



Development of microsatellite markers set for identification of Indian potato varieties



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ABSTRACT

Aim of this study was to analyze allelic variations in Indian potato varieties and to develop a dataset of SSR (simple sequence repeat) markers for varietal identification. Total of 155 SSR alleles of 12 markers were scored in 48 varieties with alleles per locus varied from 4 (STM1053) to 35 (STIKA), and polymorphic information content (PIC) ranged between 0.53 (STM1053) to 0.92 (STIKA). Total of 1492 absolute alleles frequencies were detected in the varieties ranging from 19 (Kufri Arun or Kufri Sadabahar) to 45 (Kufri Kundan) with marker-wise allele frequencies were maximum in STIKA (273) and minimum in STM5127 (73) or STM1053 (73). During the analysis two markers STM0037 and STI0030 did not show any amplification (null alleles). Allelic profiling of potato varieties showed distinctness and polymorphism of the SSR markers system. A set of minimum two-markers-set (STIKA and STU6SNRN) was developed for varietal identification up to similarity coefficient 0.89. Besides, another six-markers-set (STI0012, STPoAC58, STM0019, STM0031, STM1104 and STM1052) was also developed from the PGI (Potato Genome Identity) kit for the same purpose. These newly developed SSR markers sets of Indian potato varieties have potential roles in varietal identification, genetic fidelity testing, DUS (Distinctness, Uniformity and Stability) and molecular characterizations.

1. Introduction

The cultivated potato (*Solanum tuberosum* subsp. *tuberosum* L.) is the third most important food commodity in terms of human consumption in the world after rice and wheat. To meet the global food requirements, the crop genetic improvement is inevitable using diverse genetic materials and is more challenging as varietal preferences and stresses (biotic and abiotic) continue to change. In India, over fifty potato varieties have been developed by Indian Council of Agricultural Research (ICAR)-Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh for cultivation in the country. Nearly 80% of potato is grown in Indo-Gangetic plains while remaining area lies in hills and plateau regions of the country. The indigenous varieties have contributed substantially to increase crop area, production and productivity of potato in the country. Besides, these varieties have benefited not only this country but also several other countries as well like Afghanistan, Bangladesh, Bhutan, Bolivia, Madagascar, Nepal, Philippines and Sri Lanka, where the Indian varieties are adopted for commercial cultivation. The adaptation of potato from the temperate (Europe) to sub-tropical (India) crop has augmented national

agriculture system of the country (Kumar et al., 2011).

The International Union for the Protection of New Varieties of Plants (UPOV) has made it mandatory to meet the DUS criteria of a crop for a new genotype to be granted Plant Breeder's Rights (PBR). The UPOV has established a working group on the use of biochemical and molecular techniques (BMT) that identified the use of molecular markers for varietal identification. Emphasis was given on the potential of molecular markers that could complement or may be an alternative of phenotypic DUS testing that is carried out at present (UPOV/BMT/14/8, 2014). To assess distinctness, new variety is to be compared with all common varieties of reference collections comprising phenotypic descriptors. The DUS test takes a minimum of two seasons/years and is labour and cost intensive. Hence, a rapid and robust method based on molecular markers is highly desirable for varietal identification.

Molecular markers have been used for long time in germplasm characterization, varietal identification, genetic variation, phylogenetics and other molecular studies (Ghislain et al., 2006, 2009). Among all available markers, microsatellite or simple sequence repeat (SSR) is very suitable due to its features such as high reproducibility, high discriminatory power and easy to analyse. SSR is an excellent marker

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Table 1

Primer name, chromosome location, allelic variation and polymorphic information content of SSR markers used in analysis of Indian potato varieties.

SN	Primer name ^a	Chromosome	SSR alleles		PIC ^c
			Number	Size (bp) and absolute allele frequencies ^b	
1.	STM5127	1	6	238 (25), 239 (1), 241 (23), 250 (6), 269 (9), 272 (9)	0.746
2.	STM5114	2	8	282 (16), 280 (2), 284 (3), 287 (31), 290 (4), 295 (27), 300 (2), 304 (4)	0.748
3.	STM1053	3	4	162 (1), 168 (27), 171 (42), 172 (3)	0.530
4.	STI0012	4	13	158 (3), 161 (1), 162 (1), 163 (1), 164 (18), 167 (18), 170 (19), 173 (6), 178 (1), 182 (7), 183 (9), 185 (5), 189 (7)	0.863
5.	STP0AC58	5	14	170 (3), 190 (5), 196 (3), 202 (5), 206 (4), 208 (4), 230 (12), 232 (31), 233 (1), 234 (3), 235 (6), 240 (3), 246 (1), 247 (2)	0.816
6.	STM0019	6	10	190 (8), 191 (1), 196 (1), 201 (20), 202 (20), 203 (4), 205 (8), 206 (17), 233 (4), 235 (9)	0.842
7.	STM0031	7	13	152 (6), 153 (4), 167 (11), 168 (17), 170 (2), 172 (3), 184 (2), 185 (19), 186 (13), 187 (14), 188 (1), 192 (1), 194 (1)	0.863
8.	STM1104	8	13	165 (4), 166 (3), 167 (2), 168 (42), 169 (35), 172 (17), 173 (18), 174 (1), 175 (15), 176 (12), 177 (1), 178 (2), 179 (1)	0.828
9.	STM1052	9	15	200 (1), 207 (13), 208 (32), 216 (1), 217 (19), 220 (1), 222 (1), 223 (2), 224 (1), 225 (24), 226 (2), 246 (1), 248 (2), 249 (3), 250 (5)	0.812
10.	STM1106	10	10	151 (1), 152 (8), 153 (39), 155 (9), 156 (41), 159 (12), 182 (1), 189 (2), 192 (8), 194 (3)	0.767
11.	STM0037	11	0	Null allele (no amplification product)	–
12.	STI0030	12	0	Null allele (no amplification product)	–
13.	STIKA	–	35	185 (3), 187 (1), 190 (24), 194 (44), 195 (5), 196 (6), 198 (21), 200 (8), 202 (1), 203 (1), 214 (2), 219 (5), 220 (1), 221 (6), 222 (31), 223 (7), 224 (1), 225 (18), 226 (3), 227 (1), 230 (30), 231 (3), 234 (5), 235 (4), 236 (2), 237 (1), 241 (1), 242 (18), 243 (11), 244 (1), 246 (3), 248 (2), 253 (1), 255 (1), 257 (1)	0.920
14.	STU6SNRN	–	14	178 (10), 177 (1), 179 (22), 180 (1), 181 (41), 182 (17), 189 (42), 190 (5), 191 (1), 197 (22), 198 (37), 199 (27), 200 (7), 202 (1)	0.873
Total			155		

^a SSR markers source: SN 1-12, Ghislain et al., 2009; and SN 13-14, Provan et al., 1996.

^b Absolute allele frequencies in parenthesis represent the actual number of varieties (from total 48) in which amplification was observed.

^c PIC: Polymorphic Information Content.

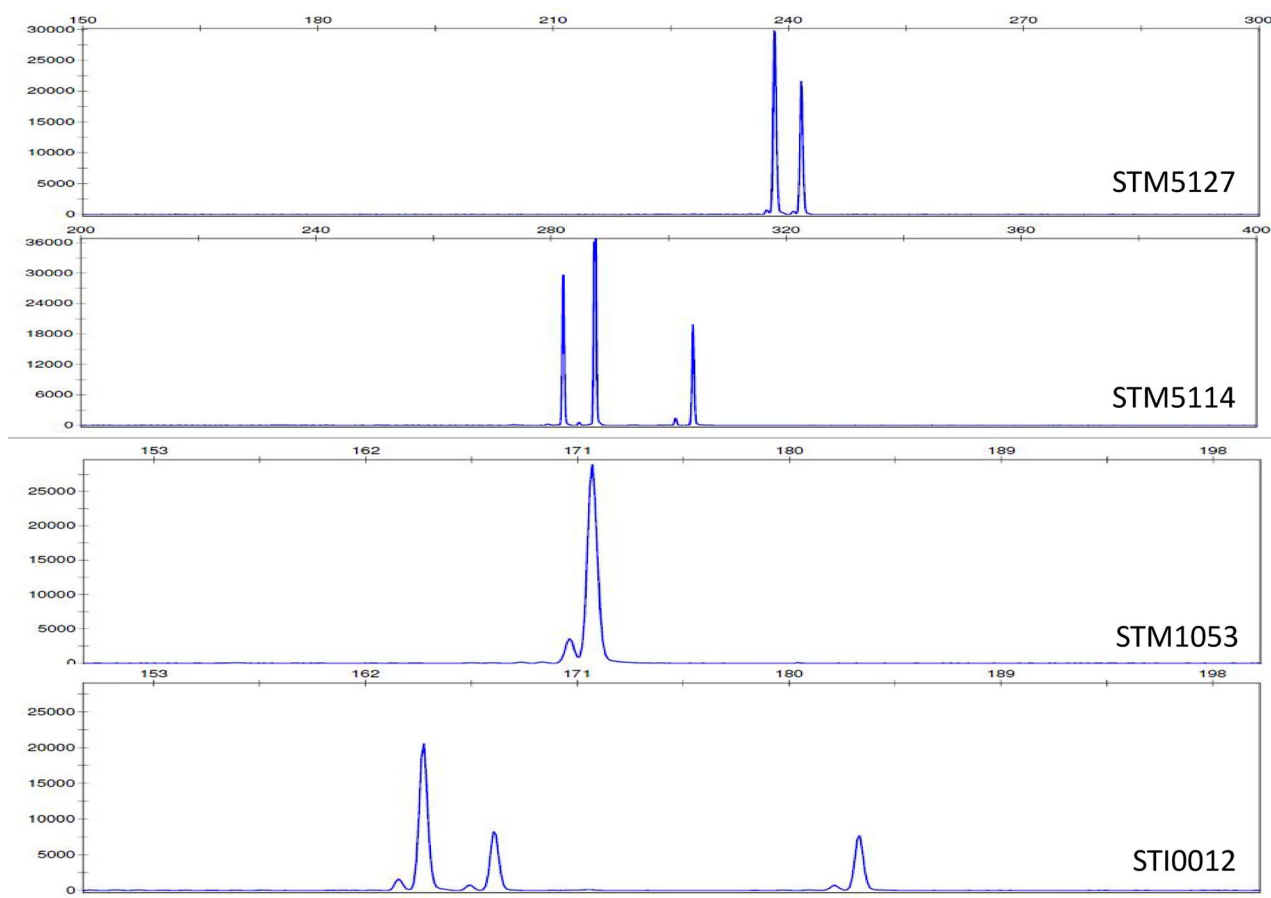


Fig. 1. (a,b,c). SSR fragments analysis of potato cv. Kufri Alankar using ‘3500 Genetic Analyzer’ (ABI). Marker name is shown on the respective images.

system to investigate closely related genotypes in germplasm management (Provan et al., 1996). Because of its co-dominant inheritance, locus specific, robustness and capable to detect high-throughput allelic variation in the plant genome, SSR has been used in many studies in potato for analyzing genetic variation (Liao and Guo, 2014; Favoretto

et al., 2011; Lung’aho et al., 2011; Spooner et al., 2007; Ispizúa et al., 2007), *in-* and *ex-situ* potato landrace cultivar (Haan et al., 2013), Russian potato collection (Gavrilenko et al., 2010), fingerprinting (Galarreta et al., 2011), construction of core collection (Ghislain et al., 2006), validation of Andigena core set (Tiwari et al., 2013),

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