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Analysing *Solanum tuberosum* L. Genetic Divergence using Molecular Marker Data

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Abstract— *Genetic polymorphism has important implications for the conservation and evolutionary studies among species as well as within genomes. Hence an enhanced understanding of intra-specific heterogeneity is anticipated which is and based on accurate database or unruffled by environmental conditions. In this context, molecular markers due to their simplicity and ubiquity have been used for genetic divergence studies of tetraploid potato. In the present study genetic diversity, marker attributes and population structure of 48 potato genotypes based on 20 SSR markers data were analysed which were able to successfully generate significant levels of DNA polymorphism to discriminate the experimental material. A total of 33 different loci were amplified that exhibited an average of 90 per cent polymorphism. The PIC value ranged from 0.11 to 0.70. PCR amplification exhibited genetic diversity was analyzed using program NTSYS-PC 2.21. Similarity coefficient or Jaccard coefficient were calculated using SIMQUAL program which varied from 0.32 to 0.92 and dendogram constructed using UPGMA cluster analysis ordered the populations of 48 genotypes into ten clusters. The maximum genetic similarity (0.92) was found between Pant Sel-09 and Pant Sel-09-04 and lowest (0.32) between Pant Sel-09-20 and Pant Sel-09-01. Most diverse groups found were cluster X and cluster II thus, can be utilized as diverse parents in potato breeding programmes.*

Keywords— ***DNA isolation; Genetic diversity; PCR; Potato; SSR.***

I. INTRODUCTION

Improving skills is a prerequisite in today's technology driven world which needs researchers to stay abreast of the latest advancements in crop research, especially staple crops like potato (*Solanum tuberosum* L.). Potato is the most important non-cereal crop and a key component to address poverty and hunger sustaining food security especially in developing countries (Tillault and Yevtushenko, 2019). Moreover, potato is considered as the fourth most important food crop in the world having potential to deal with the challenges of combating malnutrition and reassuring nutritional food security to meet the demands of ever increasing population in developing countries (Ma *et al*., 2017). Being an important cash crop, it has potential to address farmer's distress by enabling them to increase their income, thus, depleting poverty by providing more nutrition and yield per unit area of land compared to major crops (Zaheer and Akhtar, 2016). According to Zaheer and Akhtar (2016), on an average potato tuber contain 77% water, 20% carbohydrate, 3% protein, dietary fiber, vitamins and minerals. Potato covers major economic share in global agricultural market being a short duration crop with wide climate adaptability enabling its cultivation in diverse geographical borders. The worldwide demand for potato production requires constant development of new potato varieties, with improved yield, disease resistance and varied climatic resilience (Tillault and Yevtushenko, 2019). Potato production must be assured qualitatively and quantitatively at grower, processor and most importantly consumer level.

In this context, crop improvement strategy is of the utmost importance, can prove a valuable aid in both quantitative and qualitative breeding program employed for trait improvement prompting superior variety production in potato, which in turn demands wide germplasm collection, germplasm diversity know-about and their genetic relationships (Hameed *et al*., 2018). Many cultivated potato

cultivars are autotetraploid (2n=4x=48) with highly heterozygous genome having enormous genetic diversity. Potato has its origin centre in Andes of South America where diploid potato cultivars are also cultivated though they suffer from severe inbreeding depression and self-incompatibility (Xiaoyan *et al*., 2016). The evolutionary diversity of potato germplasm makes them excellent material for improving the narrow genetic base especially of cultivated potato providing enormous opportunity for breeders to choose best parents for proper breeding scheme and strategies (Anoumaa *et al*., 2017; Carputo *et al*., 2013). Genetic diversity among germplasm helps not only in choosing better performing say high yielding and resistant germplasm, but prompting them to be directly incorporated not only into breeding programmes (as a rule in conventional method) (Halterman *et al*., 2016; Dar *et al*., 2017), but also in molecular aided breeding (Carrasco *et al*., 2009). Where on one hand using conventional method during diversity analysis researcher is likely to misinterpretate the germplasm performance based on field data as it is directly affected by the environmental conditions, molecular marker on other hand are fully deprived of such limitation.

Molecular markers owing to their high resolution and accuracy in differentiating germplasm have become important tool in genetic diversity studies of agronomic and horticultural crops (Bered *et al*., 2005; Barandella *et al*., 2006). Among various marker techniques that are available, particularly promising are SSR markers (Simple Sequence Repeats), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter- Simple Sequence Repeat) etc. (Xia *et al*., 2014; Saensuk *et al*., 2016; Dumhai *et al.,* 2019; Wu *et al*., 2019). These SSRs or microsatellites are found throughout the nuclear genomes ranging from mono to hexa nucleotide in length among which di-, tri- and tetranucleotide repeats are most common choice for molecular genetic studies (Selkoe and Toonen, 2006). Different types of SSRs have been classified by source of development (Genomic SSRs, Genic SSSRs and Organellar (chloroplast and mitochondrial SSRs)), types of repeat sequence (Simple and compound with perfect and imperfect SSRs) and length of repeat motifs (Class I and II microsatellites) (Al-Samrai and Al-Kazaz, 2015). Microsatellites with tandem DNA repeats along with random genome distribution (throughout coding and non-coding regions), codominant nature, high polymorphism, high specificity with better reproducibility are promising for germplasm evaluation aiding diversity analysis and molecular assisted breeding (Qiu *et al*., 2006; Tabkhkar *et al*., 2012; Singh *et al*., 2013). As reported by various researchers a low quality DNA is enough for SSR markers for evaluating genetic diversity, moreover, if these markers could be associated with the resistance conferring trait (Barone, 2004; Gavrilenko *et al*., 2010), may furthermore assist in germplasm fingerprinting (Yang *et al*., 2015), genetic linkage mapping (Jian *et al*., 2017) and phylogenetic studies (Duan *et al*., 2018). Thus, SSRs markers have pivotal role in diversity analysis even for tetraploid species like potato offering new opportunities for selection of superior genotypes backing a sustained potato breeding program with main goal to obtain new cultivar exhibiting better yield and quality traits, along with biotic and abiotic stress resistance. The present study aimed at executing primary step of breeding program *i.e.* analyzing diversity of 48 potato genotypes based on SSR markers desired to provide the researchers with more options for designing breeding programs for producing superior potato cultivars.

II. MATERIALS AND METHODS

Experimental material

The molecular analysis was performed at Molecular lab of PCPGR (Pantnagar Center for Plant Genetic Resource), Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand in 2018. Pantnagar is geographically situated in the Tarai region at the foot hills of Himalayas at 29°N latitude and 79.3°E longitude and at an altitude of 243.83 meters above the mean sea level. The region has humid subtropical climate with the maximum temperature ranging from 30^0C to 45^0C in summer and least 3.7^0C to 12.9^0C in winter. The germplasm evaluated in this study consisted of 48 genotypes out of which 26 were developed through selection at Pantnagar named as Pant selection series and five of the germplasm consisted of advanced breeding lines i.e. J-series collected from Pantnagar itself. The study also included seventeen potentially released Kufri varieties from CPRI (Central Potato Research Institute), Shimla (Table 1).

Table 1. Detailed list of potato germplasm used in this present study.

Sl. No.	Tentative genotypes name	City	Region/State/Counry
1.	Pant Sel-09-20	Pantnagar	Uttarakhand, India
2.	Pant Sel-01-15	Pantnagar	Uttarakhand, India
3.	Pant Sel-09-07	Pantnagar	Uttarakhand, India
4.	Pant Sel-09-11	Pantnagar	Uttarakhand, India
5.	Pant Sel-09-53	Pantnagar	Uttarakhand, India
6.	Pant Sel-09-58	Pantnagar	Uttarakhand, India
7.	Pant Sel-08-11	Pantnagar	Uttarakhand, India
8.	Pant Sel-09-38	Pantnagar	Uttarakhand, India
9.	Pant Sel-09-33	Pantnagar	Uttarakhand, India
10.	Pant Sel-08-02	Pantnagar	Uttarakhand, India
11.	Pant Sel-09-57	Pantnagar	Uttarakhand, India
12.	Pant Sel-09-46	Pantnagar	Uttarakhand, India
13.	Pant Sel-09-03	Pantnagar	Uttarakhand, India
14.	Pant Sel-09-43	Pantnagar	Uttarakhand, India
15.	Pant Sel-09	Pantnagar	Uttarakhand, India
16.	Pant Sel-09-08	Pantnagar	Uttarakhand, India
17.	Pant Sel-09-04	Pantnagar	Uttarakhand, India
18.	Pant Sel-09-21	Pantnagar	Uttarakhand, India
19.	Pant Sel-08-07-01(CT)	Pantnagar	Uttarakhand, India
20.	Pant Sel-09-01	Pantnagar	Uttarakhand, India
21.	Pant Sel-09-55	Pantnagar	Uttarakhand, India
22.	Pant Sel-09-50	Pantnagar	Uttarakhand, India
23.	Pant Sel-15/5	Pantnagar	Uttarakhand, India
24.	Pant Sel-09-19	Pantnagar	Uttarakhand, India
25.	Pant Sel-01	Pantnagar	Uttarakhand, India
26.	Pant Sel-09-18	Pantnagar	Uttarakhand, India
27.	J-95-225	Pantnagar	Uttarakhand, India
28.	J-93-159	Pantnagar	Uttarakhand, India
29.	J-97-242	Pantnagar	Uttarakhand, India
30.	J-96-54	Pantnagar	Uttarakhand, India
31.	J-96-288	Pantnagar	Uttarakhand, India
32.	Kufri Surya	Central Potato Research Institute	Shimla, H.P., India
33.	Kufri sutlej	Central Potato Research Institute	Shimla, H.P., India

34.	Kufri Arun	Central Potato Research Institute	Shimla, H.P., India
35.	Kufri Frysona	Central Potato Research Institute	Shimla, H.P., India
36.	Kufri Jawahar	Central Potato Research Institute	Shimla, H.P., India
37.	Kufri Bahar	Central Potato Research Institute	Shimla, H.P., India
38.	Kufri Pushkar	Central Potato Research Institute	Shimla, H.P., India
39.	Kufri Jyoti	Central Potato Research Institute	Shimla, H.P., India
40.	Kufri Gaurav	Central Potato Research Institute	Shimla, H.P., India
41.	Kufri Giriraj	Central Potato Research Institute	Shimla, H.P., India
42.	Kufri Himalini	Central Potato Research Institute	Shimla, H.P., India
43.	Kufri Chipsona-3	Central Potato Research Institute	Shimla, H.P., India
44.	Kufri Chipsona-1	Central Potato Research Institute	Shimla, H.P., India
45.	Kufri Chipsona-2	Central Potato Research Institute	Shimla, H.P., India
46.	Kufri Ashoka	Central Potato Research Institute	Shimla, H.P., India
47.	Kufri Badshah	Central Potato Research Institute	Shimla, H.P., India
48.	Kufri Khyati	Central Potato Research Institute	Shimla, H.P., India

Genomic DNA isolation

The fresh and green leaves of 48 potato genotypes were collected and the genomic DNA was extracted by using the CTAB (cetyl trimethyl ammonium bromide) method of Doyle and Doyle (1990) with slight modifications (Deshmukh *et al*. 2007). Approximately, 2 g of leaf tissues was collected to extract the genomic DNA using the CTAB method. Genomic DNA was quantified using a NanoDrop spectrophotometer and quality of the genomic DNA was checked using electrophoresis on 1% agarose gel and later the samples stored at ─80 °C. DNA concentration was quantified by using UV spectrophotometer and the OD (optical density) was measured at 260 nm for estimating the DNA concentration. The concentration relates to the OD and calculated by equation (DNA concentration (µg/µl) = OD 260 x 50 x dilution factor/ 1000). Here, OD recorded at 260/280 nm to calculate the ratio OD206 /OD280 where, a ratio of 1.8 is best for DNA preparation. DNA was diluted to50 ng/µl and stored at 4º C for use in PCR, and concentrated stocks were stored at -80ºC for future use.

PCR amplification & Gel electrophoresis

The molecular divergence study was performed using 20 SSR primers pairs obtained from various sources evenly distributed along potato genome (Ghislain *et al*., 2001, 2004, 2009; Feingold *et al*., 2005; Kawchuk *et al*., 1996; Melbourne *et al*., 1998; Provan *et al*., 1996; Moisan-Theiry *et al*., 2005) (Table 2). The Polymerase Chain Reaction (PCR) was performed in eppendorf thermocycler. Master Mix containing dNTP mix (1.5 µL), Taq DNA polymerase (0.1 µL), forward and reverse primer 1.5 µL (50 ng/ µL), reaction buffer A 2µL (10X) and deionized water (6.6 µL) was prepared. The master mix was then distributed in each tube (11.5 µL each) and finally 1 µL of different template DNA was added in each tube. The mixture was gently mixed and centrifuged for ten seconds. The PCR amplification was achieved in thermo cycler (eppendorf thermocycler). The amplification cycles used were initial denaturation at 94 ºC for 3 minutes, followed by 35 cycles of denaturation at 94º C for 1 minute, annealing at 60-65 ºC for 45-50 sec and synthesis at 72 ºC for 1 minute culminating into final extension step of 5-7 minutes at 72ºC. Later gel electrophoresis was done where the amplified DNA product along with molecular marker was run on 2.5 % agarose gel electrophoresis and visualized under U.V. transilluminator using gel documentation system.

Table 2. Detailed description of primer sequences of SSR marker for potato

S.No.	SSR Primer	Repeat motifs	Forward sequence(5'--3') Reverse sequence (3'--5')	Map location	Annealing temp. (°C)	Size (bp)	Source
1.	STG0001	(CT)n	5'CAGCCAACATTTGTACCCCT3' 3'ACCCCCACTTGCCATATTTT5'	X1	58	137-163	Ghislain et al., 2009
2.	STG0016	(AGA)n	5'AGCTGCTCAGCATCAAGAGA3' 3'ACCACCTCAGGCACTTCATC5'	I	55	137-174	Ghislain et al., 2004
3.	STI0030	(ATT)n	5'TTGACCCTCCAACTATAGATTCTTA3' 3'TGACAACTTTAAAGCATATGTCAGC5'	XII	58	94-137	Feingold et al., 2005
4.	STI0032	(GGA)n	5'TGGGAAGAATCCTGAAATGG3' 3'TGCTCTACCAATTAACGGCA5'	V	61	127-138	Feingold et al., 2005
5.	STI0036	(AC)n	5'GGACTGGCTGACCATGAACT3' 3'TTACAGGAAATGCAAACTTCG5'	II	55	129-164	Feingold et al., 2005; Ghislain et al., 2009
6.	STI0003	(ACC)n	5'ACCATCCACCATGTCAATGC3' 3'CTCATGGATGGTGTCATTGG5'	VIII	60	137-188	Feingold et al., 2005; Ghislain et al., 2009
7.	STI0014	(TGG)n	5'AGAAACTGAGTTGTGTTTGGGA3' 3'TCAACAGTCTCAGAAAACCCTCT5'	IX	54	127-157	Feingold et al., 2005; Ghislain et al., 2009
8.	STI0023	(CAG)n	5'GCGAATGACAGGACAAGAGG3' 3'TGCCACTGCTACCATAACCA5'	X	61	172-245	Feingold et al., 2005; Ghislain et al., 2009
9.	STM1104	(TCT)n	5'TGATTCTCTTGCCTACTGTAATCG3' 3'CAAAGTGGTGTGAAGCTGTGA5'	VIII	53	178-199	Melborne et al, 1998
10.	STM0040	(AT)n	5'GCAATAATGGCCAACACTTC3' 3'TGGGAAATGTTAGTCAAAAATAGC5'	VI	58	90-120	Ghislain et al., 2004
11.	STM2005	(CTGTTG)n	5'TTTAAGTTCTCAGTTCTGCAGGG3' 3'GTCATAACCTTTACCATTGCTGG5'	XI	60	160-193	Moisan-Theiry et al., 2005
12.	STI0012	(ATT)n	5'GAAGCGACTTCCAAAATCAGA3' 3'AAAGGGAGGAATAGAAACCAAAA5'	IV	56	183-234	Feingold et al., 2005
13.	STGBSS	(TCT)n	5'AATCGGTGATAAATGTGAATGC3' 3'ATGCTTGCCATGTGATGTGT5'	VIII	53	121-150	Provan et al., 1996; Ghislain et al., 2009
14.	STM5121	(TGT)n	5'CACCGGAATAAGCGGATCT3' 3'TCTTCCCTTCCATTTGTCA5'	XII	48	297-309	Ghislain et al., 2009
15.	STM5127	(TCT)n	5'TTCAAGAATAGGCAAAACCA3' 3'CTTTTTCTGACTGAGTTGCCTC5'	I	55	248-291	Ghislain et al., 2009

16.	STM1031	(AT)n	5'TGTGTTTGTTTTTCTGTAT-3' 3’TTCAGTCAACTCCTGTTGCG-5'	V	55	236-301	Milbourne et al., 1998
17.	STM1058	(ATT)n	5'ACAATTTAATTCAAGAAGCTAGG3' 3'CCAAATTTGTATACTTCAATATGA5'	III	55	130-139	Milbourne et al., 1998
18.	STM1045	(ATC)n	5’GAAGTTTTATCAGAATCC3’ 3’ATCACCTCATCAGCAATC5’	II,III	55	130-148	Ghislain et al., 2001
19.	STM1050	(TA)n	5’GTACATATATACAATTATCTAACCG3’ 3’TTCTCTATGTTAGGCTAGAGTG5’	VI	54	150-190	Ghislain et al., 2004
20.	STM0019	(AT)n (GT)n	5’AATAGGTGTACTGACTCTCAATG3’ 3’TTGAAGTAAAAGTCCTAGTATGTG5’	VI	47	99-206	Kawchuk et al., 1996; Milbourne 1998

SSR data analysis

Amplified SSR profile of all the genotypes with each primer were documented using gel documentation system. DNA for each fragment profiles was scored in a binary fashion with 0 indicating absence and 1 indicating presence of a band for each SSR locus. Primers with null allele where an amplification product could not be detected were not considered in the analysis. Principal Component analysis was done using the software NTSYSpc version 2.2 whereas marker attributes like allele frequency (FA), allele number, polymorphic information content (PIC), Gene diversity, Effective multiplex ratio (EMR) and marker index (MI) were estimated by using the Power Marker statistics software version 3.25 (Liu and Muse 2005). Allele frequency was calculated as nu/N, where nu is number of alleles present and N is total number of genotypes (Dar *et al*., 2017). The PIC detects an allelic variability and was calculated as according to Botstein *et al*. (1980). Marker index was calculated as product of EMR and PIC (Varshney *et al*., 2007). Further the binary data were used to calculate genetic similarities based on Jaccard coefficients among the isolates using SIMQUAL program (Jaccard, 1908) and on the basis of these coefficients, dendogram was constructed using UPGMA (Unweighted Pair Group Mean Average) method to determine the genetic relationship of potato genotypes.

III. RESULTS AND DISSCUSSION

SSR polymorphism

A total of 20 SSR primers used for distinguishing potato genotypes were selected based on the quality criteria, genome coverage, and locus- specific information content as studied by Ghislain *et al*., (2009). Out of twenty SSR primers fourteen primers were polymorphic and six primers were found monomorphic (STI0003, STM0040, STM1031, STM1058, STM1045 and STM0019). A total of 33 different loci were amplified that exhibited 90 per cent polymorphism. The PIC value ranged from 0.11 to 0.70. Analysis for polymorphism in SSR markers has been provided in Table 3. All the loci amplified by the primer which were found to be polymorphic varied in size from <100bp to >300bp. Maximum number of four polymorphic bands were amplified using primer STM2005 where primers STG0016, STI0023, STI0014 and STM5127 amplified three bands each. The PCR profile of primer STM2005 and STG0016 provided in Fig. 1 and 2. Primer STM2005, STG0016, STI0023 having high polymorphism value were the most informative among multi loci SSR markers used, capable of distinguishing all the varieties studied. Primer STM2005 (highest 4 alleles) and STG0016 (3 alleles) could distinguish all the varieties except Pant Sel-09-57 and J-96-288. Primer STI0014 was comparatively less informative and could distinguish only 40 genotypes. Primer STM2005, STM0016 and primer STI0030 gave 0.70, 0.65 and 0.14 PIC respectively in our study where the same primer gave 0.78, 0.79 and 0.83 PIC respectively in study done by Solano *et al*. (2013), where primer STM0016 amplified highest number of loci. This may possibly due to difference in study material with varied genetic basis or due to narrow genetic basis of few germplasm as they all are derived through selection and few released varieties have one or other genotypes in their parentage. The number of allele ranged from 1 to 4 with an average of 2.0 as compared to other studies which may be due to lesser number of markers used.

Table 3. A summary of data analysis of polymorphism shown by SSR markers.

Sno.	Marker	Allele Frequeny	Allele no.	Gene diversity	Amplification product size	GC content (F)	GC content (R)	Annealing Tm (⁰C)	Polymorphic bands	Monomorphic bands	MI	EMR	Polymorphism (%)	PIC value
1.	STM5127	0.75	3	0.35	250-350	35	45	60.0	3	0	1.16	2	100	0.58
2.	STI0030	0.92	1	0.15	150	36	36	62.0	1	0	0.42	3	100	0.14
3.	STG0001	0.88	2	0.19	150	50	45	66.0	2	0	0.75	3	100	0.25
4.	STG0016	0.78	3	0.44	200-250	50	55	63.5	3	0	1.95	3	100	0.65
5.	STI0012	0.83	2	0.22	150-200	43	35	63.2	1	1	0.51	3	50	0.17
6.	STI0032	0.75	2	0.32	175	45	45	63.4	2	0	0.75	3	100	0.25
7.	STM2005	0.67	4	0.48	125-250	43	43	61.0	4	0	2.20	3	100	0.70
8.	STGBSS	0.66	2	0.45	150-175	36	45	64.0	2	0	0.70	2	100	0.35
9.	STM1104	0.75	2	0.27	180-200	42	48	63.5	2	0	0.42	2	100	0.21
10.	STM5121	0.94	1	0.12	175	50	45	66.0	1	0	0.33	3	100	0.11
11.	STI0023	0.67	2	0.44	150-250	55	50	64.0	3	0	1.63	3	100	0.55
12.	STI0014	0.86	2	0.23	150-250	41	43	62.5	3	0	1.20	3	100	0.40
13.	STI0036	0.89	2	0.19	150-200	55	38	63.0	2	0	0.78	3	100	0.26
14.	STI1050	0.78	2	0.31	150-200	28	41	52.5	2	0	0.75	3	100	0.25

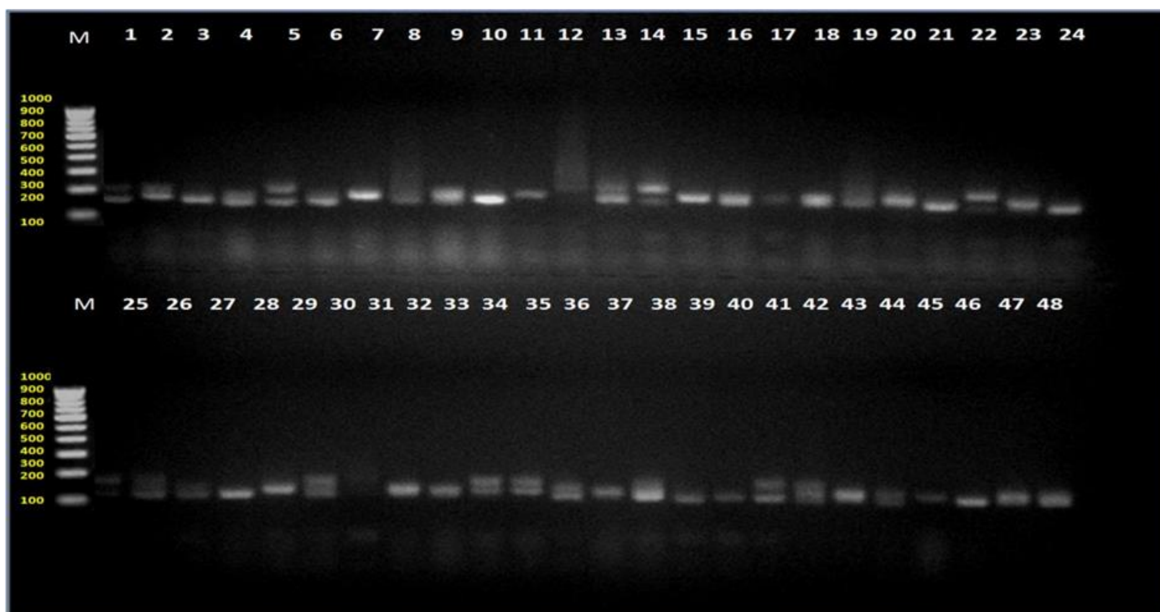

Fig.1. Amplification pattern of primer STM2005

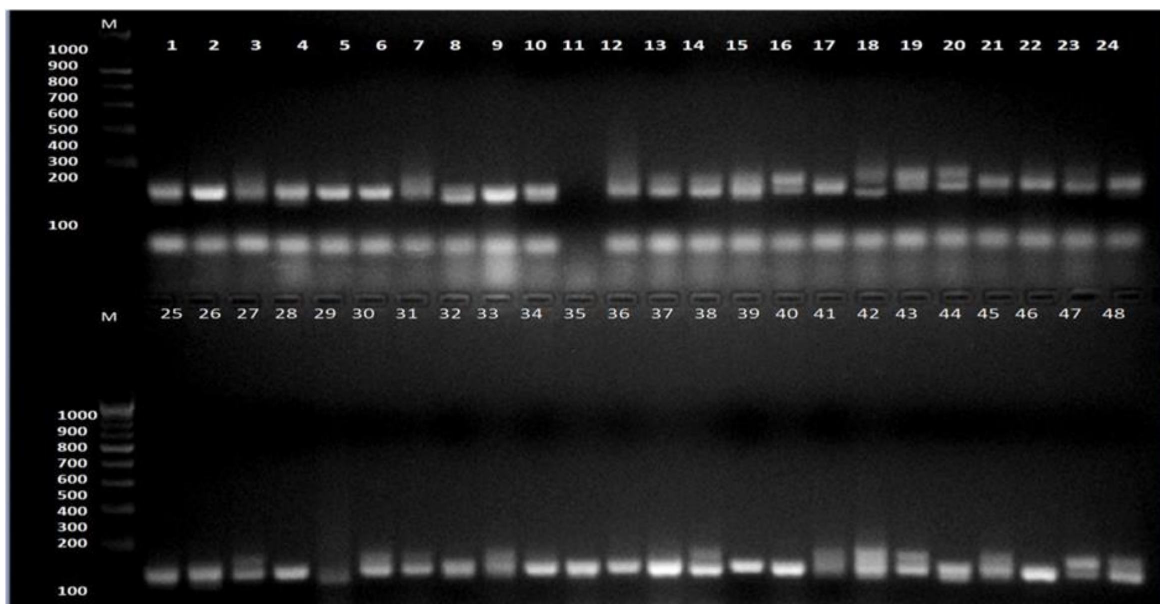

Fig.2. Amplification pattern of primer STG0016

According to Demeke *et al*. (1993), identification across database becomes easy once a fixed set of primer combination were taken in consideration. Present study in which SSR amplified a total of 33 different loci that exhibited 90 per cent polymorphism gave a better insight to which genotype are genetically more diverse. Favoretto *et al*. (2011) also found SSRs to generate three to five amplified loci. Similar results were reported by Komy *et al*. (2012) and Sharma *et al*. (2014). Many researchers (Demeke *et al*., 1993; Ghislain *et al*., 1999) have already differentiated 100 commercial potato cultivars with only twelve specific primers producing more DNA amplified polymorphism. The reported heterozygosity across screened genotypes suggested the genetic material that are distantly related and superior, can further be introduced as parents in breeding programmes (Wang *et al*., 2017; Wu *et al*., 2019).

Genetic diversity analysis

Based on the SSR marker data the Jaccard's similarity coefficients were estimated between pair of genotypes. The similarity coefficient was found to vary from 0.32 to 0.92. The highest value for genetic similarity (0.92) was found between Pant Sel-09 and Pant Sel-09-04 followed by both Pant Sel-09-04 and Kufri Jyoti with Kufri Jawahar (0.91), Pant Sel-09-11 and J-95-225 (0.91), Kufri Khyati and Kufri Badshah (0.91) and Pant Sel-09-08 and Pant Sel-09-04 (0.91). The lowest similarity value (0.32) was found between Pant Sel-09-20 and Pant Sel-09-01 followed by Pant Sel-09-57 and Pant Sel-01 (0.35), Pant Sel-09-20 and Pant Sel-15/5 (0.36) and Pant Sel-09-20 and Pant Sel-09-57 (0.38). This analysis suggests the varied germplasm collection with least to highest genetic similarity among them where high similarity suggests the possibility of germplasm belonging to same geographical area or involvement of any one similar parent in the case of Kufri varieties. Whereas, the least similar genotypes provides us with the opportunity to further utilize them in breeding program.

Cluster analysis

UPGMA based on Jaccard's similarity matrix of SSR markers ordered the populations of 48 genotypes into a single big group further dividing into ten clusters (Fig. 3). The biggest clusters were cluster IV and cluster II with maximum genotypes. Cluster II consisted of ten genotypes viz. Pant Sel-09-07, J-96-288, Pant Sel-09-11, J-95-225, Pant Sel-09-53, Pant Sel-09-46, Pant Sel-09-58, Pant Sel-09-38, Pant Sel-08-02 and Kufri Bahar. The largest cluster IV consisted of twelve genotypes viz. Pant Sel-09, Pant Sel-09-04, Kufri Jawahar, Kufri Jyoti, Kufri Chipsona-1, Pant Sel-08-07-01(CT), Kufri Chipsona-3, Kufri Pushkar, Kufri Giriraj, Kufri Himalini, Pant Sel-09-08 and Pant Sel-09-21 which varied between very low to very high yielding types (Table 4). Cluster I and cluster II showed similarity of 65 to 75 per cent where, Cluster II, III, IV and V had 70 to 74 per cent similarity between them. Cluster VI and VII was found to have about 60 to 71 per cent similarity with cluster I, II,

III, IV and V whereas Cluster VIII, IX and X were found having 51 to 58 per cent similarity between them. The most diverse groups found were Cluster X and cluster II followed by cluster IX and III with Cluster II which clearly reveals that choosing parents/genotypes from these diverse clusters may produce heterosis in segregating generations which could be utilized further for development of good and promising hybrids. In cluster analysis, all 5 advanced breeding lines or J- series scattered in five different clusters indicating presence of sufficient variability among them. Genotypes belonging to Pant-series were also found scattered in different group along with low to high yielding and late blight susceptible to resistant Kufri varieties, which are similar to the findings of Demeke *et al*. (1993) and Grover *et al*. (2009).

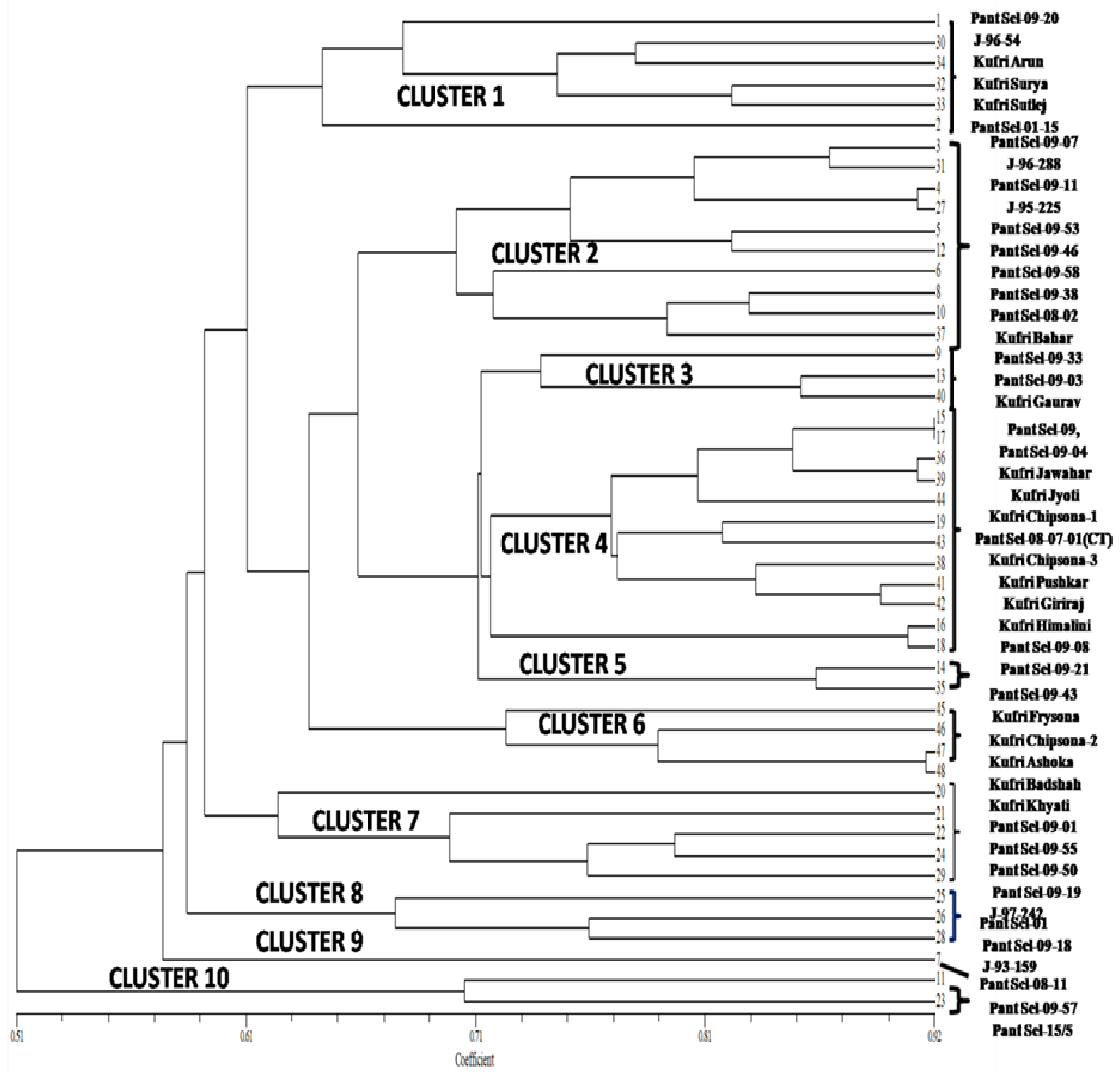

Fig.3. Dendrogram illustrating the phylogenetic relationship among 48 potato genotypes based on UPGMA cluster analysis.

Table 4. Distribution pattern of 48 potato genotypes on the basis of UPGMA cluster analysis.

Clusters Number	No. of genotypes	Genotypes
I	6	Pant Sel-09-20, J-96-54, Kufri Arun, Kufri Surya, Kufri Sutlej and Pant Sel-01-15
II	10	Pant Sel-09-07, J-96-288, Pant Sel-09-11, J-95-225, Pant Sel-09-53, Pant Sel-09-46, Pant Sel-09-58, Pant Sel-09-38, Pant Sel-08-02 and Kufri Bahar
III	3	Pant Sel-09-33, Pant Sel-09-03 and Kufri Gaurav
IV	12	Pant Sel-09, Pant Sel-09-04, Kufri Jawahar, Kufri Jyoti, Kufri Chipsona-1, Pant Sel-08-07-01(CT), Kufri Chipsona-3, Kufri Pushkar, Kufri Giriraj, Kufri Himalini, Pant Sel-09-08 and Pant Sel-09-21
V	2	Pant Sel-09-43 and Kufri Frysona.
VI	4	Kufri Chipsona-2, Kufri Ashoka, Kufri Badshah and Kufri Khyati
VII	5	Pant Sel-09-01, Pant Sel-09-55, Pant Sel-09-50, Pant Sel-09-19 and J-97-242
VIII	3	Pant Sel-01, Pant Sel-09-18 and J-93-159
IX	1	Pant Sel-08-11
X	2	Pant Sel-09-57and Pant Sel-15/5

Kufri Jyoti and K. Jawahar shared the same cluster IV which is likely because K. Jyoti is included in the parentage of K. Jawahar. However, K. Chipsona -3 having K. Chipsona-2 in parentage were found in different groups. This observation can explain the poor correlation among co-ancestries and performance of the progeny. Kufri Jawahar, Kufri Chipsona-1, Kufri Chipsona-3 (all late blight resistant varieties) along with Kufri Jyoti, Kufri Pushkar, Kufri Giriraj, Kufri Himalini (moderately susceptible to late blight resistant variety) belonged to cluster IV. It is likely that other genotypes viz. Pant Sel-08-07-01(CT), Pant Sel-09, Pant Sel-09-04, Pant Sel-09-08 and Pant Sel-09-21, belonging to the same cluster could confer resistance to late blight disease. However, late blight resistant varieties namely Kufri Badshah, K. Chipsona-2 and K. Khyati (field resistant) shared common cluster VI along with a late blight susceptible variety K. Ashoka. Although, they all were high yielding types and shown field resistant to blight disease which is similar to findings of Rocha *et al*. (2010), Tiwari *et al*. (2013) and Wang *et al*. (2017). Some of the Kufri varieties were grouped in same cluster even though they were bred from parent of wide genetic base with possible reason may be that these varieties were developed with the main aim of high yield under similar agro-climatic conditions of sub-topical plains. The genotypes viz. Pant Sel-09-20 and Pant Sel-09-01, Pant Sel-09-20 and Pant Sel-15/5 and Pant Sel-09-20 and Pant Sel-09-57 with low genetic similarity can be used for further research.

Therefore, geographical diversity of the material alone would not help in selection of genetically divergent parents. For example during field trial, genotypes namely Pant Sel-09-38, Kufri Frysona, Kufri Himalini, Pant Sel-09-04, Pant Sel-08-11, Kufri Pushkar, Pant Sel-09-50 and Pant Sel-09-43 were the best yielding genotypes but during molecular analysis, they all belonged to different clusters along with low yielding genotypes. Moreover, germplasm namely Pant Sel- 08-02, Pant Sel- 09-04, Pant Sel-09-43, Pant Sel-09-20, Pant Sel-09-11, Kufri Badshah, Kufri Ashoka, Kufri Chipsona-1 and Kufri Chipsona-2 showed high to moderate field resistance to late blight disease but no clear cut grouping was observed in resistant and susceptible genotypes by SSR primers as compared to field data indicating limited or low kinship relationship between morphological and molecular data among forty eight potato genotypes. This observation confirms that divergence is at intron and exon level both, making markers important for new hybrid development programme via combining distantly related genotypes. Molecular marker led cluster analysis provided an insight to marker's potential to carry out more comprehensive diversity analysis (Barandella *et al*. 2006; Wang *et al*. 2017; Duan *et al*. 2018; Dumhai *et al*. 2019).

IV. CONCLUSION

Evaluation of the genetic diversity of 48 potato genotypes based on 20 SSR markers gave clear idea about the genetic relationship among genotypes which resulted into grouping on the basis of the genetic distance among them aiding to deep knowledge about genetic makeup of genotypes. On the basis of PCR amplification various distantly related genotypes were identified. From this study, it may be concluded that significant diversity and variability was present among the genotypes and divergence analysis using SSR markers was proved to be better than morphological data for discrimination among genotypes. It is clear that microsatellites offer an effective means of analysing genetic distance between potato varieties which are especially useful for potato breeding program.

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