

Assessment of genetic diversity among Persian clover cultivars as revealed by RAPD markers

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ABSTRACT: Yield crop landraces are valuable sources of genetic variations that the knowledge and implication of these variations are critical in the plant breeding programs. Legume forage clover due to high forage yield, quality, nitrogen fixation and improvement of soil textures is cultivated worldwide. Persian clover (*Trifolium resupinatum* L.) is grown worldwide. Molecular markers are used efficiently for assessment of genetic diversity in crop plants. In this study 20 Persian clover cultivars collected from different areas were used. DNA extractions were carried out using minipreparation method with equal amount of leaves from 30 plants of each cultivar. DNA samples from 20 cultivars of clover was evaluated using RAPD markers. Eight primers out of 30 used primers produced repeatable bands. Cluster analysis was conducted using NTSYS software and UPGMA method based on Jaccard's similarity matrix. Primers totally produce 83 bands, of which 66 bands (%80) were polymorphic among clover genotypes. The greatest and least amplification fragments belonged to OPH₁₂ and OPG₀₆ primers, respectively and average band number of primers was estimated as 10.4 bands. According to cluster analysis and cutting dendrogram in 0.7 similarity coefficient, clover populations divided into six groups in which 'Kazerun' and 'Haftun-Isfahan' individually formed the separate clusters. According to similarity matrix, the least similarity (%36) belonged to 'Haftchin-Brujerd' and 'Kazerun' and the highest similarity belonged to 'Chegeni' and 'Haftchin-Hamedan'. Clustering based on RAPD method almost substantiated the grouping based on geographical origin. Considering the results, it is concluded that RAPD technique can be used for genetic diversity study of Persian clover as well as discrimination of its cultivars.

Key words: Diversity – Persian clover – RAPD marker – *Trifolium resupinatum*

Introduction

Assessment of genetic diversity in cultivated crops have important implications for breeding programs and for the conservation of genetic resources. Some 250 species of *Trifolium* are recognized throughout the world. They contribute nitrogen fixation and thus promote the growth of associated grass. Persian clover adapted to heavy, moist soils but is not tolerant to low winter temperatures. Under cultivation it is seeded in the fall and grows rapidly during late winter and early spring. Seed pods are inflated and light in weight, subject to distribution by wind or floating on water. Natural reseeding occurs generally. Although used mainly for grazing, Persian clover is also excellent for silage and hay. Persian clover as a self-pollinated crop, is a prolific seed producer which yields up to 675 kg/ha (Cope & Taylor, 1985). Evaluation of genetic diversity and similarity between Persian clover cultivars is a first step toward its germplasm utilization for a breeding program.

Materials and methods

Plant materials

Twenty cultivars of Persian clovers collected from different areas of Iran were used in this study: (1) 'Kermanshahi-1' and (2) 'Kermanshahi-2' from Kermanshah, (3) 'Haftun', (4) 'P513', (6) 'Sechin' and (7) 'Haftchin' from Isfahan, (5) 'Nehavandi', (8) 'Haftchin-Hamadani', (9) 'Chegini', (10) 'Doroud', (11) 'Harati', (12) 'Dehpir', (13) 'Silakhor', (14) 'Alashtar' and (15) 'Haftchin-Boroujerd' from Lorestan, (16) 'Kasaroun' from Fars, (17) 'Bazneh', (18) 'Elvijan', (19) 'Reihan' and (20) 'Tajra' from Markazi.

Random amplified polymorphic DNA markers

Genomic DNA was extracted from young leaf tissue using Dellaporta et al. (1983) procedure with modifications. Briefly, 100 mg of leaf tissue was powdered by grinding in liquid nitrogen and incubated in extraction buffer (100mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl and 1.25 % (w/v) SDS) containing 200 µl 5 M potassium acetate at 65 °C for 30 min. The slurry was extracted with 0.8 volume of chloroform – isoamyl alcohol (24:1 v/v), and the emulsion was centrifuged at 5000 rpm at 4 °C for 15 min. Extracted DNA was precipitated from supernatant with 950 g/L alcohol and washed with 700 g/L alcohol three times. After drying, DNA was dissolved in Tris-EDTA pH 8.0 (10 mM Tris, 1 mM EDTA) containing 100 µg RNAase. DNA concentration was quantified by comparing its intensity with those of DNA standard (known concentrations) on an ethidium bromide stained 1 % agarose gel.

Thirty decamer random primers obtained from Primm Co., Italy were used in PCR analysis. The PCR reaction was performed in a 25-µl volume using a Techgen thermocycler (Techgen, UK). Polymerase chain reactions based on genomic DNA performed according to Gustine & Huff (1999). PCR amplified products were separated by electrophoresis in 1.5 % agarose gel. Gels were stained with ethidium bromide (0.5 µg/ml). DNA banding patterns were visualized using Vilber Lourmat gel documentation (Model IP-008-SD, France).

For each genotype, the presence of a band (1) or its absence (0) was scored. Cluster analysis was conducted using NTSYS software and UPGMA method based on Jaccard's similarity matrix.

Results and discussion

Of 30 primers used for initial screening of polymorphism using Persian clover cultivars, 8 primers gave amplified polymorphic products (Table 1). Amplification of 20 genotypes with these primers yielded a total of 83 scorable bands, of which 17 were polymorphic (Table1). An average of 10 bands per primer and 4 bands per genotype were obtained. The highest number of bands (was obtained with primers OPH₁₂ and OPH₁₀ while the lowest number was obtained with primer OPG₀₆).

Figure 1 shows a representative amplification pattern obtained using primer OPH₁₀. A dendrogram showing genetic relationships among the 20 clover genotypes were presented in Figure 2. According to cluster analysis and cutting dendrogram in 0.7 similarity coefficient, clover populations divided into six groups in which ‘Kazerun’ and ‘Haftun Isfahan’ individually formed the separate clusters. According to similarity matrix, the least similarity (36%) belonged to ‘Haftchin Brujerd’ and ‘Kazerun’ and the highest similarity belonged to ‘Chegeni’ and ‘Haftchin Hamedan’. Clustering based on RAPD method almost substantiated the grouping based on geographical origin. Considering the results, it is concluded that RAPD technique can be used for genetic diversity study of Persian clover as well as discrimination of its cultivars.

Table 1. Percent polymorphism revealed by random primers in Persian clover

Primer	Nuclotide sequence 5' → 3'	Total number of bands	Polymorphic bands	Percentage of polymorphism
OPH ₀₄	GGAAGTCGCC	9	8	89
OPA ₀₈	GTGACGTAGG	8	7	88
OPH ₁₂	ACGCGCATGT	14	11	79
OPH ₁₀	CCTACGTCAG	14	11	79
OPB ₁₄	TCCGCTCTGG	13	10	71
OPG ₁₃	CTCTCCGCCA	13	10	71
OPH ₀₇	CTGCATCGTG	9	7	78
OPG ₀₆	GTGCCTAACC	3	2	66

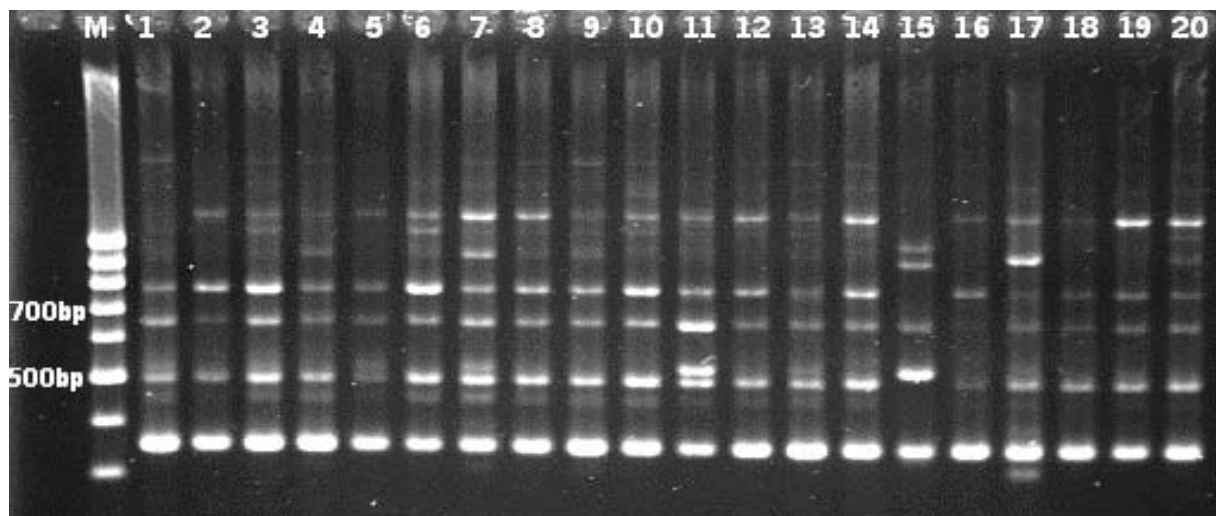


Figure 1. RAPD profile of 20 Persian clover cultivars using the primer OPH₁₀ (M = 100bp ladder, for cultivar number see Materials and methods, p. 85)

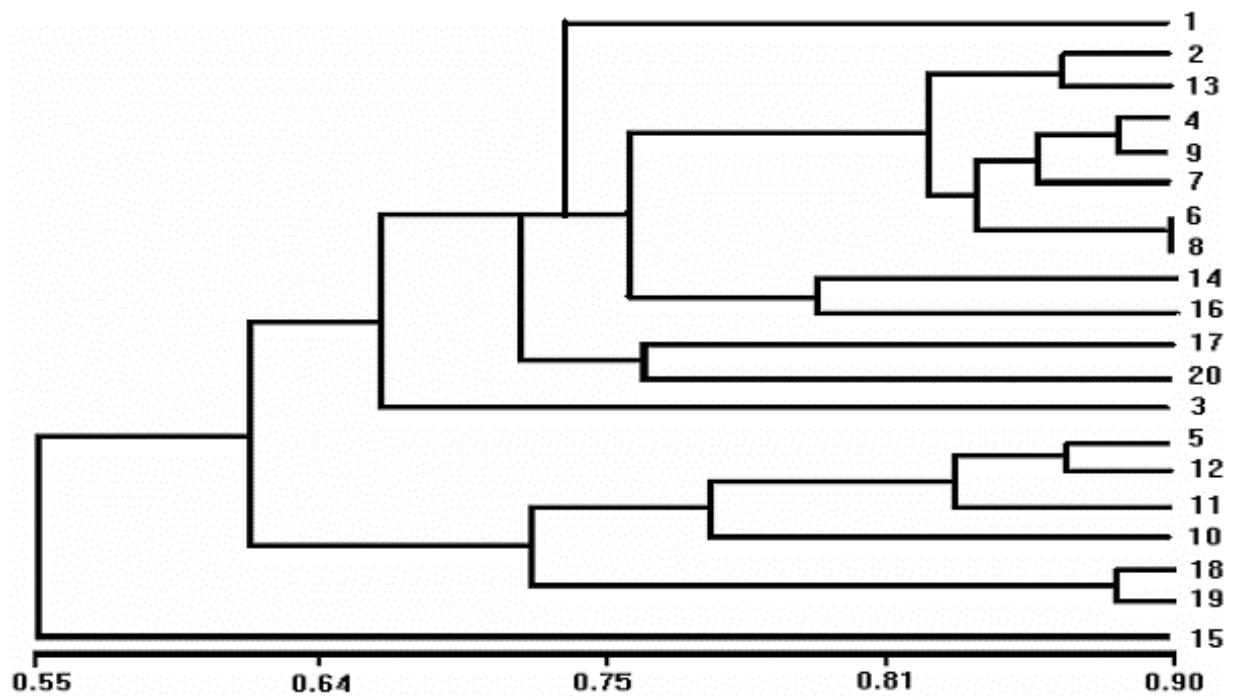


Figure 2. Dendrogram showing interrelationships of 20 Persian clover genotypes

References

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