



Molecular Characterization of Nepali Potato Cultivars Using Randomly Amplified Polymorphic DNA (Rapid) Markers

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Abstract: Randomly amplified polymorphic DNA (RAPD) was used to study the genetic diversity of four local cultivars of potato. Amplification with ten arbitrary decamer primers produced 29 different marker bands of which 69.0% were polymorphic. The size range of the amplified DNAs ranged between 370 bp and 2500 bp. On average, 17.5 alleles per genotype were amplified using the RAPD primers. With the selected primers sufficient polymorphism could be detected to allow identification of individual genotypes. A dendrogram displaying the relative genetic similarities between the genotypes showed a range of 55.2-69.0% similarity.

Keywords: RAPD, Genetic Diversity, Potato, *Solanum tuberosum*, Genetic Similarity.

1. Introduction

Worldwide, potato is the most important non-cereal food crop. Although not indigenous to Nepal, potato has rapidly become a staple crop in the country. Local cultivars cover around 80 percent of the total potato growing area³. Several local cultivars traditionally grown all over the country possess different desirable characters¹¹. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in potato improvement strategies. The identification and characterization of potato cultivars and variety selection for breeding in Nepal are based on morphological features and productivity. The identification of different cultivars based on morphological markers requires culture inspection at different stages and is not very reliable because many traits of interest have low heritability and are genetically very complex. Molecular markers based on DNA sequence are more reliable in this regard⁹. Recent developments in molecular genetics have resulted in several procedures based on DNA for detecting genetic polymorphism. RAPD technique is being used successfully to identify, characterize and estimate genetic divergence of potato cultivars^{6,10}. The aim of

this study was to assess the utility of RAPD markers by characterizing four local cultivars of potato.

2. Material and Methods

2.1 Plant materials

Four local cultivars of potato (*Solanum tuberosum* ssp. *Andigena*) Halle, Farse Red, Katchu Dallo and Local Red were obtained from the Potato Research Programme, National Agriculture and Research Centre, Khumaltar, Kathmandu. Halle and Farse Red were originally collected from the Eastern Hills, Katchu Dallo from Central Terai and Local Red from Kathmandu Valley.

2.2 Genomic DNA extraction and quantification

DNA was extracted from potato tubers using the protocol developed by Wulff¹³. The use of extraction buffer with a high NaCl content (2.5M) considerably reduced the amount of starch in the sample. The extracted DNA was separated electrophoretically on 0.8% (w/v) of agarose gel in 1x TBE buffer. Gels were stained with ethidium bromide (0.5mg/ml for 20 min.) and photographed using an Ultra-Cam gel imaging system. Concentration of DNA was determined by

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visually comparing UV-induced fluorescence emitted by ethidium bromide-DNA complexes with a 1kb ladder (Fermentas) and was found to be 12ng/ μ l.

2.3 RAPD-PCR amplification and gel electrophoresis

Twenty decamer primers (Kit A, Operon Biotechnologies GmbH, Germany) of random sequence were used. PCR amplification was performed by using the standard protocol¹². Reactions were set up in thin-walled PCR tubes with 25 μ l volumes containing 20mM Tris-HCl pH 8.3, 50mM KCl, 2mM MgCl₂, 200 μ M of each dATP, dCTP, dGTP and dTTP, 0.8 μ M primer, 1 unit Taq DNA polymerase (Roche), 24ng of template DNA and a suitable amount of sterile deionised water. Amplification conditions involved an initial denaturation step of 3 min. at 94°C followed by 40 cycles each consisting of a denaturation step of 15 Sec at 94°C, followed by an annealing step of 3 Sec at 35°C and an elongation step of 1 min. 30 Sec at 72°C. The last cycle was followed by 3 minute extension at 72°C to allow complete extension of all amplified fragments. The amplified DNA was separated electrophoretically on 1.5% (w/v) of agarose gel in 1x TBE buffer. Gels were stained with ethidium bromide (0.5mg/ml for 20 min.) and documented using an Ultra-Cam gel imaging system.

2.4 Genetic data analysis

The sizes of the amplified DNA fragments were calculated using 1Dscan EX Version 3.0 (Scanalytics, Inc.). The RAPD data were analyzed using the program Popgene Version 1.31. The presence of a fragment was scored as 1 and the absence of band as 0 by two independent readers. Bands not identified by two readers were considered non-scorable. The bivariate data were used to generate Nei's unbiased genetic similarity and genetic distance⁸. The Nei's unbiased genetic similarity between each pair of genotypes was used to construct a dendrogram using the UPGMA algorithm.

3. Result and Discussion

Out of 20 arbitrary primers screened, nine revealed clear and distinct polymorphisms in four varieties producing a total of 20 polymorphic loci. RAPD-PCR reaction with these nine primers was performed twice to confirm the reproducibility of the bands obtained. The percentage of polymorphic loci was calculated to be 69.0. Primer OPA10 is able to distinguish all the four potato genotypes. The banding patterns of four potato genotypes using primer OPA02, OPA10, OPA15 & OPA16 are shown in Figs. 1-4.

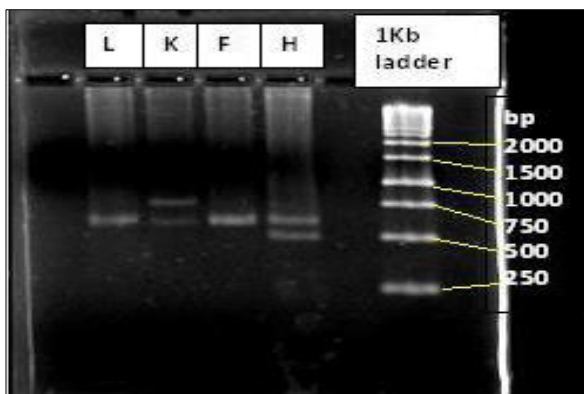


Fig. 1. RAPD patterns with primer OPA-02.
(H = Halle; F = Farse Red; K = Katchu Dallo; L = Local Red).

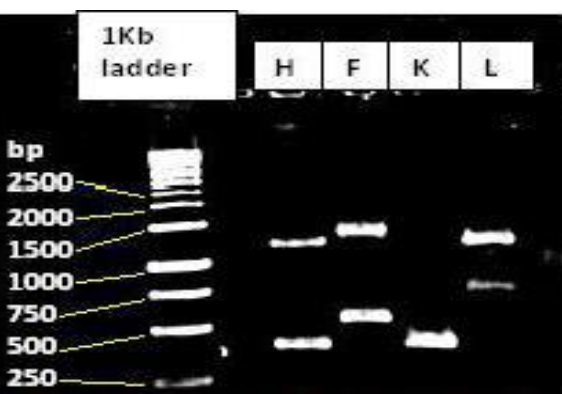


Fig. 2. RAPD patterns with primer OPA-10.
(H = Halle; F = Farse Red; K = Katchu Dallo; L = Local Red).

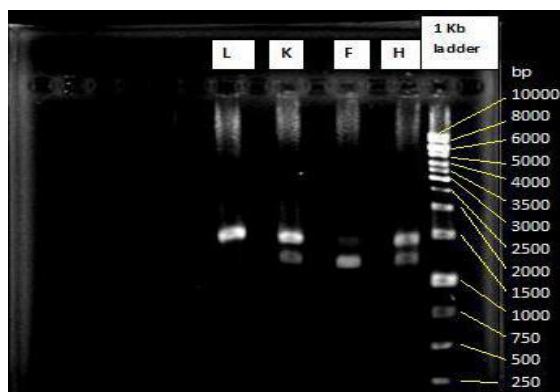


Fig. 3. RAPD patterns with primer OPA-15.
(H = Halle; F = Farse Red; K = Katchu Dallo; L = Local Red).

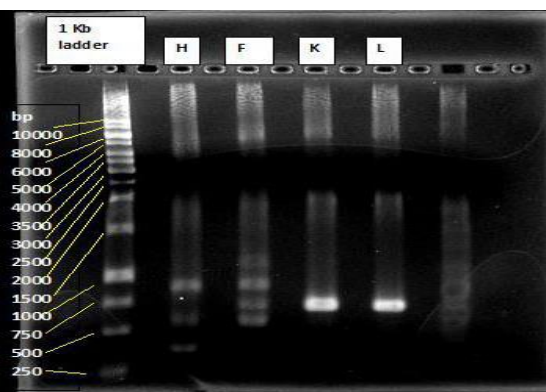


Fig. 4. RAPD patterns with primer OPA-16.
(H = Halle; F = Farse Red; K = Katchu Dallo; L = Local Red).

The size range of the amplified DNAs ranged between 370 bp and 2500 bp (Table 1). A total of 70 DNA fragments was amplified in four potato genotypes using 10 RAPD primers, giving an average of 17.5 alleles per genotype. The mean observed number of alleles was calculated to be 1.69 ± 0.47 , while the effective number of alleles⁴ was calculated to be 1.52 ± 0.39 . Genetic distances between the four genotypes were calculated (Table 2). The dendrogram was constructed using UPGMA cluster analysis (Fig. 5). In addition, Nei's gene diversity and Shannon's Information index across all investigated loci in four potato genotypes were found to be 0.293 ± 0.210 and 0.424 ± 0.294 , respectively^{5,7}. The strength of association between these variables of diversity was very high ($r = 0.968$), and the correlation coefficient was significantly different from zero ($P < 0.001$).

The relative genetic similarities between the genotypes showed a range of 55.2 to 69.0%. The Eastern Hills and Kathmandu Valley have similar ecogeographical parameters with an altitude between 2,000 and 3,000 meters. Average daily temperature in this zone varied from 10°C at the time of planting to 20°C at the time of harvesting of potato. Average

rainfall during the growing season is less than 300mm. Hence, the close resemblance of Farse Red, Local Red and Halle may be due to similar ecogeographical variables. On the contrary, Central Terai has an altitude of less than 1,000 meters. The crop is taken up mainly under conditions where the average rainfall is less than 100mm with a temperature range of 20-25°C which may be the reason for out grouping of Katchu Dallo. Some previous research in other species has found that genetic similarity is very closely associated with geographical proximity². However, correlation and regression analysis between genetic distance and ecogeographical variables for *S. fendleri* and *S. jamesii* populations showed that genetic distance was not significantly related to any of the ecogeographical variables used in the study¹. Halle and Farse Red should have shown more resemblance compared to Farse Red and Local Red, but the result is contrary to what is expected on the basis of geographical proximity. Potato first entered Nepal in the Eastern hills and to trade it moved to the Kathmandu Valley. Hence, the movement of population progressively by natural and artificial means must be the reason for the similarity between Farse Red and Local Red.

Table 1. Unique PCR bands amplified using different RAPD primers.

Primer	5' to 3' bands (size bp)	GC content (%)	Unique
OPA-01	CAGGCCCTTC	60	1080
OPA-02	TGCCGAGCTG	70	500, 830
OPA-03	AGTCAGCCAC	60	410
OPA-10	GTGATCGCAG	60	420, 560, 800, 1330, 1480
OPA-11	CAATCGCCGT	60	880, 1000, 1300
OPA-12	TCGGCGATAG	60	1300
OPA-13	CAGCACCCAC	70	1290, 1780
OPA-15	TTCCGAACCC	60	1210
OPA-16	AGCCAGCGAA	60	370, 570, 720, 900

Table 2. Nei's unbiased measures of genetic similarity (above diagonal) and genetic distance (below diagonal).

Pop ID	Halle	Farse Red	Katchu Dallo	Local Red
Halle	****	0.6552	0.5517	0.5517
Farse Red	0.4229	****	0.5517	0.6897
Katchu Dallo	0.5947	0.5947	****	0.6552
Local Red	0.5947	0.3716	0.4229	****

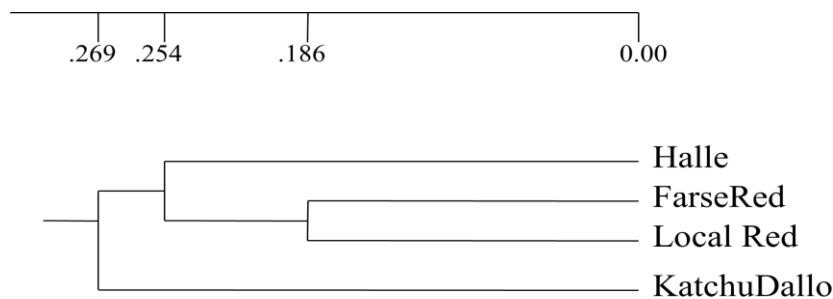


Fig. 5. UPGMA dendrogram constructed for four potato genotypes using 10 RAPD primers.

4. Conclusion

More primers should be screened to provide conclusive data on the genetic diversity of Nepali potatoes. Even so, we have pre-screened a set of OPA primers on just a few samples and showed their utility in the identification of cultivars. Further experimental investigation can now be done using this set of useful primers on major cultivated varieties and local potato cultivars of Nepal. This will give an account of genetic diversity of all Nepali potato cultivars which can be used as a complementary tool for potato breeding. RAPD analysis is the least expensive and easiest method of DNA genotyping and therefore well-suited for routine, multi-sample analysis. More molecular data are required for a better understanding of the genetic variability and subsequent improvement of potato varieties in Nepal.

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