Molecular Markers in Potato

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MOLECULAR MARKERS

1. Molecular profiling

1.1 DNA fingerprinting: A morphological marker is simply a mutation in a particular gene, which imparts a discrete, easily identifiable phenotype to an organism. Rarity of morphological markers was, however, a limiting factor for their use in genome mapping. It was, therefore, thought that biochemical markers that can be separated on the basis of relative mobility of enzyme isoforms would be more helpful in genome mapping. However, biochemical markers also suffered from similar limitations like that of morphological markers. Moreover, in both the above cases the markers were influenced by environment, stage of growth and tissues/organs used for analysis. A quantum leap towards genome mapping was only possible after introduction of DNA markers that are plenty in number and least affected by environment and stage of growth. The relative position of any marker in a chromosome is determined by the conventional principle of linkage analysis. Marker assisted selection and DNA fingerprinting (genotyping) are two important practical applications of molecular markers.

Molecular markers have been extensively used at ICAR-CPRI, Shimla for diversity analysis in potato germplasm/varieties. Both nuclear and cytoplasmic genome of the potato varieties have been studied using different markers. Initially isozyme and RAPD markers were used to characterize the Indian varieties. RAPD markers have also been used for checking genetic integrity of potato after micropropagation and long-term conservation. RAPD markers have also been used to study extent of genetic diversity within the indigenous population of the late blight pathogen *Phyotophthora infestans* and bacterial wilt pathogen *Ralstonia solanacearum*. These markers have been used to assess the diversity present within and between the late blight resistant and susceptible Indian potato cultivars. Most of the diversity was detected within variety types, with 88% of variation being within and 12% being between the resistant and susceptible cultivars. No clear groupings based on late blight resistance and susceptibility or kinship was reflected on the dendogram. More stable markers like SSR (simple sequence repeat) was also tried to study genetic diversity of potato varieties. Later a set of 24 highly informative SSR markers (two from each linkage group) called the Potato Genome Identification (PGI) kit was used to characterize the diversity among Indian potato cultivars, indigenous potato varieties, exotic potato varieties and Indian Andigena core collection. The PGI kit is being used for assessing the diversity among all the potato germplasm collection present at the institute. The utilization of inter simple sequence repeats (ISSR) markers for the varietal identification and diversity analysis has also been standardized and used on a routine basis. Cytoplasm type potato genotypes also studied using plastome-specific and chondriome-specific markers. All the potato genotypes have been into 4 cytoplasm (plastome/chondriome) types (T/β, W/α, W/γ and A/ε).

One of the most useful applications of molecular markers is genotyping, also popularly known as fingerprinting. Unlike morphological descriptors, profiles created by using molecular data are independent of environmental effect. Therefore, the International Union for the

Protection of (New) Plant Varieties (UPOV) has constituted a working group to critically examine the feasibility of using biochemical and molecular techniques (BMT) for variety identification. DNA fingerprints can be used for establishing distinctness as well as for checking uniformity and stability of a particular variety. Though DNA fingerprints are not yet mandatory for variety registration, several countries have created extensive database on DNA fingerprints of several crops including potato. DNA fingerprints of Indian potato varieties and advanced hybrids had been initially created using RAPD markers. Because of unstable nature of RAPD markers, fingerprints generated by SSR markers are now being favoured for variety identification. The DNA fingerprinting lab is accredited by the Department of Biotechnology (DBT) for the genetic fidelity test of potato as a national facility under Accredited Test Laboratory. The fingerprinting is done using '3500 Genetic Analyzer' and the profiles are analyzed using inbuilt software (Applied Biosystems).

1.2 Developed SSR markers for identification of Indian potato varieties

Analyzed allelic variation in potato varieties and developed a two-markers-set (STIKA and STU6SNRN) for varietal identification, genetic fidelity testing, DUS testing and molecular characterizations. We aimed 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). 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.

STM5127

STM5114

STM1053

STI0012

STM1052

STM1106

STIKA

STU6SNRN
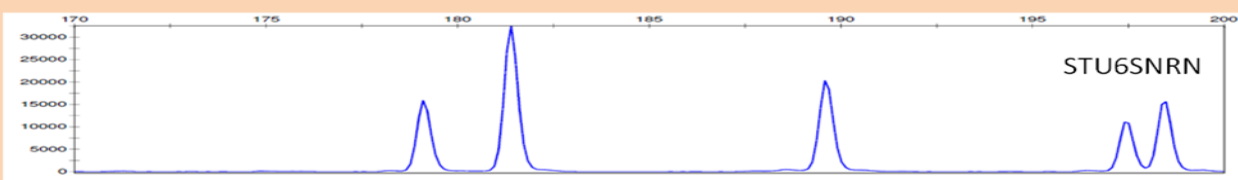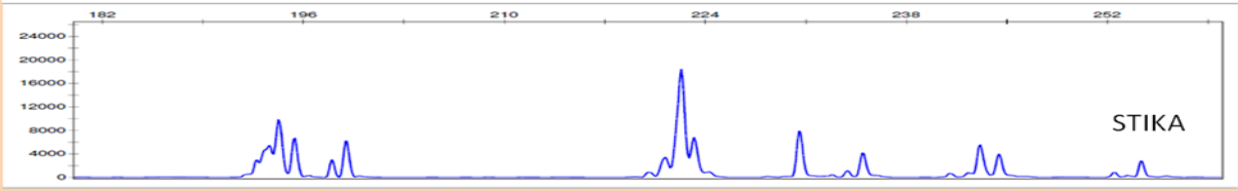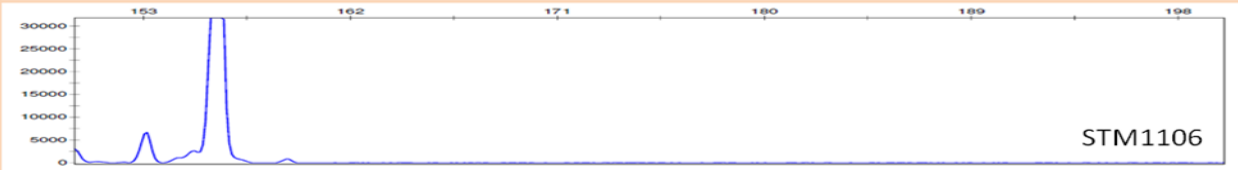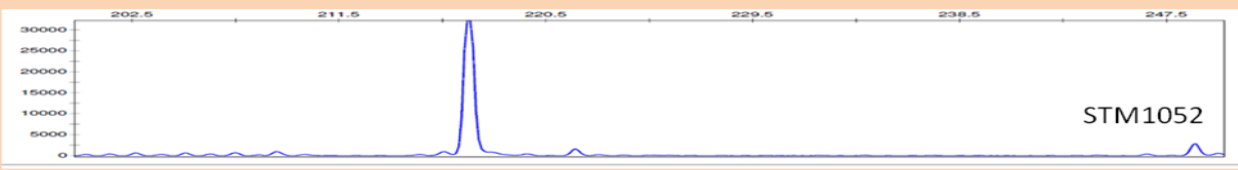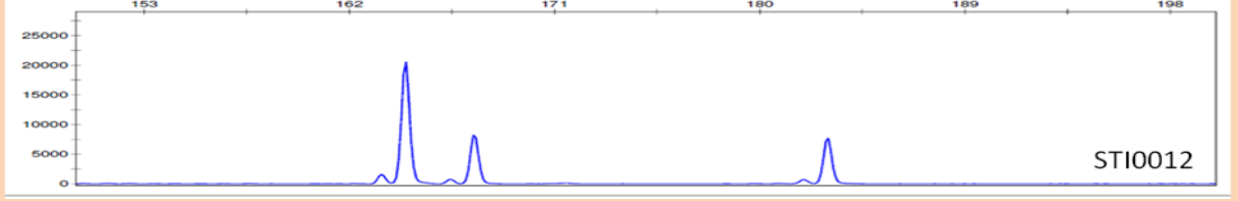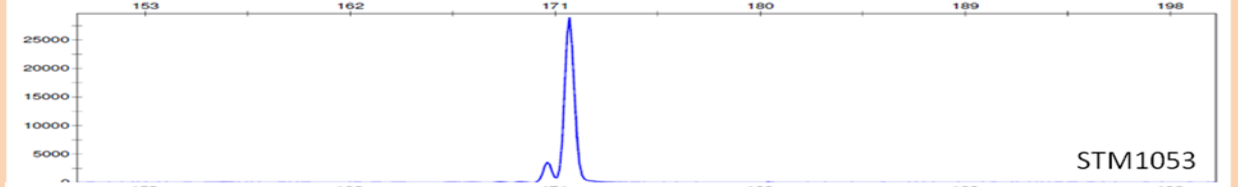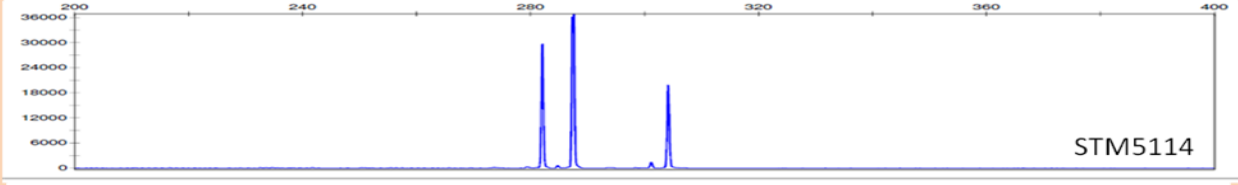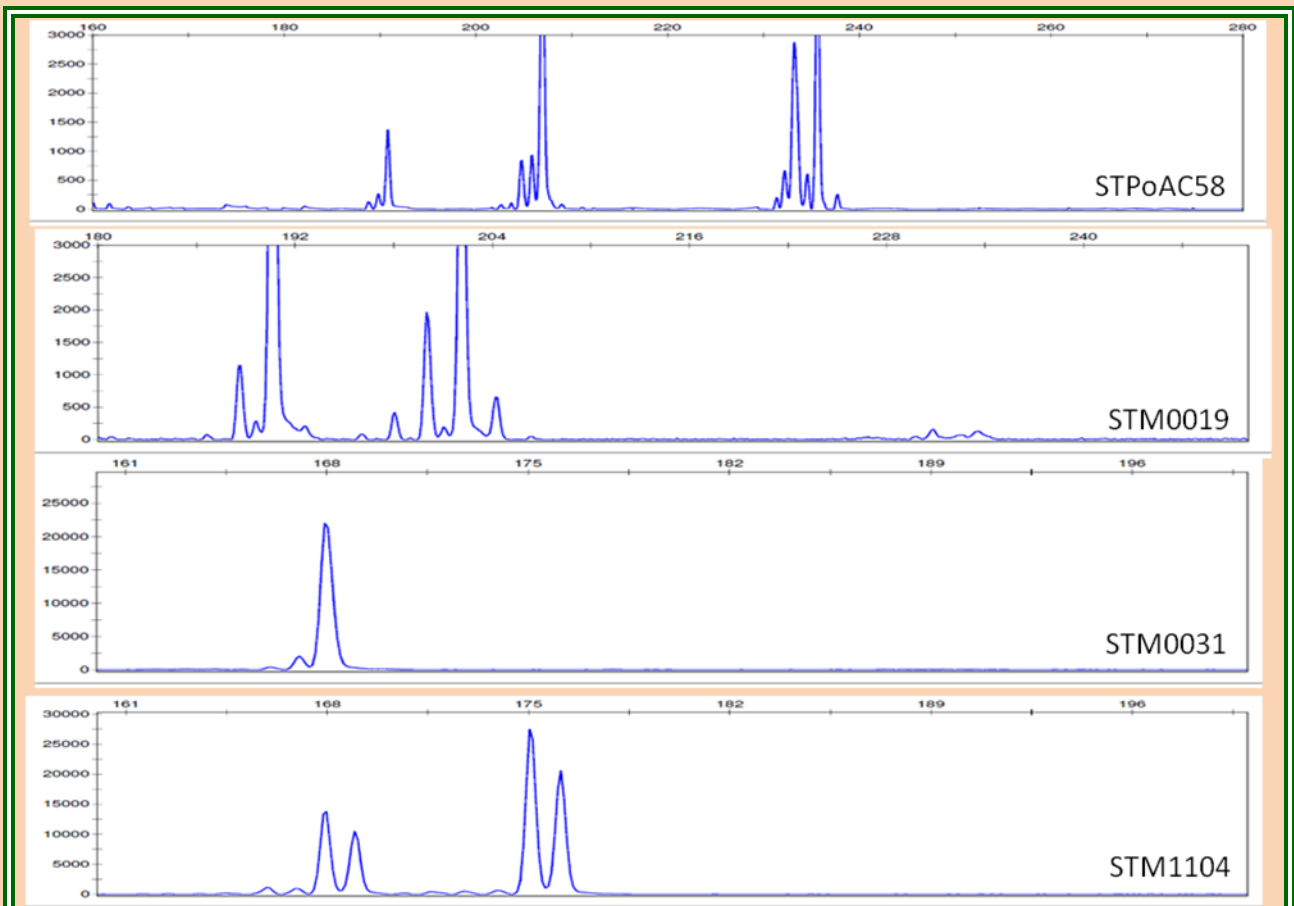
Fig. 1. SSR fragments analysis of potato cv. Kufri Alankar using '3500 Genetic Analyzer' (ABI). Marker name is shown on the respective images.
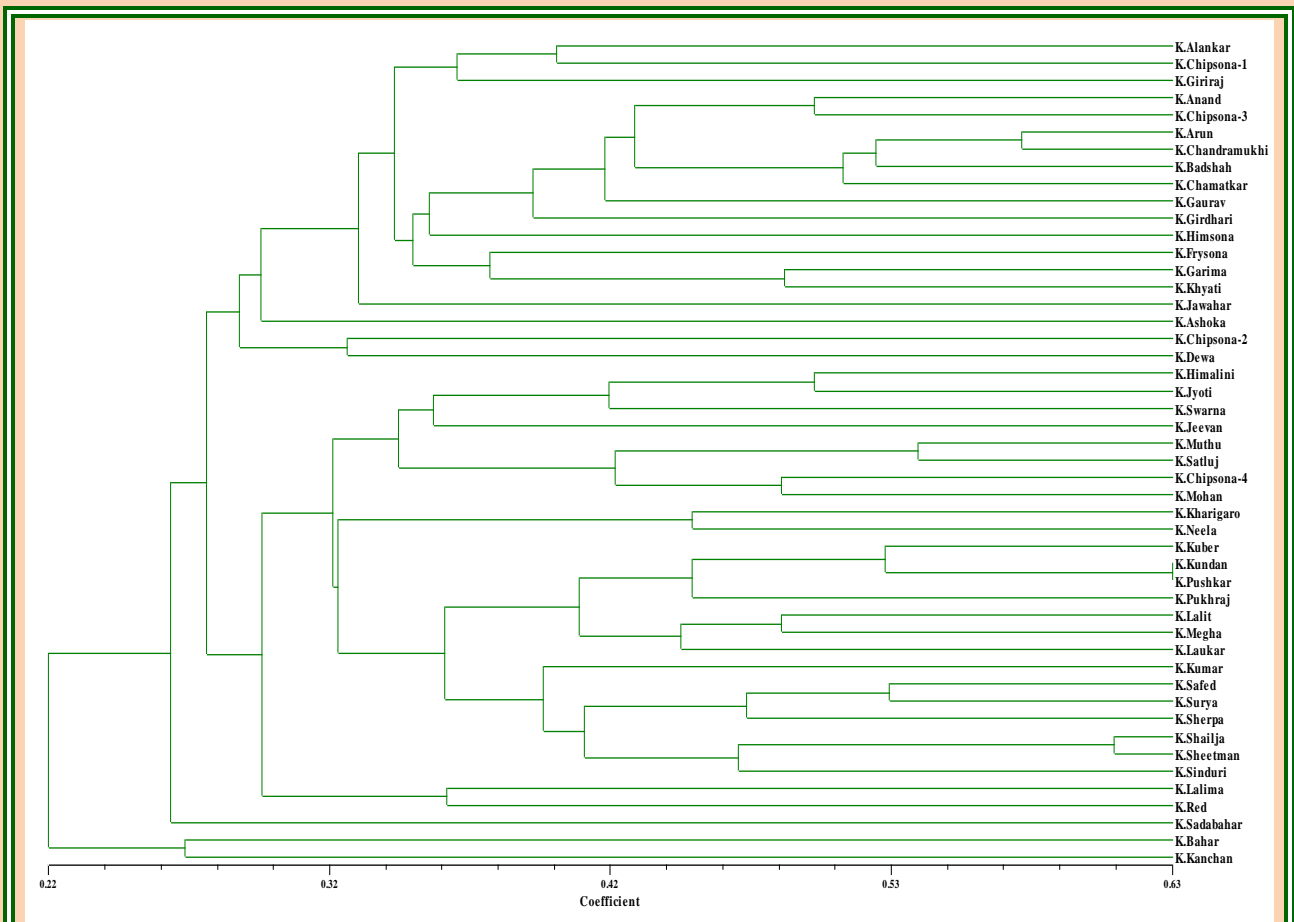
Fig. 2. Cluster analysis of 48 Indian potato varieties based on the Jaccard's coefficient by UPGMA method using 12 SSR markers.

Table 2. A database of summary of SSR allelic variations in 48 Indian potato varieties.

SN	Variety	STM5127	STM5114	STM1053	STI0012	STPoAC58	STM0019	STM0031	STM1104	STM1052	STM1106	STIKA	STU6 SNRN	Allele frequencies
1.	Kufri Kuber	238, 241	287, 295, 304	171	Nil	230, 232, 246, 247	Nil	186	168, 169, 175, 176	207, 208, 217, 250	153, 156	190, 194, 198, 222, 225, 230, 242	178, 181, 182, 189, 198, 199	34
2.	Kufri Kumar	Nil	282, 287, 295	171	Nil	230, 232	190, 206, 235	Nil	168	208	152, 153, 155, 156	190, 194, 222, 230	179, 181, 189, 198	23
3.	Kufri Kundan	238, 241, 272	287, 295, 304	168, 171	164, 167, 170	230, 232	206	168, 185, 187	168, 169, 175, 176	207, 208, 217, 248, 250	152, 153, 155, 156, 159	190, 194, 198, 221, 222, 225, 230, 243, 253	179, 181, 189, 197, 198	45
4.	Kufri Red	241, 269, 272	282, 287, 290	171	167, 183, 189	Nil	190, 205, 206, 233, 235	170, 172, 185, 186	168, 169	207, 208, 217	Nil	190, 194, 202, 223, 227, 242	179,181, 189, 198	34
5.	Kufri Safed	238, 250, 269	282, 287, 295	171	Nil	232, 235	205, 206	Nil	168, 169	Nil	155, 192	194, 198, 230, 242	179,181, 189, 198, 199	24

No.	Variety													
6.	Kufri Neela	238	287, 290, 295	168, 171	164, 170, 173, 183	230, 232	205, 206	167	172, 173	217, 225	153,156	185, 190, 194, 222, 225, 230, 235, 237	177, 178, 189, 198, 199	34
7.	Kufri Sindhuri	238, 241, 269, 271	Nil	171	Nil	232, 240	206, 235	Nil	168	Nil	153, 156	194, 198, 198, 225, 230	179,181, 189, 199	21
8.	Kufri Alankar	238, 241	282, 287, 304	171	164, 167, 183	190, 208, 233, 235	190, 191, 201, 203, 205	168	168, 169, 175, 176	217, 250	153, 156	194, 196, 198, 222, 230, 234, 243	179, 181, 189, 197, 198	39
9.	Kufri Chamatkar	Nil	280, 287	171	158, 163, 178	Nil	101, 102	168, 185	168, 169, 172, 173	Nil	153, 156	194, 196, 200, 222, 230, 243	179, 181, 182, 189, 197, 198	28
10.	Kufri Chandramukhi	Nil	Nil	168, 171	Nil	Nil	101, 102	168, 185	168, 169	208, 217, 225	153, 156	194, 198, 222	179, 181, 182, 189, 197, 198	22
11.	Kufri Jeevan	238, 241, 272	280, 287	168, 171	Nil	Nil	Nil	Nil	168, 169, 172,	208, 217, 225	Nil	194, 222, 230,	181, 189, 197, 198,	23

No.	Variety	1	2	3	4	5	6	7	8	9	10	11	12	13
									173			234	199	
12.	Kufri Jyoti	Nil	282, 287, 295	168, 171	164, 167, 185	196, 202, 230, 232	Nil	152, 153, 167, 168, 186, 187	168, 169, 172, 173	208, 225	153, 156	190, 194, 198, 221, 225, 242	178, 181, 189, 197, 198	37
13.	Kufri Khasigaro	Nil	287, 295	168, 171	173, 182	230, 232	203, 205, 206	186, 187	172, 173	208	153, 156, 192	190, 194, 203, 225, 231	178, 181, 189, 197, 198, 199	30
14.	Kufri Sheetman	238, 241, 250, 269	290, 300	171	Nil	230, 232	190, 196, 206, 235	185	165, 166, 168, 169	207, 208, 217, 249	153, 156	190, 194, 219, 225	179,181, 189, 198, 199	33
15.	Kufri Muthu	238, 241	Nil	168, 171	Nil	232	206	152, 167, 168, 186, 187	168, 169, 172, 173	208, 217, 225	152, 153, 155, 156, 159	194, 198, 214, 222, 230, 235, 241	179, 182, 190, 198, 199, 200	36
16.	Kufri Lauvkar	238, 241, 269	287, 295	Nil	164, 167, 170, 183	230, 232, 234	233, 235	185, 187	168, 172, 175	207, 208, 217, 249	152, 153, 155, 156, 159, 189, 192, 194	190, 194, 219, 222, 225, 230	178, 181, 189, 198, 199	42
17.	Kufri Dewa	Nil	287, 295	171	164, 167, 173,	170, 202, 232	101, 102	168, 172,	168, 169, 172,	Nil	189, 192	Nil	178, 181, 189,	29

9

					189			194	173, 175, 176				197, 198, 199	
18.	Kufri Badshah	Nil	Nil	168, 171	164, 167, 170, 189	208, 235	101, 102	168, 185, 187	168, 169	208, 225	153, 156	194, 196, 200, 222, 230, 243	181, 189, 197, 198	29
19.	Kufri Bahar	Nil	Nil	168, 171	167, 170, 173	Nil	101, 102	168	172, 173, 175, 176	Nil	153, 156	190, 195, 200, 222, 225, 230	180, 182, 191, 199	24
20.	Kufri Lalima	238, 241, 272	287, 290, 295	162	164, 183, 189	232	206, 233, 235	152, 153 167, 168, 170, 172	168, 169, 172, 173	208, 223, 225	Nil	190, 194, 198, 219, 222, 225, 230, 242	179, 181, 189, 199, 200	41
21.	Kufri Sherpa	238, 241	282, 287, 295	168, 171	Nil	232, 247	205, 206	Nil	168, 169	207, 208, 249	156	190, 194, 198, 225, 230	181, 189, 198, 199, 200	27
22.	Kufri Swarna	238, 239, 241	287, 295	168, 171	Nil	232, 235	Nil	167, 187	172, 173	208, 225	152, 153, 156	194, 200, 223, 242	179, 181, 189, 197, 198, 199	28

23.	Kufri Megha	238, 241	282, 287, 295	171	167, 170, 183	Nil	Nil	152, 153, 185, 186, 187	168, 169	207, 208	153, 156	190, 194, 219, 222, 225, 230, 242	181, 189, 198, 199	31
24.	Kufri Ashoka	Nil	Nil	Nil	164, 173, 183	Nil	101, 102	185	168, 169, 172	217, 223, 225	153, 156	190, 194, 196, 200, 224, 226, 243, 255	181, 189, 197	25
25.	Kufri Jawahar	Nil	287, 295	171	158, 161	Nil	101, 102	167, 168	165, 166, 168, 169	207, 208, 217	153, 156, 159, 182	190, 194, 195, 222, 223, 225, 230, 243	181, 189, 197, 199	35
26.	Kufri Sutlej	238, 269	287, 295	168, 171	Nil	232	Nil	152, 167, 186, 187	168	208, 225	152, 153, 156	194, 198, 222	182, 190, 199, 200	24
27.	Kufri Chipsona-1	Nil	Nil	168, 171, 172	164, 167, 170, 182	208, 235	101, 102	167, 168	168, 169, 175, 176	207	156	194, 198, 222, 230	181, 189, 197, 199	27
28.	Kufri Chipsona-2	Nil	Nil	168, 171	Nil	170, 202, 232, 235	101, 102	185	168, 169, 172,	220, 222, 246,	153, 156,	185, 190, 194,	178, 181, 182,	40

								173, 175, 176	248, 250	159	198, 200, 221, 226, 230, 231, 235, 243	189, 197, 198	
29.	Kufri Giriraj	Nil	Nil	168, 171	167, 170, 182, 189	190, 196, 202, 232, 240	101, 102	168, 186, 187, 188	168, 169, 174, 175	207, 217, 225	153, 156, 159	185, 194, 198, 222, 230, 234, 236, 243	179, 181, 190, 198, 199	40
30.	Kufri Pukhraj	238	282, 287	Nil	164, 170, 183	232	190, 202, 205, 206	185	168, 169	217	152, 153, 155, 156, 159	194, 198, 222, 242	181, 182, 189, 197, 198	29
31.	Kufri Anand	238, 241, 250	282, 287, 295	171	Nil	208, 235	101, 102	Nil	168, 169, 173	225	153, 156	190, 194, 222, 223, 225, 230, 243	179, 181, 189, 197, 198	29
32.	Kufri Kanchan	Nil	300	168, 171	167, 170, 183	230, 232	203, 233, 235	185, 186	168, 169	208, 217, 225	153, 156	190, 195, 219, 222, 225, 234	179, 182, 190, 199, 200, 202	33
33.	Kufri Arun	Nil	287,	Nil	Nil	Nil	101,	168,	168,	208,	153,	194, 222,	181, 182,	19

			295				102	185	169	225	156	230, 243	189	
34.	Kufri Pushkar	238, 241	282, 287, 304	168, 171	164, 170	232	206	167, 185	168, 169, 175, 176	208, 217	153, 155, 156, 159	190, 194, 198, 222, 225, 242, 243	181, 182, 189, 197, 198	35
35.	Kufri Shailja	238, 241, 269	287, 295	171	Nil	230, 232	190, 206, 235	Nil	165, 166, 168, 169	208, 225	153, 156	190, 194, 225	181, 189, 198	25
36.	Kufri Chipsona-3	238, 241, 272	282, 287, 295	168, 171	164, 167, 170, 182, 185	206, 232	101, 102	185	168, 169, 172, 173	208, 225	153, 156, 192	194, 196, 222, 223, 230, 235	179, 181, 189, 198	37
37.	Kufri Himalini	238, 241, 272	Nil	168, 171	158, 162, 167, 185	Nil	101, 102	167, 168, 186, 187	168, 169, 172, 173	208, 225, 226	153, 156, 159	190, 194, 198, 200, 223, 226, 242, 257	178, 181, 189, 197, 198	38
38.	Kufri Surya	238, 241, 250, 269	282, 287, 295	168, 171	nil	232	206	Nil	165, 168, 175	208, 217, 225	153, 156, 192	187, 194, 198, 222, 230, 236, 242	179,181, 189, 199	31

|													
39.	Kufri Girdhari	Nil	Nil	168, 171	167, 170, 183	190, 206, 232	101, 102	184, 185	168, 169	200, 207, 208	151, 153, 156, 159, 194	190, 194, 222, 225, 230	179, 181, 189, 198	31
40.	Kufri Himsona	Nil	282, 287, 295	Nil	164, 170, 185	170, 206, 230, 232	101, 102	185	168, 169, 175, 176, 177, 178	208, 226	156, 192	194, 198, 222, 230	179, 181, 189, 198, 199	32
41.	Kufri Khyati	Nil	nil	168, 171	Nil	232, 240	190, 203, 205, 206	152, 153, 167, 168, 186, 187	168, 169, 175, 176	208, 225	153, 155, 156	194, 195, 222, 230, 242	181, 182, 189, 197, 198	33
42.	Kufri Sadabahar	241, 272	287, 295	171	164, 167, 170	Nil	Nil	Nil	Nil	217, 225	153, 156, 159	221, 230	179,181, 189, 198	19
43.	Kufri Frysona	Nil	284, 295	168, 171, 172	164, 170, 182, 185	190, 206, 232	101, 102	187	168, 169, 175, 176, 178, 179	207, 208, 224, 225	153, 156, 194	194, 196, 200, 223, 231, 242, 244, 248	178, 181, 182, 189, 197, 198, 199	43
44.	Kufri Chipsona-4	238, 241, 269, 272	282, 287, 295	168, 171, 172	Nil	230, 232	206	186, 187	168, 173	208, 225	153, 156, 192	194, 198, 221, 242, 248	179, 182, 189, 198, 199,	33

No.	Name													
													200	
45.	Kufri Garima	Nil	284, 295	168, 171	170, 173, 182	Nil	101, 102	168, 185	168, 169, 175, 176	208, 225	152, 153, 155, 156, 159	194, 195, 220, 222, 230, 242, 246	181, 182, 189, 197, 198	34
46.	Kufri Gaurav	Nil	284	168, 171	164, 167, 170, 182	Nil	101, 102	184, 185	168, 169, 172, 173	217, 225	153	194, 198, 222, 242, 246	179, 181, 182, 189, 198	28
47.	Kufri Lalit	238, 241, 250	282, 287, 295	171	164, 167, 170, 189	232, 234	Nil	185	168, 169, 172, 173	208	Nil	190, 194, 222, 230, 242	178, 181, 189, 197, 198, 199	30
48.	Kufri Mohan	238, 241, 250	282, 287, 295	168, 171	Nil	232	190, 235	Nil	168, 173	207, 208	153, 156	194, 214, 221, 234, 242, 246	182, 190, 198, 199, 200	28
	Total allele frequencies	73	89	73	96	83	92	94	153	108	124	273	234	1492

Note: SSR fragments analysis was performed on '3500 Genetic Analyzer' (ABI). 'Nil' represents either no or vey minor non scorable amplification.

1.3 Molecular diversity of Andigena potato core collection

Molecular diversity was validated in the core collection of 77 Andigena accession (*Solanum tuberosum* Gp. Andigena) using 24 SSR markers. Molecular diversity of the Andigena core collection based on the microsatellite data appears to have quite distinct genotypes. The core collection of 77 accessions was prepared based on 21 morpho-agronomic traits of total 740 accessions.
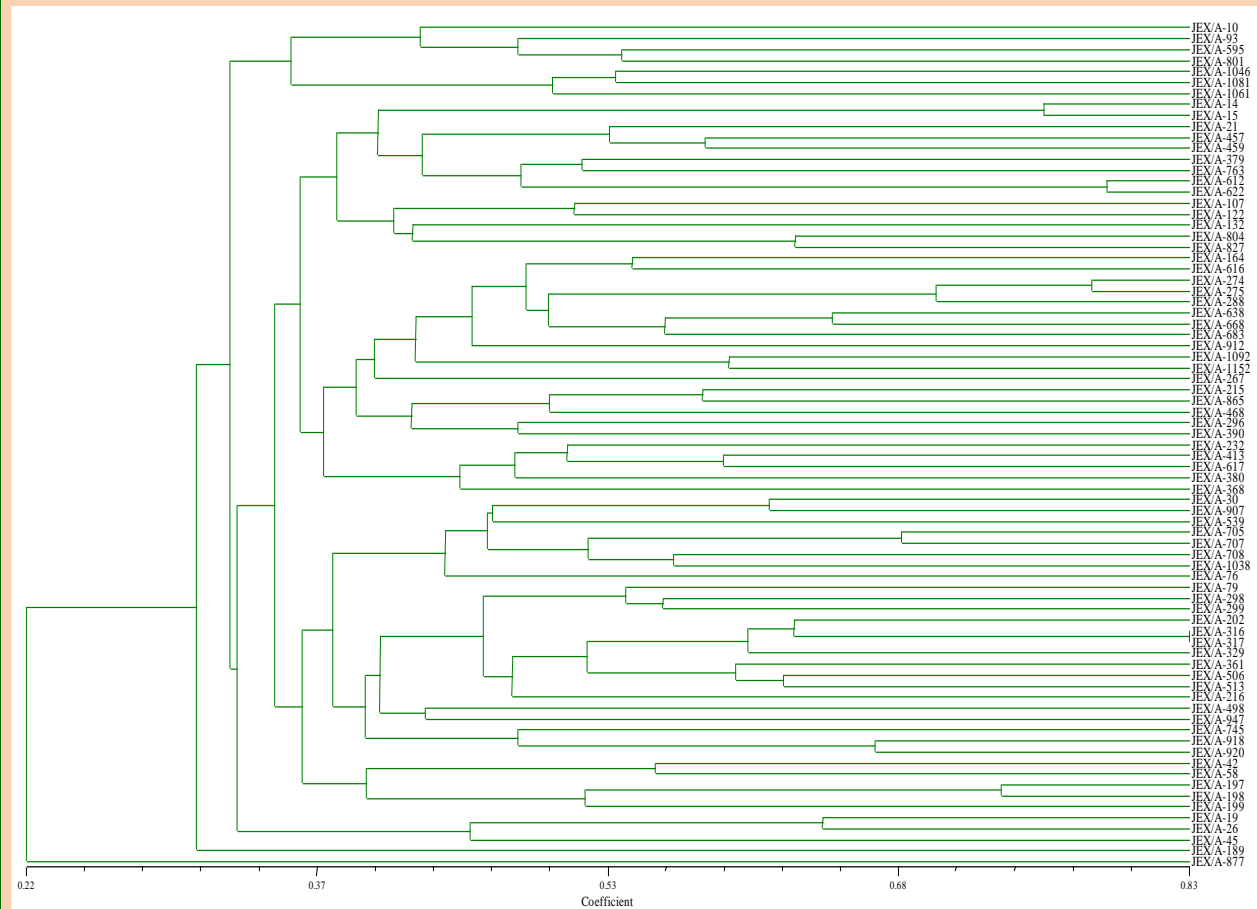

Fig. 3. Dendrogram based on the Dice similarity coefficient showing the core Andigena core collection of 77 accessions.

## 1.4 Genetic stability of tissue culture potato plants

Genetic fidelity of *in vitro* propagated potato microtubers was assessed using molecular markers (RAPD, ISSR, SSR and AFLP). Our study suggests that genetic stability of *in vitro* conserved microplants for three-years on MS medium supplemented with 40 g/L sorbitol and 7 g/L agar at low temperature (7±1°C) under controlled conditions is a safe method for conservation of true-to-type potato genotypes. Further, genetic and epigenetic changes (DNA methylation) were examined by molecular markers (AFLP- Amplified fragment length polymorphism; and MSAP-methylation-sensitive amplified polymorphism) in the tissue-culture propagated somatic hybrids.

Study showed that *in vitro* propagated somatic hybrids could be multiplied 'true-to-type' upto 30th cycles of sub-culturing from micropropagated plants for their future use in the potato breeding.
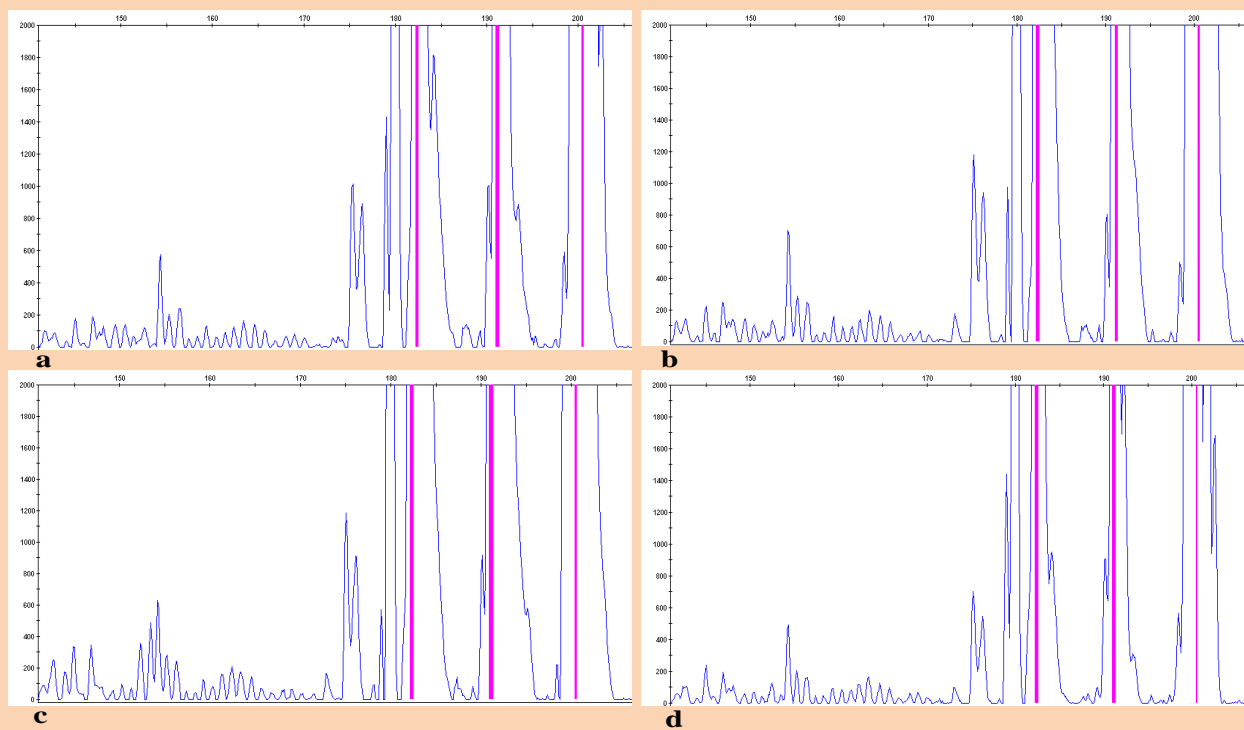
Fig. 4. SSR profiles generated by marker STU6 of potato genotypes a) Kufri Sindhuri *in vitro*, b) Kufri Sindhuri T1, c) Kufri Sindhuri T2, and c) Kufri Sindhuri T3.
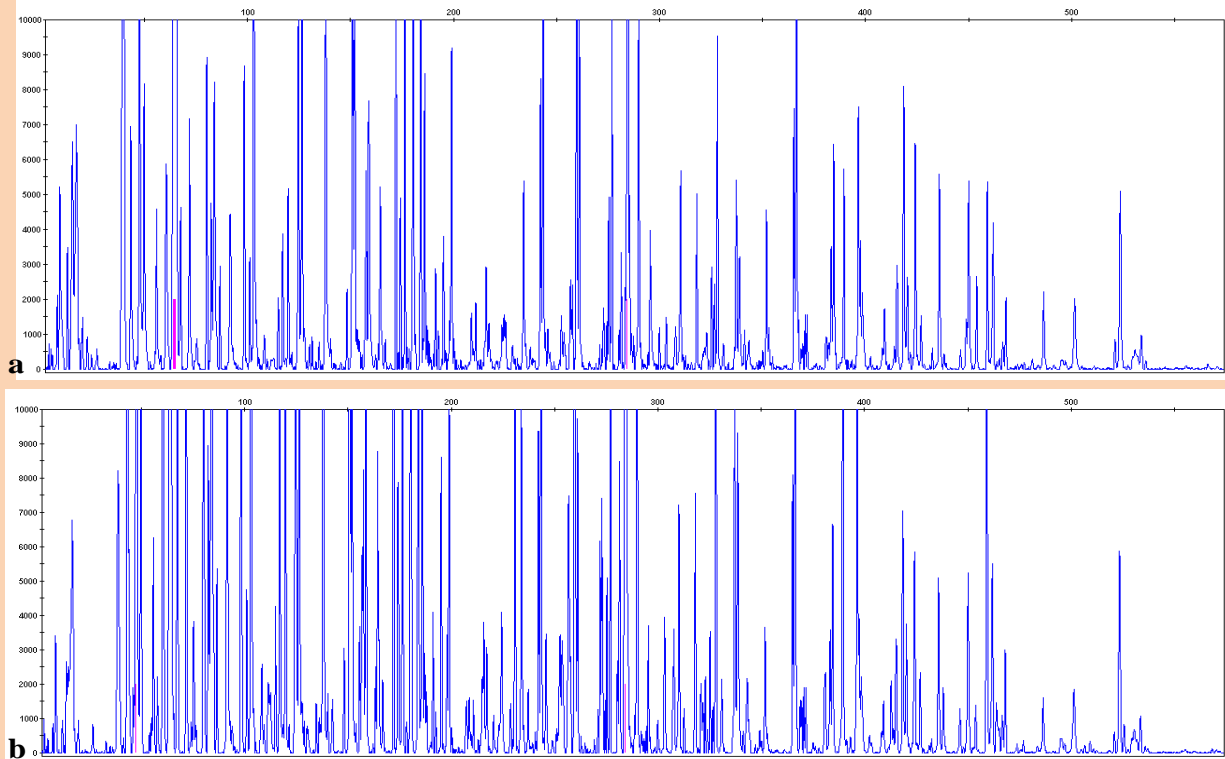
Fig. 5. AFLP profiles of the primer pairs E11+M1 showing genetic similarity of the somatic hybrid mother plant P7 (a) and its regenerant (b).

1.5 SSR allelic diversity of wild potato species

Allelic variation in wild potato (*Solanum*) species was analysed using 14 simple sequence repeat (SSR) markers. SSR allelic profiles showed high polymorphism and distinctness among the wild species. Cluster analysis using SSR allelic profiles of 82 accessions grouped showed five major clusters (I-V) based on the Dice similarity coefficient using Neighbour-Joining clustering method. Distinct allelic variations were observed among the accessions irrespective of the origin country, series and species. Our study suggests that SSR-based molecular characterization of wild potato species is accession specific and development of an allelic dataset for all the accessions would strengthen their utilization in potato research in future.

Fig. 6. Wild potato (*Solanum*) species were used in SSR analysis.
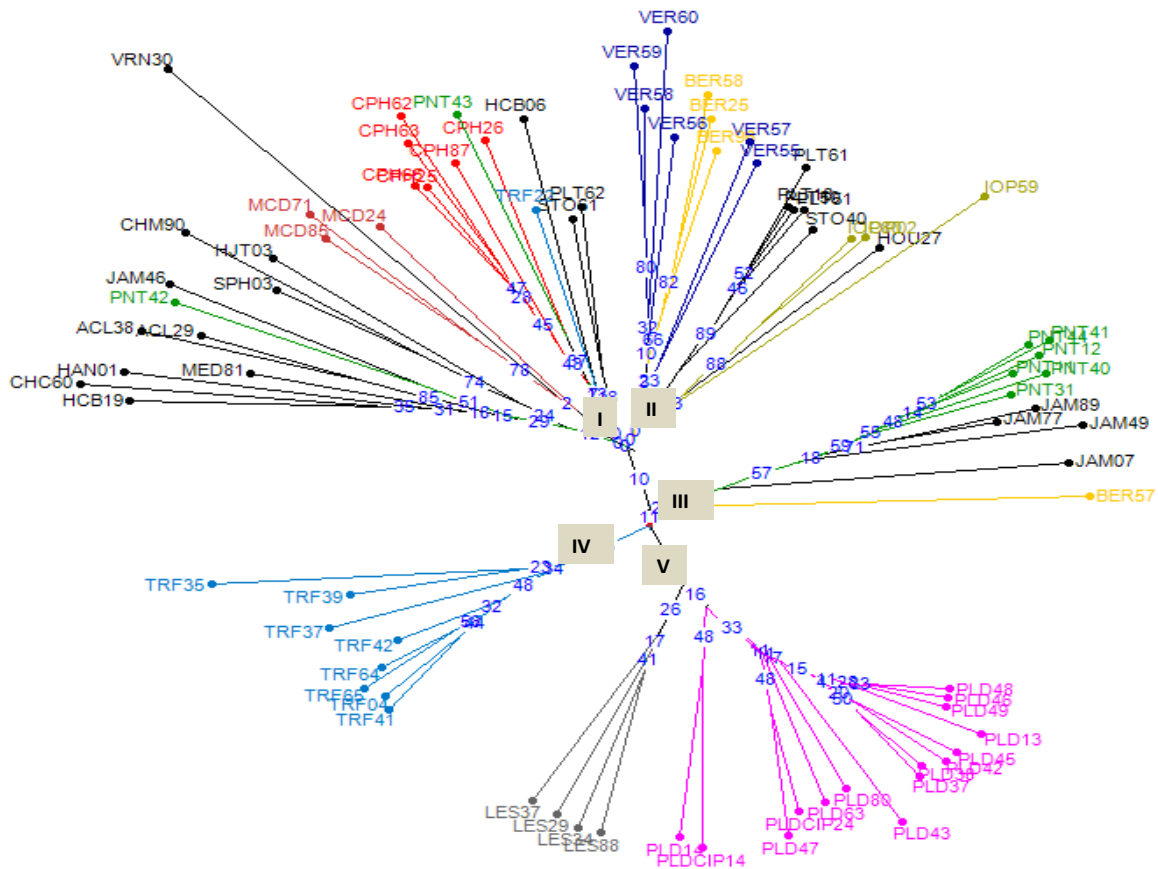
Fig. 7. Diversity analysis in wild species using 14 SSR markers based on the Dice coefficient using Weighted Neighbour-Joining tree construction method.

2. Marker assisted selection

Marker assisted selection (MAS) is particularly useful in case of introgression breeding where one or a few useful genes from an otherwise undesirable wild background have to be transferred to a commercial cultivar. In usual practice, this is achieved by recurrent back crossing and selection cycles. Markers tightly linked to the gene of interest can be used in such cases to identify progenies with minimum ingress of wild genome, thereby decreasing number of breeding generations as well as recurrent selection. MAS also provides a uniform and objective scoring of offspring and avoids cumbersome phenotypic scoring until the end of the breeding programme. Tightly linked markers for many qualitative and quantitative traits of potato have been published and are available for MAS. A published SCAR marker tightly linked to extreme resistance to PVY has been validated here and it was used to develop triplex parental line and for breeding potato varieties with combined resistance to PVY and late blight. Similarly, diversity analysis of late blight resistant and susceptible cultivars using RAPD markers revealed a particular fragment $OPC16_{915}$ that was found in most of the resistant potato varieties for which

SCAR marker has been developed. Very recently, two markers linked to high carotenoid content in potato β Carotene hydrogenase (bch) and Zeaxanthin epoxidaseq (*zea*) have been validated using 150 potato germplasm lines including the 50 Indian potato cultivars and 68 indigenous varieties. These markers are being used in screening and selecting the parents in breeding programs for developing nutrition rich potatoes. At the institute, breeding of multiple disease resistance is actively undergoing at the institute. Recently, a new potato variety Kufri Karan has been released through MAS. This variety has high resistance to late blight, viruses and moderate resistance to potato cyst nematode.

Severe inbreeding depression of cultivated potato precludes development of homozygous parental line. Therefore, a SCAR marker was validated and used to develop a unique parental line having PVY extreme resistance gene (Ry_{adg}) in triplex state through marker assisted pre-breeding which has been registered as INGR 10143 by the Plant Germplasm Registration Committee of ICAR-NBPGR, New Delhi. Further, we have identified several potato germplasm lines with PVY resistance using molecular markers. Potato germplasm have been identified with extreme resistance to PVY using molecular markers by Ry_{adg} gene [SCAR (RYSC3) and CAPS (ADG2/*Bbv*I)] and ELISA. The Ry_{adg} gene derived from *Solanum tuberosum* ssp. *andigena* provides extreme resistance to PVY.
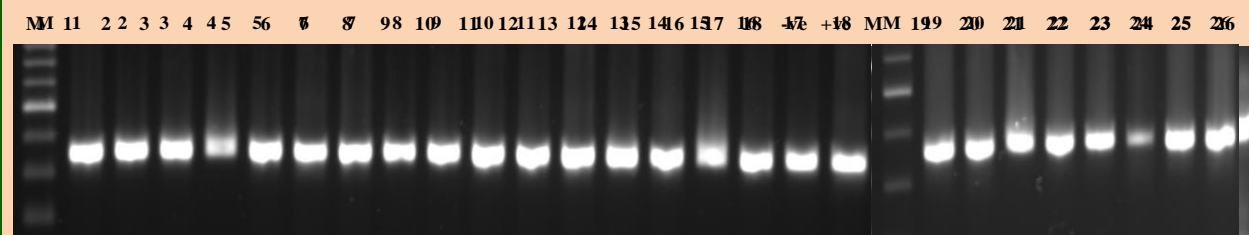
Fig. 8. MAS using SCAR marker RYSC3 (321 bp, above) and CAPS marker ADG2/*Bbv*I (355 bp, below) in PVY resistant potato germplasm.

Recently we have development of CAPS marker for nitrogen use efficiency in potato. We analyzed sequence variations in candidate genes involved in N metabolism and developed molecular marker for improving NUE in potato crop using two contrasting potato varieties Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient). Identified 12 '*N*-homologous genes' (NCBI acc. KU965581-KU965592) to the known N metabolic pathways genes. In addition, cleaved amplified polymorphic sequence (CAPS) marker was developed for the nitrate reductase (NR) gene for use in molecular breeding.
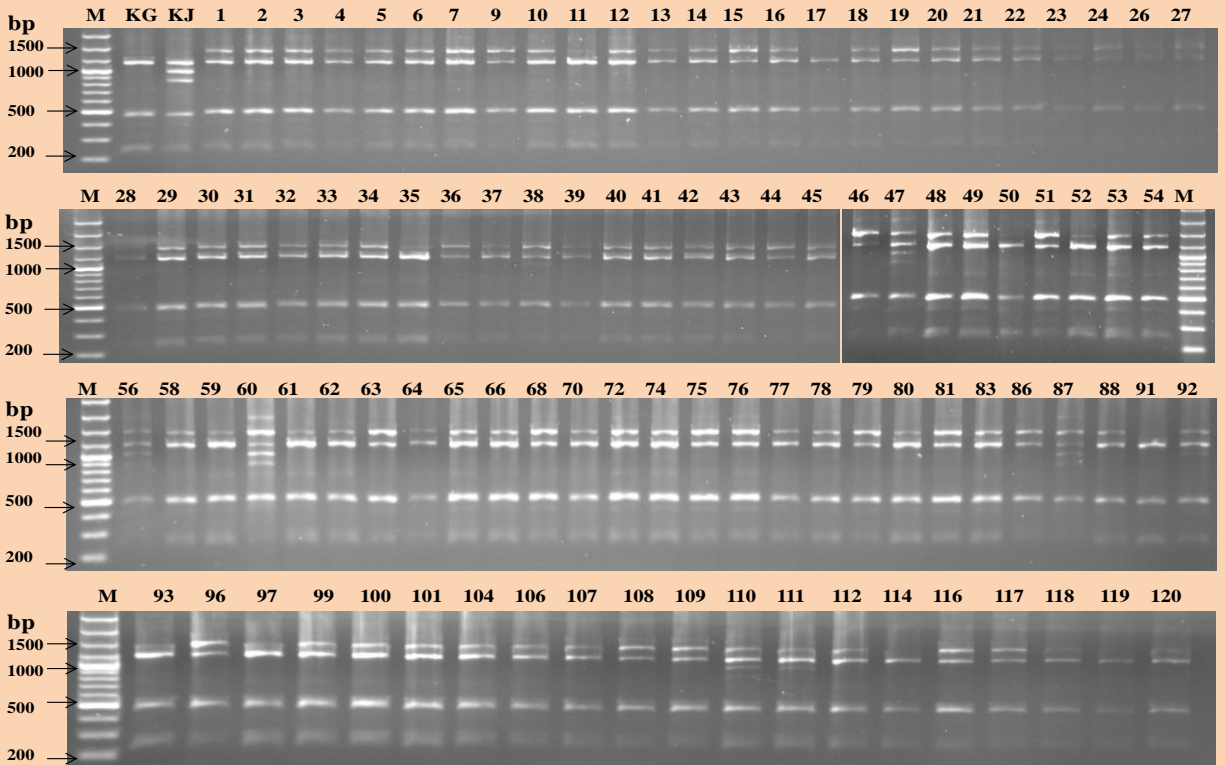
Fig. 9. Validation of CAPS marker for the NR gene in a segregating population of 123 lines (Kufri Jyoti x Kufri Gaurav). (PCR products: 500, 900, 1000 and 1200 bp were restriction digestion by *Dra*I enzyme.

Table 1. Molecular markers of late blight resistance (*R*) genes/QTLs for MAS in potato

Gene/ QTL	Chr.	Marker/primer	Marker type	Primer sequence (5' → 3')	Reference
R1	5	R1-1205	SCAR	CACTCGTGACATATCCTCACTA GTAGTACCTATCTTATTTCTGCAAGAAT	Sokolova et al. 2011
		BA47f2	SCAR	TAACCAACATTATCTTCTTTGCC GAATTTGGAGAGGGGTTTGCTG	Gebhardt et al. 2004
		CosA	SCAR	CTCATTCAAAATCAGTTTTGATC GAATGTTGAATCTTTTTGTGAAGG	Gebhardt et al. 2004
		R1F/R (76-2sf2/76-2SR)	AS	CACTCGTGACATATCCTCACTA CAACCCTGGCATGCCACG	Ballvora et al. 2002
		GP76	SCAR	ATGAAGCAACACTGATCAA TTCTCCAATGAACGCAAACT	Oberhagemann et al. 1999
		SPUD237 (*Alu*I)	CAPS	TTCCTGCTGATACTGACTAGAAAACC AGCCAAGGAAAAGCTAGCATCCAAG	De Jong et al. 1997
		GP21 (*Alu*I)	CAPS	AGTGAGCCAGCATAGCATTACTTG GGTTGGTGGCCTATTAGCCATGC	De Jong et al. 1997
		GP179	SCAR	GGTTTTAGTGATTGTGCTGC AATTTCAGACGAGTAGGCACT	Meksem et al. 1995
R3 (*R3a* & *R3b*)	11	R3-1380	SCAR	TCCGACATGTATTGATCTCCCTG AGCCACTTCAGCTTCTTACAGTAGG	Sokolova et al. 2011
		SHa-F/ SHa-R	AS	ATCGTTGTCATGCTATGAGATTGTT CTTCAAGGTAGTGGGCAGTATGCTT	Huang et al. 2005
		*R3b*F4/ *R3b*R5	AS	GTCGATGAATGCTATGTTTCTCGAGA ACCAGTTTCTTGCAATTCCAGATTG	Rietman 2011
RB/Rpi-blb1	8	RB-629/638	SCAR	AATCAAATTATCCACCCCAACTTTTAAAT CAAGTATTGGGAGGACTGAAAGGT	Sokolova et al. 2011
		RB-1223	SCAR	ATGGCTGAAGCTTTCATTCAAGTTCTG CAAGTATTGGGAGGACTGAAAGGT	Pankin et al. 2011
		CT88 (Primer 1/ primer 1')	SCAR	CACGAGTGCCCTTTTCTGAC ACAATTGAATTTTTAGACTT	Colton et al. 2006
Rpi-abpt	4	*R2*-F1/ *R2*-R3	AS	GCTCCTGATACGATCCATG ACGGCTTCTTGAATGAA	Kim et al. 2012
		Th2	CAPS	AGGATTTCAGTATGTCTCG TCCATTGTTGATTGCCCCT	Park et al. 2005b
Rpi-ber1	10	CT214 (*Dde*I)	CAPS	GAACGCGAAAGAGTGCTGATAG CCCGCTGCCTATGGAGAGT	Tan et al. 2010
		TG63 (*Bme*1390I)	CAPS	TCCAATTGCCAGACGAA	Tan et al. 2010

				TAGAGAAGGCCCTTGTAAGTTT	
		Q133	SCAR	ATCATCTCCTCAAAGAATCAAG ATCTCCCCATTGACAACCAA	Tan et al. 2010
Rpi-mcd1	4	TG339 (*Mnl*I)	CAPS	GCTGAACGCTATGAGGAGATG TGAGGTTATCACGCAGAAGTTG	Tan et al. 2010
Rpi-phu1	9	GP94 (OPB07+TG/GT)	RAPD	GAAACGGGTG + TG/GT	Śliwka et al. 2006
QTL_*phu-stn*	3	OPA17	RAPD	GACCGCTTGT	Wickramasinghe et al. 2009
		OPA03	RAPD	AGTCAGCCAC	Wickramasinghe et al. 2009
		GP198F/ R	SCAR	GTAATTTGCGAGGAAGGAGAAG TCACTTTGGTGCTTCTGTCG	Wickramasinghe et al. 2009
		GP198F-1/R	AS	TTTGCTTACTCTTGTTGTATG TCACTTTGGTGCTTCTGTCG	Wickramasinghe et al. 2009
Rpi-sto1	8	Ssto-448	SCAR	GTGGAACGCCGTCCATCCTTAG TGCATAGGTGGTTAGATGTATGTTTGATTA	Sokolova et al. 2011
Rpi-avl1		N2527	AS	GAAACACAGGGGAATATTCACC CCATRTCTTGWATTAAGTCATGC	Verzaux 2010
Rpi-cap1	11	CP58 (*Msp*I)	CAPS	ATGTATGGTTCGGGATCTGG TTAGCACCAACAGCTCCTCT	Jacobs et al. 2010
Rpi-dlc1	9	GP101 (*Alu*I)	CAPS	GGCATTTCTATGGTATCAGAG GCTTAACATGCAAAGGTTAAA	Golas et al. 2010
		S1d5-a	AS	CGCCTCTTTCTCTGAATTTC GATCTGGGATGGTCCATTC	Golas et al. 2010
Rpi-mcq1	9	TG328 (*Alu*I)	CAPS	AATTAAATGGAGGGGGTATC GTAGTATTCTAGTTAAACTACC	Smilde et al. 2005
Rpi-snk1.1 and *Rpi-snk1.2*	4	Th21 (*Mbo*I)	CAPS	ATTCAAAATTCTAGTTCCGCC AACGGCAAAAAAGCACCAC	Jacobs et al. 2010
Rpi-ver1	6	CD67 (*Hpy*CH4IV, *Ssi*I)	CAPS	CCCCTGCAAATCCGTACATA CCATACGAGTTGAGGGATCG	Jacobs et al. 2010
Rpi-vnt1.1, and *Rpi-vnt1.3*	9	TG35(*Hha*I/*Xap*I)	CAPS	CACGGAGACTAAGATTCAGG TAAAGGTGATGCTGATGGGG	Pel et al. 2009
		NBS3B	AS	CCTTCCTCATCCTCACATTTAG GCATGCCAACTATTGAAACAAC	Pel et al. 2009

Adapted from Tiwari et al. (2013) Molecular markers for late blight resistance breeding of potato: an update. *Plant Breeding* 132: 237–245. (see detailed reference also)

Table 3. Molecular marker of virus resistance genes in potato

Gene	Virus	Marker name	Marker type	Primer sequence (5' → 3')	Reference
Plrv.1 (QTL)	PLRV	$Nl27_{1164}$	SCAR	F: TAGAGAGCATTAAGAAGCTGC	Marczewski *et al.* (2001a)
				R: TTTTGCCTACTCCCGGCATG	
Plrv.4 (QTL)		$UBC864_{600}$	ISSR	ATGATGATGATGATGATG	Marczewski *et al.* (2004)
		$UBC864AC_{600}$	SCAR	ATGATGATGATGATGAC	Marczewski *et al.* (2004)
Rl$_{adg}$		$E35M48_{192}$	AFLP	F: GACTGCGTACCAATTCACA	Velásquez *et al.* (2007)
				R: GATGAGTCCTGAGTAACAC	
Rlr$_{etb}$		$C2\_At1g42990_{1100}$ (*Alu*I)	COSII	F: ATGACCCCGTCGATAAGAAGCG R: ACCTCACAGCTGCATCTCTATTCCTC	Kelley *et al.* (2009)
Ry$_{adg}$	PVY	$ADG1_{356}$	RGL	F: CACACTCTCGTATCAGTTTGA	Hämäläinen *et al.* (1998)
				R: ATTTAATAGCGTGACAGTCAAC	
		$ADG2_{354}$	RGL	F: ATACACTCATCTAAATTTGATGG	Hämäläinen *et al.* (1998)
				R: ACTTAACTGCATCATGTTCAAG	
		$ADG2_{310}$ (*Bbv*I)	CAPS	F: ATACTCTCATCTAAATTTGATGG	Sorii *et al.* (1999)
				R: ACTGAACAGCATCATGTTCAAG	
		$RYSC3_{321}$	SCAR	F: ATACACTCATCTAAATTTGATGG	Kasai *et al.* (2000)
				R:AGGATATACGGCATCATTTTTCCGA	
Ry$_{sto}$*	PVY	M5	AS	F: GACTGCGTACATGCAGTG	Brigneti *et al.* (1997)
				R: GATGAGTCCTGAGTAACAA	
		M45	AS	F: GACTGCGTACATGCAGCT	Brigneti *et al.* (1997)
				R: GATGAGTCCTGAGTAAGGA	
		M17	AS	F: GACTGCGTACATGCAGTG	Brigneti *et al.* (1997)
				R: GATGAGTCCTGAGTAACAT	
		M6	AS	F: GACTGCGTACATGCAGCT	Brigneti *et al.* (1997)
				R: GATGAGTCCTGAGTAAGAA	
Ry$_{sto}$	PVY	$GP122_{406}$ (*Eco*RV)	CAPS	F: CAATTGGCTCCCGACTATCTACAG	Heldák *et al.* (2007)
				R: ACAATTGCACCACCTTCTCTTCAG	
		$STM0003_{111}$	SSR	F: GGAGAATCATAACAACCAG	Song *et al.* (2005)
				R: AATTGTAACTCTGTGTGTGTG	
				R: GATGAGTCCTGAGTAACGA	
		$YES3-3A_{341}$	STS	F: TAACTCAAGCGGAATAACCC	Song *et al.* (2005)
				R:AATTCACCTGTTTACATGCTTCTTGTG	
		$YES3-3B_{284}$	STS	F: TAACTCAAGCGGAATAACCC	Song *et al.* (2005)
				R: CATGAGATTGCCTTTGGTTA	
Ry-f$_{sto}$	PVY	$UBC857_{980}$	ISSR	ACACACACACACAC(CT)G	Flis *et al.* (2005)

		GP122$_{718}$ (*Eco*RV)	CAPS	F: TATTTTAGGGGTACTTCTTTCTTA	
				R: GATACTTCCAACCGCTTCAC	
		GP204$_{800}$ (*Taq*I)	CAPS	F: CATAGATGGCTCAAACAACTC	Flis *et al.* (2005)
				R: GTGGAAACATGGCTTACC	
		GP269$_{650}$ (*Dde*I)	CAPS	F: TCGCAATGAAAGATAAGC	Flis *et al.* (2005)
				R: TGTGATAAAGAGTGTAGCAGTC	
		GP81$_{400}$	STS	F: GCAGCGTTTCCTACAAT	Flis *et al.* (2005)
				R: AGAGACTAATGCTGAAAAT	
		GP122$_{564}$ (*Eco*RV)	CAPS	F: TATTTTAGGGGTACTTCTTTCTTATGTT	Witek *et al.* (2006)
				R: CTGTCAAAAAAATTCACTTGCATAACTAC	
Ry$_{chc}$	PVY	38-530 (OPC-01)	RAPD	TTCGAGCCAG	Hosaka *et al.* (2001)
Ny-1	PVY	SC895$_{1139}$	SCAR	F: GGTAGCTCTTGATCTCGTCTT	Szajko *et al.* (2008)
				R: GTAGCTCTTGATCACCCATTT	
		GP41$_{443}$	SCAR	F: GTTGGTACCAGGCTTGTT	Szajko *et al.* (2008)
				R: CATTCGGTGCTTTAGGAT	
		C2_At3g16840$_{1100}$ (*Taq*I)	COSII	F: TCCAGTGTCCAAAGAAAGAAAA	Szajko *et al.* (2008)
				R: ATGCTCATGTCCCCGAAACC	
Rx	PVX	IPM3 (*Dde*I)	CAPS	F: AGTAGTTTCAGGCTAGTG	Bendahmane *et al.* (1997)
				R: CAACATCACTTGATCAGAC	
		IPM4 (T*aq*I)	CAPS	F: GTACTGGAGAGCTAGTAGTGATCA	Bendahmane *et al.* (1997)
				R: ACCACTGGCAAATGGCCATACGA	
		IPM5 (*Dde*I)	CAPS	F: AGCTCCATTCGTGACGAT	Bendahmane *et al.* (1997)
				R: AGCTTCGATAATTCTAAATTTG	
Nb	PVX	GM339$_{330}$	Allele Sp.	F: GGTAGTTGGACGAGCATAT	Marano *et al.* (2002)
				R: CTCACTTTTAGACCAGATTT	
		GM637$_{220}$	Allele Sp.	F: GCAGAAGATCGGATAGCAAAC	Marano *et al.* (2002)
				R: GTAACGAGTTGAAGTTACTGA	
		GP21 (*Alu*I)	CAPS	F: GGTTGGTGGCCTATTAGCCATGC	De Jong *et al.* (1997)
				R: AGTGAGCCAGCATAGCATTACTTG	
		SPUD237 (*Alu*I)	CAPS	F: TTCCTGCTGATACTGACTAGAAAACC	De Jong *et al.* (1997)
				R: AGCCAAGGAAAAGCTAGCATCCAAG	
Ns	PVS	OPE15$_{550}$	RAPD	ACGCACAACC	Marczewski *et al.* (1998)
		OPJ13$_{500}$	RAPD	CCACACTACC	Marczewski *et al.* (1998)
		OPG17$_{450}$	RAPD	ACGACCGACA	Marczewski *et al.* (1998)
		OPH19$_{900}$	RAPD	GTCAGGGCAA	Marczewski *et al.* (1998)
		SCG17$_{321}$	SCAR	F: ACGACCGACACTCAAATTTGTACAAGAAA	Marczewski *et al.* (2001b)
				R: GATGCCCCGACAGAGGAAG	
		SCG17$_{448}$ (*Mun*I)	CAPS	F: ACGACCGACACTCAAATTTGTACA	Marczewski *et al.* (2001b)
				R: ACGACCGACAAGAGGACCAAGGGAATAAC	

		UBC811$_{660}$	ISSR	GAGAGAGAGAGAGAGAC	Marczewski (2001)
		UBC811$_{950}$	ISSR	GAGAGAGAGAGAGAGAC	
		SC811$_{260}$ (*Mbo*I)	CAPS	F: CGAACAAAATACGTAATGCATTGAATAA	Witek *et al.* (2006)
				R: GACCTATATCAGTCCCTTCTAATCCACTAT	
Rm	PVM	GP283$_{320}$ (*Dde*I)	CAPS	F: CCCTCCCCATGAAAAAGGTA	Marczewski *et al.* (2006)
				R: GCAACTTCCTGTCCGAATGT	
		GP250$_{510}$ (*Xap*I)	CAPS	F: AGTTCAACACCAGTAGGAC	Marczewski *et al.* (2006)
				R: GACATCAAGTTACCTATGAC	
Gm	PVM	SC878$_{885}$	SCAR	F: GGATGGATGGATGAGGAGGAAACT	Marczewski *et al.* (2006)
				R: CCGACTAGCGATTTGGATGC	

Adapted from Tiwari et al. (2012) Marker-assisted selection for virus resistance in potato: options and challenges. Potato J 39 (2): 101-117 (see detailed reference also)

3. QTL Mapping

Molecular markers are now indispensable for linkage mapping of plants. Once the genome of a plant species is densely mapped with DNA markers, it is possible to identify tightly linked markers with every conceivable trait; either monogenic qualitative characters or quantitative characters. Such markers can, thereafter be used for screening large population of the progeny even at the seed stage instead of growing the population for phenotype expression. DNA markers tightly linked with several monogenic traits have been identified in potato. Attempts have also been made to identify markers linked with major QTLs of quantitative characters. At CPRI, we mapped late blight resistant QTLs in wild potato species *Solanum chacoense* with AFLP markers and identify two major QTLs on potato chromosome 9 and 10. A mapping population consisting of 126 F_1 progenies from the cross of *S. spegazzinii* (susceptible) and *S. chacoense* (resistant) was genotypes and phenotyped for late blight resistance. Major QTLs linked to late blight resistance have been identified and are being used in marker assisted selection (MAS) and breeding.
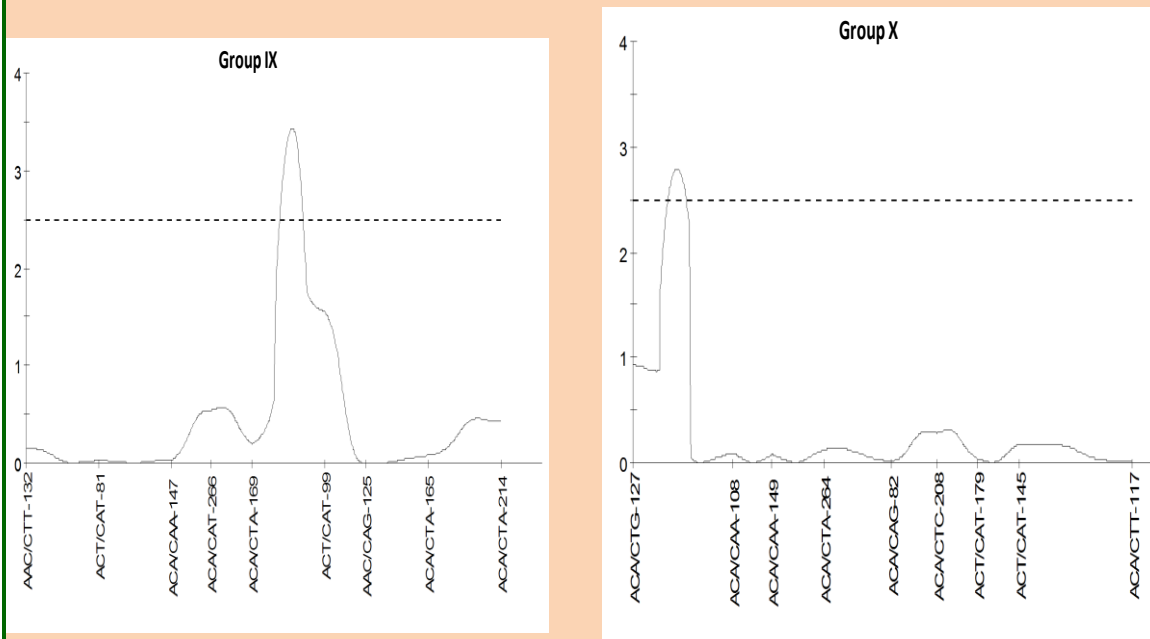
Fig. 10. QTL mapping for late blight resistance in potato wild species