2%). The analyzed diversity among the different carotenoid containing Indian potato cultivars would be of great help to breeders as many of these are used as parents to develop new and improved potato varieties.

KEYWORDS: Microsatellite, gene pool, yellow flesh, similarity coefficient

INTRODUCTION

Potato is a significant source of energy with 80% water and 20% dry matter of which more than 75% is the carbohydrate/ energy (Patil *et al*., 2016). Though, potato is considered as energy rich food, it also consists of wide range of bioactive phyto-chemicals including anthocyanin and carotenoids highly desirable in human diet. In the last decade, an increased interest has been raised on the study of potatoes as an excellent source of dietary phyto-nutrients, such as phenolics, flavonoids, folates, carotenoids and anthocyanin (Ezekiel *et al*., 2013) and importance is given around the world for breeding specialty potatoes and potatoes with increased nutritional content (Brown, 2005).

Carotenoids are effective antioxidants with important health promoting functions such as provitamin A activity, immune system booster, suppresser of cardiovascular disease or cancer and also help in prevention of atherosclerosis (Lachman *et al*., 2016). In plant, they help in photosynthesis and are one of the major constituents contributing to total antioxidant capacity of potato tubers through their ability to neutralize free radicals by donor electron, without becoming active free radicals. Major antioxidant carotenoids in the potato are lutein and zeaxanthin (42-66%) and β carotene is either absent or present in traces (Brown, 2005). It is a genetic factor and is least effected by environmental and cultivation practices (Kotikova *et al*., 2007). In general, yellow fleshed tubers contain more carotenoids than the white fleshed ones and is controlled by a single locus (*Y/y*) mapped on to chromosome 3 of potato (Bonierbale *et al*., 1988). The total carotenoid content

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in potato tubers ranges from 0.5 to 1 mg/kg fresh matter (f.m.) in white fleshed and to 20 mg/Kg in yellow fleshed potatoes (Brown, 2005). It is reported that some of the cultivated diploid potato contain even >2000 µg/100g potato fresh weight and predicted that high carotenoid in cultivated tetraploid is derived from *S. phureja*, a diploid species endemic to the Andean mountains (Brown *et al.*, 1993). Looking at the increasing importance of phyto-nutrients in potato mainly carotenoids, very recently we had characterised major Indian potato cultivars for their total carotenoid content (Patil *et al.,* 2020). Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources (Patil *et al.,* 2012). The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Therefore, the present study was carried out to estimate the genetic diversity present among the different carotenoid containing cultivars based on the highly informative SSR markers.

MATERIALS AND METHODS

Plant material and DNA extraction

Forty one Indian potato cultivars, comprising 24 low and 17 medium carotenoid containing cultivars (Singh *et al.*, 2018; Patil *et al.,* 2020), were grown under disease free tissue culture conditions for 27 days. Total genomic DNA was extracted by following modified CTAB procedure (Doyle and Doyle, 1987) and its quantity and quality was determined with NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA) and 0.8% agarose gel electrophoresis respectively. The DNA was than diluted to 5 ng/µl for the diversity assay using SSR markers.

Simple Sequence Repeats analysis

Polymerase Chain Reaction (PCR) for SSR analysis was carried out using robust and highly informative microsatellite-based genetic identity kit for potato (Ghislain *et al.*, 2009; Tiwari *et al.*, 2013) and these markers containing 24 set of SSR primers (2 SSRs/linkage group) were synthesized from Sigma-Aldrich. PCR was carried out in a reaction volume of 12 µl containing 5µL EmeraldAmp® GT PCR Master Mix (Takara Bio) along with 5 µl (5ng/µL) genomic DNA, 1 µl (5 pM) each primer and samples were carried through 33 cycles on GeneAmp® PCR system 9700 (Applied Biosystems, USA) thermal cycler by using the following temperature sequence: 94 °C for 1 min, 1 min at annealing temperature (T_m) (**Table 2**) and 72 °C extension for 1 min. Cycles were preceded by denaturation for 4 min at 94 °C and followed by a final extension at 72 °C for 7 min. Amplified products were initially checked on agarose gel electrophoresis (2.5% in Tris-borate buffer). Finally the separation of the PCR products were done on BioAnalyser 2100 (Agilent Technologies, USA) using DNA 1000 separation kit (having size range of 25-1000bp and resolution capacity of ±5bp). The separated PCR products were shown in electrophorograms, which were further used for the diversity analysis using NTSYS-pc (version 2.02j) Software.

Data analysis

DNA amplification with each primer was assayed twice. DNA fragment 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). Similarities were graphically expressed

using the group average agglomerative clustering to generate dendrogram. The analysis was done using the software package NTSYS-pc (version 2.02j) (Rohlf, 2001). Principal Coordinate (PCO) analysis from the binary data was performed to graphically recapitulate associations among the cultivars using NTSYS-pc. The degree of polymorphism between low and medium total carotenoid containing cultivars was calculated for each primer from the binary data matrix by using Shannon's index of phenotypic diversity from the following equations (Paul *et al*., 1997): $H_0 = -\Sigma P_i \ln P_i$, where, P_i is the frequency of phenotype i and H_0 is genetic diversity within low and medium carotenoid containing cultivars (groups) detected by a particular primer. The genetic diversity detection capacity of particular marker, $H_{0(P)} = 1/3[H_{0(L)} + H_{0(M)}]$, where, $H_{0(L)}$ is average diversity in low and $H_{0(M)}$ in medium carotenoid groups revealed by a particular primer. $H_{Sp} = -\Sigma P_i \ln P_i$, where, H_{Sp} is diversity in all the 42 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 calculate as $H_{0(P)}/H_{Sp}$ and was compared with that between populations, $(H_{Sp} - H_{0(P)})/H_{Sp}$.

RESULTS AND DISCUSSION

All the 41 Indian potato cultivars were grouped into 24 low carotenoids (≤ 100 µg/100g f.w.) containing and 17 medium (100 - 350 µg/100g f.w.) carotenoid containing varieties based on their total carotenoid content (Singh *et al.*, 2018; Patil *et al.*, 2020) (**Table 1**). These were screened with highly informative and robust 24 microsatellite markers and analyzed both separately as well as combining all of them. A total of 177 alleles were obtained with 99.43% polymorphism and 7.37 average number of alleles per locus and 4.11 mean number of alleles per genotype. The higher mean of allele per locus may be due to multi loci marker like STM0019, that amplifies more than one locus of tetraploid potato genome (Sharma and Nandineni, 2014) or due to the consideration of stutter bands that are artifacts in the technique that occur by DNA slippage during PCR amplification (Bhardwaj *et al*., 2016). Tiwari *et al*. (2013) used the same set of microsatellite markers to identify diversity in 77 Indian andigena potato core collection and obtained 214 alleles of which 208 were reported to be polymorphic (97.2%). Microsatellite markers show better polymorphism than RAPD and ISSR markers (Vanishree *et al*., 2016). The set of primers used in both cases were part of highly informative 24 microsatellite locus specific markers containing potato genome identification (PGI) kit reported by Ghislain *et al*. (2009) for fast molecular characterization in potato. Five SSR markers namely STG0010, STI0012, STI0014, STM0031 and STI0032 gave the highest number of scorable alleles with

Table 1: Major Indian potato cultivars and their total carotenoid content

Low Group (≤ 100 mg/100 g f.w.)#	Medium Group (101 - 350 µg/100 g f.w.)#
Kufri Safed, Kufri Neela, Kufri Sheetman, Kufri Jeevan, Kufri Naveen, Kufri Chamatkar, Kufri Dewa, Kufri Badshah, Kufri Bahar, Kufri Sherpa, Kufri Megha, Kufri Ashoka, Kufri Chandramukhi, Kufri Pukhraj, Kufri Chipsona-I, Kufri Giriraj, Kufri Anand, Kufri Pushkar, Kufri Surya, Kufri Chipsona-III, Kufri Girdhari, Kufri Khyati, Kufri Frysona, Kufri Gaurav (24 varieties)	Kufri Kumar, Kufri Kundan, Kufri Sindhuri, Kufri Jyoti, Kufri Muthu, Kufri Lauvkar, Kufri Lalima, Kufri Swarna, Kufri Sutluj, Kufri Jawahar, Kufri Chipsona-II, Kufri Kanchan, Kufri Shailja, Kufri Himalini, Kufri Himsona, Kufri Sadabahar, Kufri Chipsona-IV, Gulmerg special* (17 varieties)

#The Indian Potato cultivars are categories based on their carotenoid content into Low - (≤100 mg/100 g f.w.), Medium (101 - 350 mg/100 g f.w.), High (≥ 350 mg/100 g f.w.) according to Patil *et al*. (2020)
*Indigenous potato variety

Table 2: The polymorphism and diversity found among the low and medium carotenoid containing potato cultivars

	Marker[$]	Primer sequence	T_m	Ch	Low Carotenoid			Medium Carotenoid			All cultivars			$H_{0(P)}$	$H_{0(P)}/H_{Sp}$	$(H_{Sp} - H_{0(P)})/H_{Sp}$
					Total Alleles	Poly-morphic alleles	$H^*_{0(L)}$	Total Alleles	Poly-morphic alleles	$H^{\#}_{0(M)}$	Total Alleles	Poly-morphic alleles	$H^+_{0(Sp)}$			
1	STG0001	F: CAGCCAACATTTGTACCCCT R: ACCCCCACTTGCCATATTTT	58	XI	9	9	2.59	7	7	2.09	9	9	2.53	2.34	0.92	0.08
2	STG0010	F: CGATCTCTGCTTTGCAGGTAR: GTTCATCACTACCGCCGACT	60	III	10	10	2.92	10	10	3.01	10	10	2.99	2.97	0.99	0.01
3	STG0016	F: AGCTGCTCAGCATCAAGAGAR: ACCACCTCAGGCACTTCATC	55	I	9	9	3.11	9	9	2.92	9	9	3.08	3.02	0.98	0.02
4	STG0025	F: TGGAATCCGAATTACGCTCTR: AGGTTTTACCACTCGGGCTT	56	X	7	7	2.37	7	7	2.26	7	7	2.39	2.32	0.97	0.03
5	STI0001	F: CAGCAAAATCAGAACCCGATR: GGATCATCAAATTCACCGCT	60	IV	9	9	2.64	9	9	2.51	10	10	2.61	2.58	0.99	0.01
6	STI0003	F: ACCATCCACCATGTCAATGCR: CTCATGGATGGTGTCATTGG	60	VIII	7	7	2.31	7	7	2.13	7	7	2.28	2.22	0.97	0.03
7	STI0004	F: GCTGCTAAACACTCAAGCAGAAR: CAACTACAAGATTCCATCCACAG	60	VI	6	6	1.57	6	6	1.81	6	6	1.71	1.69	0.99	0.01
8	STI0012	F: GAAGCGACTTCCAAAATCAGAR: AAAGGGAGGAATAGAAACCAAAA	56	IV	10	10	3.21	10	10	3.00	10	10	3.20	3.11	0.97	0.03
9	STI0014	F: AGAAACTGAGTTGTGTTTGGGAR: TCAACAGTCTCAGAAAACCCTCT	54	IX	10	10	3.08	9	9	2.91	10	10	3.23	3.00	0.93	0.07
10	STI0030	F: TTGACCCTCCAACTATAGATTCTTCR: GACAACTTTAAAGCATATGTCAGC	58	XII	7	7	2.41	7	7	2.41	7	7	2.44	2.41	0.99	0.01
11	STI0032	F: TGGGAAGAATCCTGAAATGGR: TGCTCTACCAATTAACGGCA	61	V	10	10	3.33	10	10	3.33	10	10	3.41	3.33	0.98	0.02
12	STI0033	F: TGAGGGTTTTCAGAAAGGGAR: CATCCTTGCAACAACCTCCT	61	VII	7	7	2.13	7	7	1.80	7	7	2.06	1.97	0.95	0.05
13	STM0019	F: AATAGGTGTACTGACTCTCAATGR: TTGAAGTAAAAGTCCTAGTATGTG	47	VI	8	8	2.43	8	8	2.33	8	8	2.46	2.38	0.97	0.03
14	STM0031	F: CATACGCACGCACGTACACR: TTCAACCTATCATTTTGTGAGTCG	53	VII	10	10	3.55	9	9	3.26	10	10	3.52	3.41	0.97	0.03
15	STM0037	F: AATTTAACTTAGAAGATTAGTCTC R: ATTTGGTTGGGTATGATA	52	XI	4	4	1.23	4	4	1.21	4	4	1.23	1.22	0.99	0.01
16	STM1052	F: CAATTTCGTTTTTTCATGTGACACR: TGGCGTAATTTGATTTAATACGTAA	50	IX	9	9	3.04	9	9	3.12	9	9	3.14	3.08	0.98	0.02
17	STM1053	F: TCTCCCCATCTTAATGTTTCR: CAACACAGCATACAGATCATC	53	III	3	3	0.68	3	3	0.79	3	3	0.76	0.74	0.97	0.03
18	STM1064	F: GTTCTTTTGGTGGTTTTCCTR: TTATTTCTCTGTTGTTGCTG	55	II	6	6	1.77	6	6	2.00	6	6	1.93	1.89	0.98	0.02

	Marker$	Primer sequence	T_m	Ch	Low Carotenoid			Medium Carotenoid			All cultivars			$H_{0(P)}$	$H_{0(P)}/H_{Sp}$	$(H_{Sp} - H_{0(P)})/H_{Sp}$
					Total Alleles	Poly-morphic alleles	$H^*_{0(L)}$	Total Alleles	Poly-morphic alleles	$H^{\#}_{0(M)}$	Total Alleles	Poly-morphic alleles	$H^+_{0(Sp)}$			
19	STM1104	F: TGATTCTCTTGCCTACTGTAATCGR: CAAAGTGGTGTGAAGCTGTGA	53	VIII	5	5	1.53	5	5	1.51	5	5	1.53	1.52	0.99	0.01
20	STM1106	F: TCCAGCTGATTGGTTAGGTTGR: ATGCGAATCTACTCGTCATGG	51	X	9	9	3.01	9	9	3.12	9	9	3.14	3.07	0.98	0.02
21	STM5114	F: AATGGCTCTCTCTGTATGCTR: GCTGTCCCAACTATCTTTGA	60	II	5	4	1.05	5	4	1.27	5	4	1.18	1.16	0.98	0.02
22	STM5121	F: CACCGGAATAAGCGGATCTR: TCTTCCCTTCCATTTGTCA	48	XII	3	3	0.90	3	3	0.97	3	3	0.94	0.94	0.99	0.01
23	STM5127	F: TTCAAGAATAGGCAAAACCAR: CTTTTTCTGACTGAGTTGCCTC	55	I	5	5	1.79	5	5	1.68	5	5	1.75	1.74	0.99	0.01
24	STPoAc58	F: TTGATGAAAGGAATGCAGCTTGTGR: ACGTTAAAGAAGTGAGAGTACGAC	57	V	8	8	2.75	8	8	2.73	8	8	2.77	2.74	0.99	0.01
	Total/Average				176	175	2.31	172	171	2.26	177	176	2.34	2.28	0.98	0.02

$H^*_{0(L)}$ - Genetic diversity among varieties with low total carotenoid content, $H^{\#}_{0(M)}$ - Genetic diversity among varieties with medium carotenoid content, $H^+_{0(Sp)}$ - 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, $(H_{Sp} - H_{0(P)})/H_{Sp}$ – Diversity present between the population, $The potato genome identity (PGI) kit as described by Ghislain *et al.* (2009) and Tiwari *et al.* (2013), Ch - Potato chromosome

10 and two markers STM1053 and STM5121 gave the least with only 3 alleles. Hubert *et al.*, 2015 reported the diversity among the Indian potato cultivars for their reduced cold induced sweetening using 10 SSR markers and 11 varieties. Similarly, Patil *et al.,* 2020 assessed the genetic diversity present between the late blight resistant and susceptible Indian potato cultivars using SSR markers. Lowest number of scorable fragments were found in Kufri Jawahar (46) whereas, highest was found with Kufri Himalini and Kufri Lalima (81). A total of 176 alleles were obtained with 24 low carotenoid containing varieties, with 99.43% polymorphism whereas, a total of 172 alleles were obtained with 17 medium carotenoid containing varieties with 99.42% polymorphism. STM0031 gave the highest diversity among the low carotenoid containing potato varieties (3.55) whereas, STI0032 gave the highest diversity among medium group with (3.26) (**Table 2**). The average proportion of diversity present within the Indian potato cultivars (Low + Medium) was found to be 98% ($H_{0(P)}/H_{Sp}$) whereas, the proportion of diversity between the cultivars of low and medium carotenoid containing varieties was found to be very low (2%) ($H_{Sp} - H_{0(P)}/H_{Sp}$). Similar results were obtained by Patil *et al.* (2020) using SSR markers, who reported only 6% diversity between the late blight resistant and susceptible groups as compared to 94% within populations.

Cultivars were grouped based on the Nei and Lie coefficient for genetic similarities and dendrogram obtained on the basis of agglomerative clustering revealed 2 major clusters and 4 minor clusters (Fig. 1). The coefficient of diversity varied from as low as 24% between Kufri Lauvkar (medium) and Kufri Sheetman (low) to as high as 70% between Gulmarg special, an indigenous variety with medium carotenoid content

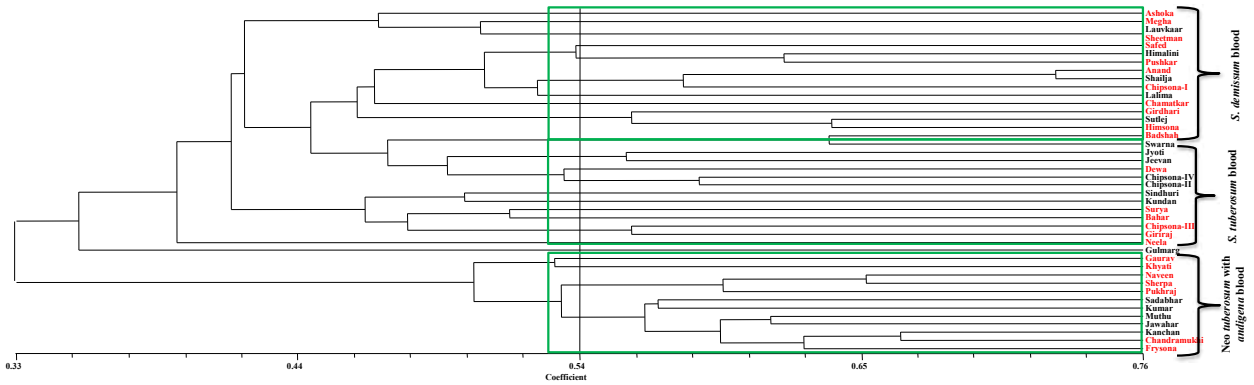

Fig 1: Dendogram depicting the diversity among the low (red) and medium (black) total carotenoid containing Indian potato cultivars

often used as parent material in crossing and all other Indian potato cultivars. The Indian potato were clustered in a way that all varieties with *S. demissum* blood clustered together and contained higher number of low total carotenoid containing varieties including cultivars containing Phulwa blood like Kufri Sheetman, Kufri Chamatkar, Kufri Deva and Kufri Safed. In contrast, cultivars with Kathadin blood like Kufri Kundan and Kufri Sindhuri, similarly cultivars with Kufri Jyoti blood like Kufri Shailja, Kufri Jawahar and Kufri Swarna contained medium total carotenoid. Gulmarg special, an indigenous potato cultivar is placed in a separate cluster as per expectation. The other two clusters were formed with *S. tuberosum* blood and neo tuberosum with Andigena blood with lesser number of low carotenoid containing cultivars. This may be due to carotenoid content in tetraploids is derived from diploid potato species from Andean region, which are the progenitors of present-day tetraploid cultivars (Burgos *et al*., 2009). The genetic diversity findings are in line with Chimote *et al*. (2007) who also classified 32 Indian potato cultivars into 3 major clusters and 5 minor clusters using 5 SSR markers. Sharma and Nandineni (2014) also reported 3 major clusters using 19 SSR markers on 47 Indian potato cultivars. Forty-three Indian potato cultivars were clustered into 3 main, Andigena, *S. demissum* derived and pure tuberosum groups using 11 different ISSR markers (Vanishree *et al*., 2016) and genome specific markers (Patil *et al*., 2012). The same clustering is also reported by using the mitochondrial markers (Chimote *et al*., 2008). All these results implies that the genetic diversity among the Indian potato cultivars has been largely fixed may be because of the strategies adapted in breeding programs in India, where maximum new cultivars developed have other cultivars in their parentage directly or indirectly. Therefore, the frequency of a variety containing relatively high total carotenoid has also decreased (**Table 1**). There is urgency of bringing the new blood into the future potato varieties by including the distant and unrelated parents in the breeding programs to add the high nutritional characters. The association among the clusters formed by potato cultivars was further confirmed by principal coordinate (PCO) analysis. No particular pattern of distribution of low and medium carotenoid containing groups was found varieties of each group were not scattered randomly but found together in small clusters.

CONCLUSION

Characterization of Indian potato cultivars for their total carotenoid content and quantification of genetic diversity present within the varieties by various molecular markers would be of immense help for the breeders to improve inheritable qualitative character like total carotenoid content in potato through selection of efficient and diverse parents. The present study demonstrates the successful utilization of SSR markers (PGI kit) to assess the genetic variability within and between the Indian potato cultivars with low and medium carotenoid content. The specific alleles identified for carotenoid content may be further verified to utilized to be utilized in breeding programs.

ACKNOWLEDGEMENT

The work was funded jointly by Department of Biotechnology, Government of India and European Union under PotBio: Generating biomarkers for breeding healthy potatoes - an Indo-European collaborative project involving India, the Netherlands, Germany, Spain, and UK.

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