Tr. J. of Agriculture and Forestry
23 (1999) 627-630


# RAPD Analysis of Genetic Variation in Barley

Gülruh ALBAYRAK
Istanbul University, Faculty of Science, Biology Department, 34459 Vezneciler, Istanbul-TURKEY
Nermin GÖZÜKIRMIZI
Istanbul University, Faculty of Science, Biology Department, 34459 Vezneciler, İstanbul-TURKEY

Received: 08.06.1998

**Abstract:** The variation between wild barley lines (*Hordeum murinum, Hordeum bulbosum* and *Hordeum vulgare spontaneum*) originated from Turkey were investigated comparatively at the molecular level with the Random Amplified Polymorphic DNA (RAPD) technique. Leaf DNAs extracted from different barley lines were amplified with randomly chosen primers in a Polymerase Chain Reaction (PCR). Rates of polymorphisms between the 23 lines were determined in pairwise comparisons. The lowest value of polymorphism was determined to be 0.3% between wild barley lines numbered 50327 and 50354, and between 48101 and 50365 while the highest polymorphism value was determined to be 60% between wild barley lines numbered 40659 and 49047. The results can be used in the selection of commercially important traits (such as resistance against disease, drought etc.) present in wild barley lines. Thus, development of high quality barley cultivars will be possible.

## Arpada Genetik Varyasyonun RAPD Analizi

**Özet:** Türkiye kökenli yabani arpa hatları (*Hordeum murinum, Hordeum bulbosum* ve *Hordeum vulgare spontaneum*) arasındaki çeşitlilik moleküler düzeyde Rastgele Çoğaltılmış Polimorfik DNA (RAPD) tekniği kullanılarak karşılaştırmalı olarak araştırılmıştır. Farklı arpa hatlarından ekstre edilen yaprak DNA'ları Polimeraz Zincir Reaksiyonu'nda (PCR) rastgele seçilmiş primerler ile çoğaltıldı. 23 hat arasındaki polimorfizm oranı çiftler halinde karşılaştırmalı olarak tayin edildi. En yüksek polimorfizm oranı %60 olarak 40659 ile 49047 nolu yabani arpa hatları arasında bulunurken en düşük polimorfizm oranı ise %0.3 olarak 50327 ile 50354, ve 48101 ile 50365 nolu yabani arpa hatları arasında bulundu. Elde edilen sonuçlar, yabani arpa hatlarında mevcut olan ekonomik öneme sahip karakterlerin (hastalıklara, kuraklığa dirençlilik vb.) seçilmesinde kullanılabilir. Böylece yüksek kaliteli arpa kültürlerinin geliştirilmesi mümkün olabilecektir.

## Introduction

A general problem in genetic research at molecular level is identification of specific DNA sequence differences underlying phenotyping variations. Polymerase Chain Reaction (PCR), developed with that aim, has an extraordinary sensitivity. PCR, used for detection of nucleic acids at very low levels in biological samples, is a method detecting amplified sequences between the annealing sites of two synthetic DNA primers (1).

Recently Williams et al. (2) developed an RAPD method that employs only one and random primers in a PCR to rapidly generate polymorphic markers that can be used to create genetic linkage that are amplified from one parent but not the other. RAPD markers generated by a PCR can be used to differentiate between morphologically indistinguishable strains and vraieties (3, 4). DNA polymorphisms in the results of RAPD analyses are independent of environmental conditions and have several advantages (5).

Genetic improvement of any organism depends upon the existence, nature and extent of the genetic variability available for manipulation (6). Gene banks have an important role in the maintenance of plant genetic resources for breeding purposes (7). Recently developed RAPD markers provide a rapid, inexpensive and effective system for identification and selection of individuals and accessions in the gene banks (2, 7). This method also helps to establish genetic relations among the collected samples (8).

Turkey is the most important gene pool for the wild species, *Hordeum murinum, Hordeum bulbosum* and *Hordeum vulgare spontaneum.* Among the species used in this study, *Hordeum murinum* and *Hordeum bulbosum* are naturally found in all regions of the country, but *Hordeum vulgare spontaneum* is not found in Thrace or the Central Black Sea coast (9).

We present polymorphic features of wild barley lines from Turkey established by fingerprinting using RAPD markers.

## Materials and Methods

### Plant material

Twenty three wild barley lines (Table 1) were obtained from the Aegean Agricultural Research Institute Gene Bank, Menemen-İzmir, TURKEY. DNAs were isolated from 20-day-old seedlings for each line according to the protocol described by Walbot (10).

Table 1. 23 wild barley lines from the Aegean Agricultural Research Institute Gene Bank, Menemen-İzmir, TURKEY.

| Record No | Botanical Name |
|---|---|
| TR 4968 | *Hordeum murinum* |
| TR 48883 | *Hordeum murinum* |
| TR 4937 | *Hordeum bulbosum* |
| TR 40659 | *Hordeum bulbosum* |
| TR 50327 | *Hordeum bulbosum* |
| TR 50335 | *Hordeum bulbosum* |
| TR 50354 | *Hordeum bulbosum* |
| TR 50387 | *Hordeum bulbosum* |
| TR 50401 | *Hordeum bulbosum* |
| TR 31623 | *Hordeum vulgare spontaneum* |
| TR 40799 | *Hordeum vulgare spontaneum* |
| TR 48101 | *Hordeum vulgare spontaneum* |
| TR 48125 | *Hordeum vulgare spontaneum* |
| TR 48132 | *Hordeum vulgarespontaneum* |
| TR 49047 | *Hordeum vulgare spontaneum* |
| TR 49053 | *Hordeum vulgare spontaneum* |
| TR 49059 | *Hordeum vulgare spontaneum* |
| TR 49075 | *Hordeum vulgare spontaneum* |
| TR 49085 | *Hordeum vulgare spontaneum* |
| TR 50365 | *Hordeum vulgare spontaneum* |
| TR 50379 | *Hordeum vulgare spontaneum* |
| TR 50392 | *Hordeum vulgare spontaneum* |
| TR 50400 | *Hordeum vulgare spontaneum* |

### Primers

The three primers which formed polymorphic bands between the 38 evaluated oligonucleotide primers were:

TUB38; 5' GCACTGACCTCCCACATTCC 3' (synthesised in TÜBİTAK, TURKEY)

OPA01; 5' CAGGCCCTTC 3' (Operon Technologies CA/USA)

OPC20; 5' ACTTCGCCAC 3' (Operon Technologies CA/USA)

### Amplification conditions for RAPD

The reaction procedure was a modification of that described by Welsh and McClelland (7). In a final reaction volume of 25 µl, 25 ng genomic DNA, 15 ng primer, 2 mM $MgCl_2$, 100 µM of each dATP, dGTP, dCTP, dTTP (Boehringer-Mannheim) and 0.02 units of Taq DNA polymerase (Boehringer-Mannheim) were mixed in a buffer, 10 mM Tris-HCl, 50 mM KCl, 0.1 µg/ml gelatine, and were overlaid with mineral oil. Two step PCR amplification was performed: (1) Four cycles at 95°C for 1.5 min, 34°C for 1.5 min and 72°C for 3 min, and (2) forty cycles at 95°C for 1 min, 34°C for 1 min and 72°C for 2 min with a final extension step at 72°C for 10 min.

The amplification products were analysed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide.

### Calculation of polymorphism

Polymorphism was calculated according to the method of Nei and Li (11), and the results are shown in Table 2.

## Results and Discussion

The three primers of arbitrary sequences produced 44 amplified fragments, 13 for TUB38, 11 for OPA01 and 20 for OPC20. They were taken for the analysis of polymorphism (Fig. 1). In pairwise comparison with the RAPD markers obtained, a polymorphism spectrum between 0.3% and 60% was found (Table 2).

The primers were enough to reveal polymorphism between the species and lines. Similarly, Hu and Quiros (12) demonstrated that as few as four primers could be enough to distinguish Brassica cultivars with RAPD markers. In many cases, however, different primers are necessary to obtain comparable RAPD results (8). By prescreening 24 10-mer primers for their informativeness Koller et al. (13) found one primer that detects enough genetic variation among 11 apple cultivars to allow for complete differentiation.

The highest polymorphism value (60%) was determined between two wild barley lines numbered 40659 belonging to *Hordeum bulbosum* and 49047 belonging to *Hordeum vulgare spontaneum.* The lowest

Table 2. Polymorphism values (%) in pairwise comparisons, among 23 wild barley lines.

| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. 50387 | - | | | | | | | | | | | | | | | | | | | | | | |
| 2. 50354 | 12 | - | | | | | | | | | | | | | | | | | | | | | |
| 3. 50335 | 15 | 4 | - | | | | | | | | | | | | | | | | | | | | |
| 4. 50327 | 12 | 0.3 | 4 | - | | | | | | | | | | | | | | | | | | | |
| 5. 49085 | 19 | 16 | 12 | 16 | - | | | | | | | | | | | | | | | | | | |
| 6. 49047 | 19 | 39 | 12 | 39 | 32 | - | | | | | | | | | | | | | | | | | |
| 7. 50392 | 1 | 1 | 1 | 1 | 21 | 14 | - | | | | | | | | | | | | | | | | |
| 8. 50379 | 19 | 26 | 12 | 26 | 25 | 31 | 0.6 | - | | | | | | | | | | | | | | | |
| 9. 49075 | 15 | 22 | 7 | 22 | 22 | 46 | 6 | 16 | - | | | | | | | | | | | | | | |
| 10. 49059 | 17 | 18 | 15 | 18 | 35 | 49 | 6 | 29 | 19 | - | | | | | | | | | | | | | |
| 11. 48883 | 7 | 23 | 9 | 23 | 14 | 25 | 1 | 35 | 30 | 25 | - | | | | | | | | | | | | |
| 12. 4968 | 1 | 17 | 1 | 17 | 1 | 21 | 1 | 27 | 27 | 26 | 5 | - | | | | | | | | | | | |
| 13. 50400 | 17 | 39 | 10 | 39 | 35 | 28 | 14 | 41 | 46 | 50 | 25 | 23 | - | | | | | | | | | | |
| 14. 49053 | 1 | 19 | 1 | 19 | 1 | 12 | 1 | 16 | 16 | 12 | 9 | 15 | 5 | - | | | | | | | | | |
| 15. 40659 | 12 | 11 | 4 | 11 | 16 | 60 | 26 | 55 | 50 | 46 | 22 | 15 | 51 | 9 | - | | | | | | | | |
| 16. 48125 | 19 | 37 | 12 | 37 | 5 | 19 | 1 | 10 | 21 | 35 | 29 | 21 | 21 | 16 | 52 | - | | | | | | | |
| 17. 50401 | 12 | 31 | 23 | 31 | 26 | 47 | 1 | 40 | 37 | 12 | 34 | 15 | 38 | 9 | 20 | 40 | - | | | | | | |
| 18. 31623 | 1 | 19 | 1 | 19 | 1 | 23 | 1 | 22 | 22 | 19 | 17 | 15 | 15 | 9 | 9 | 21 | 9 | - | | | | | |
| 19. 4937 | 1 | 14 | 1 | 14 | 1 | 32 | 26 | 36 | 12 | 7 | 12 | 17 | 27 | 4 | 29 | 34 | 4 | 12 | - | | | | |
| 20. 48132 | 19 | 34 | 12 | 34 | 5 | 39 | 1 | 15 | 26 | 30 | 22 | 15 | 40 | 0.6 | 47 | 9 | 34 | 9 | 4 | - | | | |
| 21. 48101 | 19 | 28 | 12 | 28 | 5 | 20 | 1 | 7 | 18 | 31 | 25 | 16 | 23 | 12 | 26 | 7 | 36 | 17 | 8 | 11 | - | | |
| 22. 50365 | 19 | 28 | 12 | 28 | 29 | 27 | 14 | 20 | 29 | 42 | 25 | 16 | 29 | 12 | 46 | 7 | 36 | 17 | 8 | 37 | 0.3 | - | |
| 23. 40799 | 17 | 26 | 10 | 26 | 23 | 35 | 9 | 17 | 21 | 34 | 22 | 16 | 33 | 12 | 41 | 9 | 35 | 17 | 8 | 14 | 3 | 9 | - |

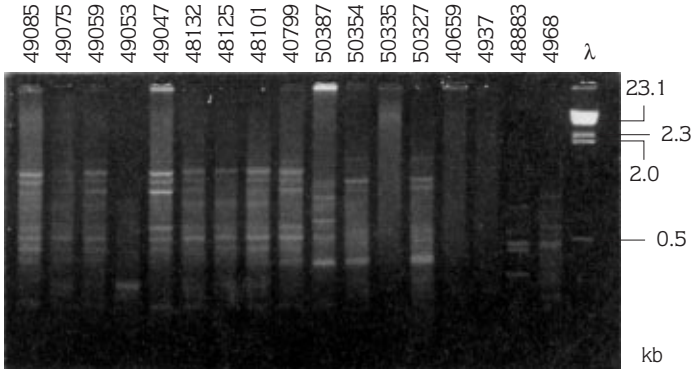


Figure 1. RAPD markers among 23 wild barley lines revealed by the OPC20 primer (5'ACTTCGC-CAC3'). Amplification products were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

value of polymorphism, which was 0.3%, was determined both between the wild barley lines numbered 50327 and 50354 of the *Hordeum bulbosum,* and between the wild barley lines numbered 48101 and 50365 of the *Hordeum vulgares pontaneum.* Polymorphism values between wild lines of *Hordeum vulgare* which were obtained from Ankara University, Osman Tosun Gene Bank were determined to be between

3% and 15% in the study of Gürel et al. (14). These two studies show that Turkey has a great potential to be a gene bank for wild barley lines.

The polymorphic values of wild barley lines shown in this study, have important implications for the improvement of high quality barley lines. In addition, RAPD fingerprint data could be compiled with gene bank data, thereby selection based on genetic linkage between DNA bands and loci of commercially important traits could be established. This technique has many advantages, compared with the isoenzyme systems (15) and RFLPs (16).

## References


1. Benito, C., Figuerias, A.M., Zaragoza, C., Gallego, F.J., de laPena, A. Rapid identification of Triticeae genotypes from single seeds using the polymerase chain reaction. Plant Molecular Biology, 21: 181-183, 1993.
2. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18 (22): 6531-6535, 1990.
3. Welsh, J., Peterson, C., McClelland, M. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Research, 19: 303-306, 1991.
4. Goodwin, P., Annis, S.L. Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. Appl. Environ. Microbiol. 57: 2482-2486, 1991.
5. Mori, M., Hosaka, K., Umemura, Y., Kaneda, C. Rapid identification of Japanese potato cultivars by RAPDs. Jpn. J. Genet, 68: 167-174, 1993.
6. Waugh, R., Powell, W. Using RAPD markers for crop improvement. Tibtech, 10: 186-191, 1992.
7. Welsh, J., McClelland, M. Fingerprinting using PCR with arbitrary primers. Nucleic Acids Research, 18 (24): 7213-7218, 1990.
8. Tinker, N.A., Fortin, M.G., Mather, D.E. Random Amplified Polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. 85: 976-984, 1993.
9. von Bothmer, R., Jacobsen, N., Baden, C., Jørgensen, R.B., Linde-Laursen, I. An ecogeographical study of the genus *Hordeum*. Descriptions of the species 92-9043-207-1. Internatioal board for plant genetic resources, Rome, 1991.
10. Walbot, V. Preparation of DNA from single rice seedlings. Rice Genet. Newslett. 5: 149-151, 1988.
11. Nei, M., Li, W.H. Mathematical model for studying genetic variation in terms of restriction endonucleases. Pro. Natl. Acad, Sci. USA. 76: 5269-5273, 1979.
12. Hu, J., Quiros, C.F. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Reports, 10: 505-511, 1991.
13. Koller, B., Lehmann, A., McDermott, J.M., Gessler, C. Identification of apple cultivars using RAPD markers. Theor. Appl. Genet., 85: 901-904, 1993.
14. Gürel, A.F., Arı, Ş., Gözükırmızı, N. Identification of variation in barley at the molecular level. XV. International Botanical Congress, Yokohama, Japan, 179, 1993.
15. Jørgensen, R.B. Relationships in the barley genus (*Hordeum*): An electrophoretic examination of proteins. Hereditas, 104: 273-291, 1986.
16. Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann R.G. Construction of an RFLP map of barley. Theor. Appl. Genet. 83: 250-256, 1991.