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SIMPLE SEQUENCE REPEAT (SSR) ANALYSIS OF MULTI-COLOURED POTATO HYBRID PROGENIES

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ABSTRACT: The aim of this study was to analyze multi-coloured flesh potato hybrids using simple sequence repeat (SSR) markers and identify diagnostic SSRs for genetic fidelity purpose. The parents MP/4-816 (female) and CP4242 (male) were hybridized and 108 progenies (MSP/17-136 to MSP/17-243) were produced. The 110 genotypes (2 parents and 108 progenies) were analysed by 59 known potato SSR markers. Of which, only one marker STI0003 (PIC: 0.84) was identified or diagnostic purpose, which clearly distinguished the hybrid progenies. Allelic profile of STI0003 showed total eight alleles (118, 122, 125, 130, 139, 145, 149 and 152 bp), of which distinct alleles were 118, 130 and 149 bp in MP/4-816, and 130, 149 and 152 bp in CP 4242, and their progenies contained 2 to 7 alleles. The study suggests that STI0003 is a diagnostic marker for further characterization of these multi-coloured potato hybrids.

KEYWORDS Multi-colour potato, hybrids, SSR, identification

INTRODUCTION

Potato is a wholesome food in term of its high nutritive value, energy, dry matter per unit area and time compared to cereals, and its capacity to ensure food and nutritional security of the world (Chakrabarti *et al.*, 2017). The Indian potato breeding has been largely focussed on developing white-fleshed varieties having biotic and abiotic stress tolerance/resistance quality and other desirable tuber traits. Mostly red-skinned potatoes are preferred in eastern part of the country like Bihar, West-Bengal and North-East, whereas white and light yellow fleshed varieties are preferred all over the country. On the other hand, multi-coloured potato varieties with red/purple/yellow flesh colours has several health benefits on account of rich source of antioxidants (anthocyanins & carotenoids) and micronutrients like Fe and Zn (Luthra *et al.*, 2018). Hence, now focus has been reoriented at the institute to develop coloured potato varieties, which can benefit the people and ensure both food and nutritional security.

Cultivated and wild/semi-cultivated species of potato are rich source of genetic resources (Chakrabarti *et al.*, 2017). Hence, there is enough opportunity to develop coloured potato varieties in near future. While developing varieties, it is also important to ensure genetic fidelity of genotypes by molecular markers like RAPD, ISSR, SSR, ALFP etc, so that variety can be identified in future. A large number of markers have been applied in potato for molecular characterization of potato species (Ghislain *et al.*, 2009), somatic hybrids (Chandel *et al.*, 2015), varietal identification (Tiwari *et al.*, 2018), wild species (Tiwari *et al.*, 2019) and genetic diversity (Provan *et al.*, 1996) so on. Hence, the aim of this study was to develop diagnostic SSR marker for identification of potato clones with multi-coloured flesh tubers.

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MATERIAL AND METHODS

In the present investigation, indigenous advanced stage hybrid MP/4-816 (female parent) and exotic germplasm accession CP4242-Bora Valley (male parent) were hybridized at ICAR-CPRI, Regional Station, Modipuram, Meerut, UP during 2015-16 and seedlings raising (2016-17) and F_1C_1 stage (2017-18) were grown at ICAR-CPRI, Regional Station, Modipuram, Meerut. Phenotyping data of breeding materials were recorded based on the standard procedures described by Luthra et al. (2018) and reported here. The 108 progenies (MSP/17-136 to MSP/17-243) along with parents MP/4-816 and CP4242 were selected for DNA analysis. Leaf samples of 110 genotypes (2 parents and 108 progenies) were analysed using 59 simple sequence repeat (SSR) markers. The annealing temperature was 55°C for the SSR markers (STG0016, STM0001, STM0003, STM0004, STM0007, STM0010, STM0011, STM0013, STM0015, STM0017, STM0019, STM0023, STM0031, STM0032, STM0037, STM0046, STM1001, STM1003, STM1007, STM1024, STM1036, STM1041, STM1043, STM1046, STM1052, STM1057, STM1058, STM1064, STM1069, STM1097, STM2002, STM2003, STM2004, STM2005, STM2020, STM2021, STM2023, STM2026, STM3003, STM3012, STM3018, STM51217), except some STG0025 (56°C), STI0001 (60°C), STI0003 (60°C), STI0004 (60°C), STI0012 (56°C), STI0014 (54°C), STI0030 (58°C), STI0032 (61°C), STI0033 (61°C), STG0001 (58°C), STG0010 (60°C), STM1053 (53°C), STM1104 (53°C), STM1106 (51°C), STM5114 (60°C), STM5121 (50°C) and STPoAC58 (57°C) (Provan *et al.*, 1996; Ghislain *et al.*, 2009). Initially SSR markers were selected for profiling of total 110 samples using 59 SSR markers. Further, only one marker could be selected for Genetic Analyzer analysis based on the two or more clear bands on the gel among the lines. Other markers showed either one or non-scorable and overlapping bands on the gel, hence not carried further. DNA isolation was performed using DNeasy Plant Mini kit (Qiagen, the Netherlands) and quality was checked on agarose gel (1%) and NanoDrop (Thermo Fisher Scientific, USA). Polymerase chain reaction (PCR) included total of 10 µl reaction mix which included 100 ng DNA, 1 µl (10 pM) each primer (forward and reverse), 1 U Taq polymerase, PCR buffer with 2.5 mM $MgCl_2$ and 200 µM dNTP and Milli-Q water (Qiagen). The PCR cycles included denaturation at 95°C/5 min; 35 cycles of 94 °C/45 sec, annealing (except above) at 55 °C /45 sec, and 72 °C for 1 min; and extension at 72 °C/7 min in GeneAmp PCR System (Applied Biosystems, CA, USA). The amplified PCR products were resolved on high resolution agarose gel (3%). Of which only selected SSR markers were synthesized with FAM labelled and PCR amplified. The amplified PCR products were analysed with a 500-bp 'GS 500 ROX' standard on '3500 Genetic Analyzer' using GeneMapper® Software Version 4.1 (Applied Biosystems, CA, USA). SSR fragments were recorded from the Genetic Analyzer based on the repeat motifs, whereas on gel it was scored by comparing with the known DNA ladder. PCR reactions were repeated twice and only reproducible and distinct SSR markers were selected for further analysis. Scoring was done on the basis of presence (1) or absence (0) of SSR fragments. Cluster analysis was performed with unweighted Neighbour Joining using the DARwin software using bootstrap value 100.

RESULTS AND DISCUSSION

The tubers phenotypes of the 108 progenies of cross MP/4-816 × CP4242 indicated wide variation for tuber skin and flesh colour as compared to parents MP/4-

816 (light yellow ovoid tubers with shallow eyes and light yellow flesh) and CP4242 (purple oblong tubers with shallow eyes and purple flesh (**Fig. 1**). The tuber skin colour of the progenies varied from pink, purple to yellow and flesh colour varied form cream, yellow, pink to purple with contrasting secondary colour of pink and purple variegation different than both the parents. The tuber shape of the progenies also segregated from ovoid to oblong. However, eyes depth (shallow or fleet) of the progenies was almost similar to both the parents (shallow eyes) (Luthra et al. 2018).

A total of 59 SSR markers were analysed in all 110 samples (2 parents and 108 progenies). The PCR products were checked on high-resolution agarose gel (3%) to visualize polymorphic bands in the parents and progenies. However, only one SSR marker (STI0003) was identified as diagnostic SSR marker for the identification of the multi-colour fleshed progenies. The SSR profile of STI0003 (PIC: 0.84) showed polymorphism in 110 genotypes **(Fig. 2)**. Further, SSR profile of FAM dye-labelled STI0003 marker showed good polymorphism using '3500 Genetic Analyzer' and the distribution of alleles in the parents and progenies is depicted in **Fig. 3** and summarised in **Tables 1** and **2**. The allelic profiles of STI0003 marker showed total eight alleles (118, 122, 125, 130, 139, 145, 149 and 152 bp) in the 110 genotypes (**Table 2**). Of which, very distinct STI0003 alleles in parent MP/4-816 were 118, 130 and 149 bp; whereas alleles in CP 4242 were 130, 149 and 152 bp, and progenies contained 2 to 7 alleles (e.g. MSP/17-13: 130 and 149 bp).



Fig. 1. Tuber traits of parents (MP/4-816 & CP4242) and progenies (MSP/17-147 & MSP/17-212)

Table 1. Allelic profile of diagnostic SSR marker (STI0003) for identification of multi-coloured fleshed potato hybrids

SSR marker	SSR motif	Sequence (5′□3′)	Ta (°C)	Allele size (bp)	No. of genotypes having alleles	Allele frequency	PIC
STI0003	(ACC)n	F: ACCATCCACCATGTCAATGC R: CTCATGGATGGTGTCATTGG	60	118	44	0.095	0.84
				122	29	0.062	
				125	60	0.129	
				130	105	0.227	
				139	34	0.073	
				145	30	0.064	
				149	86	0.186	
				152	74	0.160	
Total					460	1	

Note: Allele frequency=Number of allele counts in genotypes present/Total number of allele counts in all genotypes i.e. 460 (e.g. 44/460 = 0.095 so on)

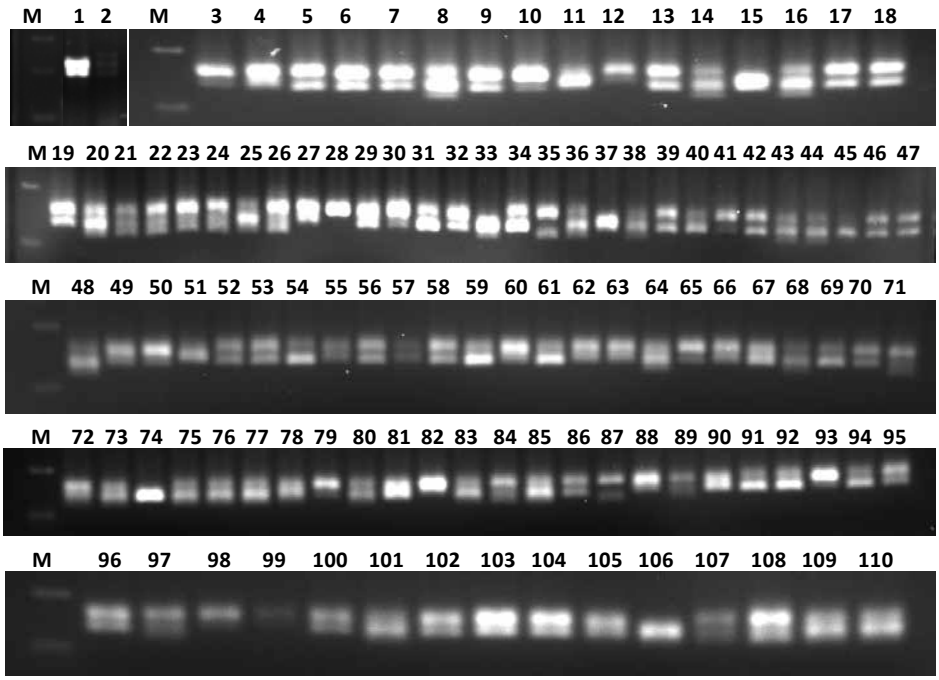

Fig. 2. Gel profile of 110 genotypes (2 parents and 108 progenies) analysed by SSR marker (STI0003). Gel images: M=DNA ladder 50 bp; 1=MP/4-816; 2=CP4242; 3-110: MSP/17-136 to MSP/17-243.

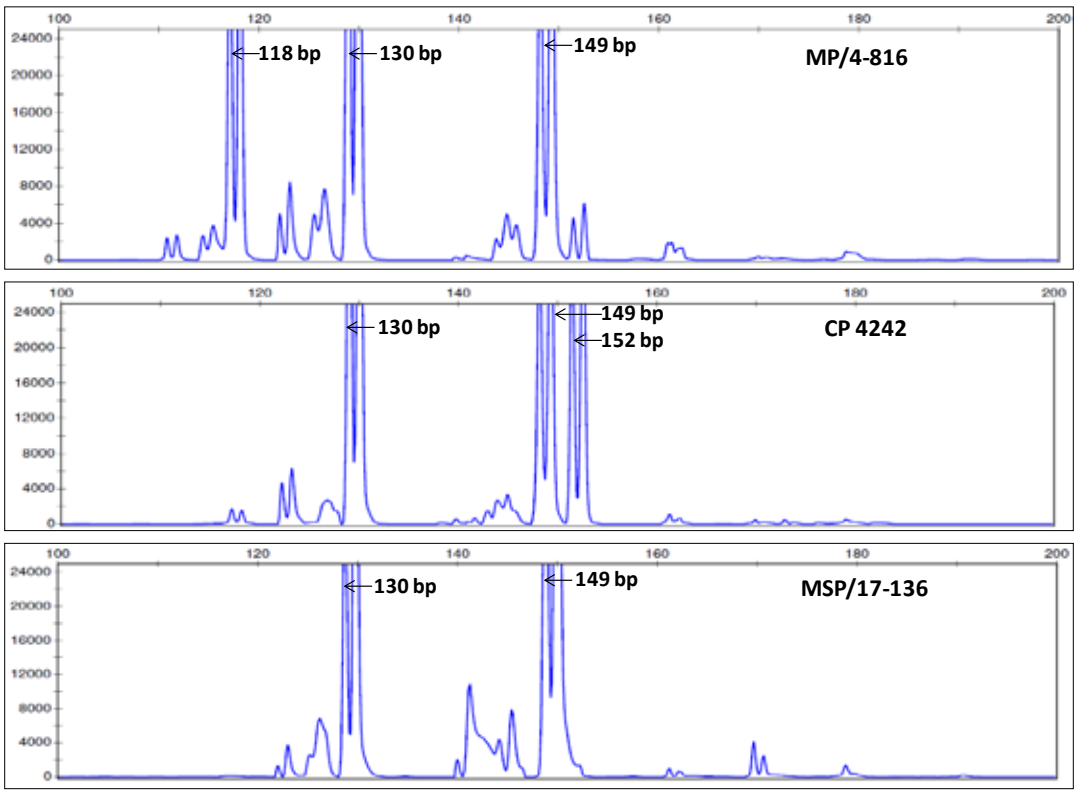

Fig. 3. Molecular profile of potato progenies by FAM labelled SSR marker STI0003 using '3500 Genetic Analyzer' (ABI). Parents: MP/4-816 and CP4242; and their progeny MSP/17-136.

Table 2. SSR allelic profiles of the parents and progenies for multi-coloured flesh potatoes

Sr. No.	Sample	SSR (STI0003) allele size (bp)								Allele #
		118	122	125	130	139	145	149	152	
					Parents					
1.	MP/4-816	1	1	1	1	1	1	1	1	8
2.	CP 4242	0	1	1	1	0	0	1	1	5
					Progenies (MP/4-816 × CP 4242)					
3.	MSP/17-136	0	1	1	1	1	1	1	0	6
4.	MSP/17-137	0	0	1	1	1	1	1	1	6
5.	MSP/17-138	0	1	1	1	0	1	1	1	6
6.	MSP/17-139	0	1	1	1	1	1	1	0	6
7.	MSP/17-140	0	1	1	1	1	1	1	0	6
8.	MSP/17-141	1	0	1	1	0	0	1	1	5
9.	MSP/17-142	0	1	1	1	1	1	1	0	6
10.	MSP/17-143	0	1	1	1	1	1	1	1	7
11.	MSP/17-144	0	0	1	1	1	1	0	1	5
12.	MSP/17-145	0	0	0	1	1	1	1	1	5
13.	MSP/17-146	0	1	1	1	0	1	1	1	6
14.	MSP/17-147	1	1	1	1	0	0	1	1	6
15.	MSP/17-148	0	1	1	1	1	0	0	0	4
16.	MSP/17-149	1	1	1	1	0	0	1	1	6
17.	MSP/17-150	0	1	1	1	0	1	1	1	6
18.	MSP/17-151	0	1	1	1	0	1	1	1	6
19.	MSP/17-152	0	1	1	1	1	1	1	0	6
20.	MSP/17-153	1	0	1	1	0	0	0	1	4
21.	MSP/17-154	0	1	1	1	1	1	1	0	6
22.	MSP/17-155	1	1	1	1	0	1	1	1	7
23.	MSP/17-156	1	1	1	1	1	1	1	0	7
24.	MSP/17-157	1	0	1	1	0	1	1	1	6
25.	MSP/17-158	1	1	1	1	0	0	1	1	6
26.	MSP/17-159	1	1	1	1	0	1	1	1	7
27.	MSP/17-160	0	1	1	1	1	1	1	1	7
28.	MSP/17-161	0	0	0	0	1	1	1	1	4
29.	MSP/17-162	0	1	1	1	1	1	1	0	6
30.	MSP/17-163	0	0	0	1	1	1	1	0	4
31.	MSP/17-164	0	0	1	1	0	0	0	1	3
32.	MSP/17-165	0	0	0	1	0	0	1	0	2
33.	MSP/17-166	1	1	1	1	0	0	0	0	4
34.	MSP/17-167	0	1	1	1	0	0	0	1	4
35.	MSP/17-168	1	0	0	0	0	1	1	1	4
36.	MSP/17-169	1	1	1	1	0	0	1	1	6

Sr. No.	Sample	SSR (STI0003) allele size (bp)								Allele #
		118	122	125	130	139	145	149	152	
37.	MSP/17-170	0	1	1	1	1	0	0	0	4
38.	MSP/17-171	1	1	1	1	0	1	1	0	6
39.	MSP/17-172	1	1	1	1	0	1	1	1	7
40.	MSP/17-173	0	1	0	1	1	0	1	1	5
41.	MSP/17-174	0	0	0	1	0	0	1	1	3
42.	MSP/17-175	0	0	0	1	0	0	1	1	3
43.	MSP/17-176	1	0	0	1	0	0	1	0	3
44.	MSP/17-177	1	0	0	1	0	0	0	1	3
45.	MSP/17-178	0	0	1	1	0	0	1	0	3
46.	MSP/17-179	1	0	1	1	0	0	1	1	5
47.	MSP/17-180	0	0	0	1	0	0	1	1	3
48.	MSP/17-181	1	0	0	1	0	0	0	1	3
49.	MSP/17-182	0	0	0	1	1	1	1	1	5
50.	MSP/17-183	0	0	0	1	0	1	1	0	3
51.	MSP/17-184	0	0	0	1	1	0	0	1	3
52.	MSP/17-185	0	0	0	1	0	0	1	1	3
53.	MSP/17-186	0	0	0	1	0	0	1	1	3
54.	MSP/17-187	0	0	1	1	0	0	1	1	4
55.	MSP/17-188	0	0	0	1	1	0	1	1	4
56.	MSP/17-189	0	0	1	1	0	0	1	1	4
57.	MSP/17-190	1	0	0	1	0	0	1	0	3
58.	MSP/17-191	0	0	0	1	0	0	1	1	3
59.	MSP/17-192	0	0	1	1	0	0	1	0	3
60.	MSP/17-193	0	0	0	1	1	0	1	0	3
61.	MSP/17-194	0	0	1	1	0	0	1	0	3
62.	MSP/17-195	0	0	0	1	1	0	1	1	4
63.	MSP/17-196	0	0	0	1	1	0	1	1	4
64.	MSP/17-197	1	0	1	1	0	0	1	0	4
65.	MSP/17-198	0	0	0	1	0	0	1	1	3
66.	MSP/17-199	0	0	0	1	1	0	1	1	4
67.	MSP/17-200	0	0	1	1	1	0	1	0	4
68.	MSP/17-201	1	0	0	1	0	0	0	1	3
69.	MSP/17-202	0	0	1	1	0	0	1	0	3
70.	MSP/17-203	0	0	0	1	0	0	1	0	2
71.	MSP/17-204	1	0	0	1	0	0	1	0	3
72.	MSP/17-205	1	0	0	1	1	0	1	1	5
73.	MSP/17-206	1	0	0	1	0	0	1	0	3
74.	MSP/17-207	0	0	1	1	0	0	0	0	2
75.	MSP/17-208	1	0	0	1	0	0	0	1	3

Sr. No.	Sample	SSR (STI0003) allele size (bp)								Allele #
		118	122	125	130	139	145	149	152	
76.	MSP/17-209	1	0	0	1	0	0	0	1	3
77.	MSP/17-210	1	0	1	1	0	0	0	1	4
78.	MSP/17-211	1	0	0	1	1	0	1	1	5
79.	MSP/17-212	0	0	0	1	1	0	1	1	4
80.	MSP/17-213	1	0	1	1	0	0	1	1	5
81.	MSP/17-214	0	0	1	1	1	0	1	0	4
82.	MSP/17-215	0	0	0	0	1	1	1	0	3
83.	MSP/17-216	1	0	1	1	0	0	1	1	5
84.	MSP/17-217	1	0	0	1	0	0	1	1	4
85.	MSP/17-218	1	0	1	1	0	0	0	1	4
86.	MSP/17-219	0	0	1	1	0	0	1	1	4
87.	MSP/17-220	1	0	0	0	0	0	1	1	3
88.	MSP/17-221	1	0	0	0	0	0	0	1	2
89.	MSP/17-222	1	0	0	1	0	0	1	0	3
90.	MSP/17-223	0	0	1	1	1	0	1	0	4
91.	MSP/17-224	0	0	1	1	0	0	0	1	3
92.	MSP/17-225	0	0	1	1	0	0	0	1	3
93.	MSP/17-226	0	0	0	1	0	1	1	0	3
94.	MSP/17-227	0	0	1	1	0	0	0	1	3
95.	MSP/17-228	0	0	0	1	0	0	1	1	3
96.	MSP/17-229	0	0	1	1	0	0	1	1	4
97.	MSP/17-230	1	0	0	1	0	0	1	1	4
98.	MSP/17-231	0	0	0	1	0	0	1	1	3
99.	MSP/17-232	0	0	0	1	0	0	1	1	3
100.	MSP/17-233	0	0	0	1	1	0	1	0	3
101.	MSP/17-234	1	0	0	1	0	0	0	1	3
102.	MSP/17-235	0	0	0	1	0	0	1	1	3
103.	MSP/17-236	0	0	1	1	0	0	1	1	4
104.	MSP/17-237	0	0	1	1	0	0	1	1	4
105.	MSP/17-238	1	0	1	1	0	0	1	0	4
106.	MSP/17-239	1	0	1	1	0	0	0	0	3
107.	MSP/17-240	1	0	0	1	0	0	1	1	4
108.	MSP/17-241	1	0	0	1	0	0	1	1	4
109.	MSP/17-242	1	0	1	1	0	0	0	1	4
110.	MSP/17-243	1	0	0	1	0	0	0	1	3
Allele #		**44**	**29**	**60**	**105**	**34**	**30**	**86**	**74**	**462**

Note: Presence: 1; Absence: 0

In terms of allelic richness, allele size of 130 bp was found in maximum (105) genotypes followed by 149 bp (86) and 152 bp (74). Cluster analysis using STI0003 marker data showed genetic distinctness in 110 genotypes is depicted in **Fig. 4**. Other 58 markers were either very faint or monomorphic or non-scorable amplification or non-amplification type in our tested samples (data not reported).

SSR markers have been used in potato for various applications like genetic diversity (Provan *et al.*, 1996; Ghislain *et al.*, 2009), varietal characterization (Tiwari *et al.*, 2018), wild species (Tiwari *et al.*, 2019), core collection characterization (Tiwari *et al.*, 2013). Our results are in agreement with allelic profile of this marker (Tiwari et al. 2013, 2018, 2019). Interestingly, this marker (STI0003) profile can be used for identification of varieties developed from this progenies. Many of the advanced stage hybrids are in pipeline to develop new varieties in future. Studies showed that SSR has excellent discriminatory power for molecular characterization of potato species, land races characterization (Provan *et al.*, 1996). SSR profiles in terms of allele size and number could be variable due to scoring technology used in the study. Earlier, we have used SSR markers for characterization of somatic hybrids (Chandel *et al.*, 2015), varietal identification (Tiwari *et al.*, 2018), wild species (Tiwari *et al.*, 2019). This study identifies STI0003 as diagnostic marker for identification of parents MP/4-816 and CP4242, and their segregating progenies.

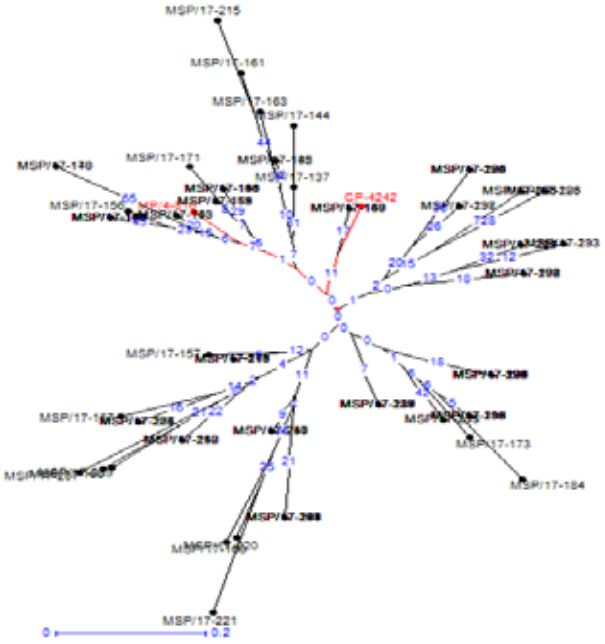

Fig. 4. Cluster analysis of 110 genotypes using STI0003 marker data based on unweighted Neighbour Joining method using the DARwin software with 100 bootstrap value.

CONCLUSION

This investigation identified SSR marker (STI0003) for characterization and identification of potato clones developed by crossing MP/4-816 (118, 130 and 149 bp) and CP4242 (130, 149 and 152 bp). Nevertheless, future study would be required to investigate genetic stability and linked markers with the flesh colour of the potato genotypes.

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CONFLICTS OF INTEREST

The authors confirm that this manuscript has no conflicts of interest

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