Potato, popularly known as 'The king of vegetables', has emerged as the fourth most important food crop in India after rice, wheat and maize with a global annual production of approximately 300 million tons. In the world scenario, India became the second largest producer of potato. India produced 42.34 million t from 1.86 million ha with an average yield of 22.72 t/ha of Potato during 2010-11. Though, during the recent past the productivity of potato in India has registered a noticeable increase, but can this level be sustained or enhanced in future, is a matter of concern today. Knowledge on genetic divergence is essential for sustained genetic improvement of a crop. However, only fragmented information is available on genetic divergence of Indian potato genotypes. Molecular markers due to their simplicity, quickness and informativeness have replaced the traditional D2 analysis used for studies on genetic divergence. In this study we used RAPD markers for genetic divergence analysis. Ten primer pairs were used to study the genetic diversity of 30 genotypes. Dendogram was constructed using the NTSYSpc software based on a similarity matrix.

MATERIALS AND METHODS

30 potato genotypes (Solanum tuberosum L.) were collected from AICRP on Potato, BCKV, Kalyani for this study. Three tubers of each cultivar were planted in pots and maintained in a greenhouse until after leaf formation and then some amount of leaf was taken to the Molecular Laboratory, AICRP on Tuber Crops at Director of Research building BCKV, Kalyani for molecular analysis.

The CTAB method was used for leaf DNA extraction, as proposed with modifications. Ten RAPD primers were selected on the basis of previous works to evaluate the molecular polymorphism among the potato cultivars. PCR reaction was performed using Veriti 96 Well Thermal Cycler (Applied Biosystem). For the statistical analysis, each RAPD locus was distinguished as a dominant marker, according to the presence or absence of bands, which were scrutinized visually. This data was used in the construction of a binary data matrix, where the value 1 (one) means presence of bands and the value 0 (zero) their absence. The genetic similarity matrix of
Table-1. RAPD primers, with their respective base sequences, PIC Polymorphic Information Content and number of Polymorphic markers in 30 potato

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Tm (˚C)</th>
<th>Number of Bands</th>
<th>Number of Polymorphic Bands</th>
<th>PIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPG 08</td>
<td>TCACGTCACC</td>
<td>25.0</td>
<td>7</td>
<td>7</td>
<td>0.268</td>
</tr>
<tr>
<td>OPG10</td>
<td>AGGGCGTGCT</td>
<td>27.0</td>
<td>7</td>
<td>7</td>
<td>0.336</td>
</tr>
<tr>
<td>OPG13</td>
<td>CTCTCCGCCA</td>
<td>27.0</td>
<td>8</td>
<td>8</td>
<td>0.371</td>
</tr>
<tr>
<td>OPG17</td>
<td>ACGACGACA</td>
<td>25.0</td>
<td>8</td>
<td>8</td>
<td>0.352</td>
</tr>
<tr>
<td>OPG19</td>
<td>GTCAAGGCCA</td>
<td>25.0</td>
<td>8</td>
<td>7</td>
<td>0.224</td>
</tr>
<tr>
<td>OPJ13</td>
<td>CCACACTACC</td>
<td>25.0</td>
<td>7</td>
<td>7</td>
<td>0.360</td>
</tr>
<tr>
<td>OPM02</td>
<td>ACAACGCCCTC</td>
<td>25.0</td>
<td>7</td>
<td>7</td>
<td>0.202</td>
</tr>
<tr>
<td>OPM07</td>
<td>CCGTGACTCA</td>
<td>25.0</td>
<td>10</td>
<td>9</td>
<td>0.265</td>
</tr>
<tr>
<td>OPM12</td>
<td>GGGACGTGGG</td>
<td>27.0</td>
<td>8</td>
<td>8</td>
<td>0.290</td>
</tr>
</tbody>
</table>

Fig.1 RAPD pattern produced from DNA amplification of the following potato genotypes with Primer OPG 13

Fig.2 Dendrogram representing the genetic similarities obtained based on RAPD markers of 30 potato genotypes generated by NTSYS pc software
the 30 potato cultivars was calculated using the Jaccard coefficient and a cluster analysis was performed using the NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) software based on the estimates of genetic similarity dendrograms by the unweighted pair-group method using arithmetic averages (UPGMA). NTSYSpc was used also to estimate the cophenetic value. The total number of polymorphic bands was carried out. The polymorphic information content (PIC) was also calculated, determined by the equation: PIC = 1 - Σ pij², where pij is the frequency of allele p of locus i in primer j.

RESULTS AND DISCUSSION

Using RAPD primer molecular diversity and polymorphisms studies was done on 30 potato genotypes. Ten RAPD Primers (10-mer) were initially screened on 30 popular potato genotypes for their ability to amplify polymorphic fragment of DNA. Out of them only nine primers viz. OPG08, OPG10, OPG13, OPG17, OPG19, OPJ13, OPM02, OPM07, OPM12 showed distinct polymeric DNA profiles. Some total of 70 bands were obtained from these primers with an average of 7.7 bands per primer. The polymorphic bands ranged from seven in the primers OPM02, OPG19, OPJ13, OPG10 and OPG08 to nine bands in primer OPM07. The PIC value ranged from 0.371 for primer OPG13 to 0.202 for OPM02 (Table-1). Primer OPG13 was considered very informative due to its high efficiency in detecting polymorphisms among the evaluated plants. Our results agreed with the results of Rocha et al., 2010. The DNA picture of 30 potato genotypes using OPG13 primer is shown in figure 1. The number of polymorphic bands was considered appropriate to assess the genetic divergence of potato genotypes. The reason may be more amount of GC content (60-70%) of the primers used in this study. Increased number of bands with increasing GC content of the primer. The amplified DNA profiling was scored according to the presence and absence of bands. Researchers reported on genetic diversity in potato cultivar by RAPD and SSR markers and they notice that, genomic DNA of 16 potato cultivars was amplified with 25 RAPD primers that generated 92 polymorphic bands. The cultivar identification using RAPD markers is well documented in studies of molecular characterization. By the dendrogram (Fig.2) based on RAPD markers the cultivars were allocated. Dendrogram generated using NTSYS-pc version 2.1 separated these 30 potato genotypes into 2 major groups. The largest group (I) includes 29 and the smallest group (II) is having only one genotype Kufri Sutlej.

The first main group is again divided into four subgroups. In the first subgroup (I) there are eight genotypes viz; PH-4, Kufri Khyati, Kufri Shailaja, MM-12, LB-3, Kufri Ashoka, V2-645, K-22 which are close to 80% similarity. In the subgroup(II) which is the largest subgroup in group(I) include genotypes Kufri Pushkar, Kufri Himalini, LB-5, EM-1, PH-3 and Kufri Chipsona-3, V4-956, PH-2, G-4, Kufri Bahar, V1-121 as well as Kufri Chipsona-1 respectively. This group is having near about close to 76% similarity with each other. In this subgroup it was found two duplicates of each other which are V4-956 and PH-2. The third subgroup contains the genotypes like Kufri Jyoti, PH-1, Kufri Pukhras, Kufri Sadabahar, V5-2051 and V3-950 having just close to 80% similarity. While in subgroup (IV) the genotypes settled are Atlantic, LB-4 and Kufri Surya. A high level of genetic variation was found between PH-4 and Kufri Sutlej which is having near about 40% genetic dissimilarity. Our results agreed with the results. Again we found genetic variation between subgroup (I) and subgroup (IV). We observed about 45% genetic variation between these groups. The use of RAPD markers to genetically fingerprint plants which are morphologically similar or indistinguishable has been established as a reliable, efficient and very informative tool. We have been able to separate 30 genotypes of potato into 7 subgroups based on genetic variation found using 9 RAPD primers. But, as these genotypes were collected from the single source we didn't found that much variation up to that level. The results obtained with RAPD markers were not consistent in this study, justified by the low correlation (0.63) between the similarity matrices. Similarly, the dendrograms generated by RAPD and SSR markers in potato were not correlated.

It has been suggested that the large number of varieties may have been derived from crosses made between PH-4, Kufri Sutlej, Kufri Khyati, Kufri Surya, etc. Researchers reported that, RAPD technique can be successfully applied to determine the genetic fidelity of potato plant. A limited study has been made on genetic divergence in potato either at tetraploid. Other researchers reported that an understanding of the nature and magnitude of variability among the genetic stock is of prime importance to the breeders. One possible explanation for this result could be the kind of information revealed by each molecular marker. RAPD markers are
randomly distributed in the genome sampling preferentially intergenic regions. Hence, there is thirst to analyze the genetic diversity of parental materials. We can get so much information about genetic diversity in Indian potato genotypes from the present molecular study we did.

REFERENCES


