

# Phylogenetic Relationships of some Wild and Cultivated Barley Accessions Using Seed Storage Protein Markers

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## Abstract

Barley is of renewed interest throughout the world because of its nutritional value and low glycemic index. It has been suggested that biochemical accomplished with molecular markers can be used to solve population diversity of barley. Hordeins are the storage proteins of the wild and cultivated barley samples. For this reason, we analyzed genetic diversity in the storage protein hordein encoded loci in seeds from 106 accessions of wild and cultivated of barley accessions from different countries. Cluster analysis by using Nei genetic distance and UPGMA methods all studied wild and cultivated barley accession divided into 6 main groups. Grouping of Azerbaijan wild and cultivated barley accession in the same cluster showed that Azerbaijan cultivated barley accessions was clearly distinguished from its wild accessions. The presence of high level of diversity among the tested genotypes grouped into divergent clusters indicated their suitability for further research can be done in this direction by selecting superior barley genotypes.

**Keywords:** wild and cultivated barley, seed storage protein, phylogenetic relationships

## Introduction

Cereals such as wheat, rice, barley, maize, and oats account for the majority of agricultural output. Barley (*Hordeum vulgare* L.) is the world's fourth most widely cultivated grain and one of the top ten agricultural plants. It is used in the production of food, feed, and malt. Barley has strong resilience to abiotic stresses, and in order to conserve genetic variety and reduce genetic loss, we must preserve and identify genetic diversity in both wild and cultivated barley.

Storage proteins in barley grains are categorized into two groups based on their solubility: globulins and prolamins (Gubatz S, Shewry PR, 2011). The prolamins found in barley grains are known as hordeins, and they are only found in the cells of

the starchy endosperm (Yupsanis T, 1990 ) & (Shewry P.R., 1993). Hordeins, the main storage proteins in barley seed and it contain 35 to 50% nitrogen of whole seed. It shows significant inter-genotypic variability and have been used as cultivar identification markers in studies of genetic diversity in collections (Doll H. and Brown A.H.D., 1979) & (Shewry P.R., 1983) & (Shewry P.R. and Milfin B.J., 1982) & (Heisel S.E., 1986. )& (Vapa, 1996). Hordeins were classified into four groups in polypeptide families (A, B, C, and D) based on electrophoretic mobility in sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and amino acid composition (Shewry P.R. and Milfin B.J., 1985).

Monomeric prolamins (according to wheat gliadins) benefit from a high level of diversity in both cultivated and wild barley species (Pan ZF, 2007). These proteins can be classified as  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\omega$  areas (Eshghi & Akhundova, 2009).

The whole hordein map, contains a large number of alleles, may be utilized as a fundamental technique to distinguish cultivars, identify barley seeds, and determine heritability, as well as breeding programs.

The aim of this work was to explore the phylogenetic relationships of wild and cultivated barley accessions using biochemical markers as hordeins as a first step towards their further utilization in breeding program.

## **Materials and methods**

In this study, 106 wild and cultivated barley accessions from different countries which had been

provided by National Azerbaijan Genbank were investigated, focusing on phylogenetic analysis in hordein. For extraction, electrophoresis and identification of hordein areas, (Poperelya and Mujarinko's., 2001) method was used. Hordeins were extracted from mature grains with 0.25 ml solution containing 6.9% acetic acid and 5% 2-mercaptoetanol and 16% urea and 0.01% pyronine. 0 and 1 coefficients were calculated for all the genotypes, depending on the presence (1) or absence (0) of the bands. It was also used in obtaining other results as well as similarity coefficients matrix of Nei (1978). Furthermore, in order to classify the accessions, cluster analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic Means). Calculating similarity coefficients matrix and dendrogram was done by PowerMarker program.

## Result and discussion

The genetic distance between parents is directly proportional to the extent of heterosis found in progenies. Experiments on genetic variability provide a base of information regarding trait wise variation in the experimental material (Cheres et al., 2000).

Figure 1 shows a dendrogram obtained using the UPGMA method based on the Nei (1978) genetic distance index. Through cluster analysis, 106 genotypes from 15 barley populations included in the study were divided into 6 main groups based on hordein. The first cluster consist of 22 cultivated and wild barley samples from Azerbaijani origin. The placing of cultivated and wild accessions within a cluster is proof that Azerbaijani barley genotypes are composed of its ancestral wild relatives. At the same time, in the current group, genotypes 37 with 33, 41 with 40 and 39, 32 with 24, 28, and 7, 47 with 46 and 42, 45 with 44 and 43 were completely identical based on hordein proteins. In other words, the current samples were not identified from each other on the base of hordein. The second cluster also consisted of only 12 barley samples originated Azerbaijan. In this group, genotypes 29 and 26, 23 and 16, 20 and 21, 38 and 31 and 30 were found to be genetically similar. The third cluster consists of genotypes from France and Germany. Samples 61, 62, 63, 64 and 65 of French origin were identical in this group. Most of the barley genotypes studied are grouped in the 4th cluster. The barley samples from Germany, Czech, Kazakhstan, Poland, USA, Swiss and Russian were grouped in this cluster. Placing of Europe origin barley samples in the current group is reminiscent of the gene flow between these countries. The fifth cluster consisted of barley samples No. 73, 74 and 75 from Denmark, No. 79, 80, 81 and 82 from Georgian, No. 104, 105 and 106 from Canada and No. 72 from Czech Republic. The 6th cluster consists of cultivated barley samples of originated from Azerbaijani, Turkish and Hungary.

The cluster analysis revealed that considerable variation existed among genotypes that could be implicated in selection of wheat for the development or improvement of cultivars and germplasm.

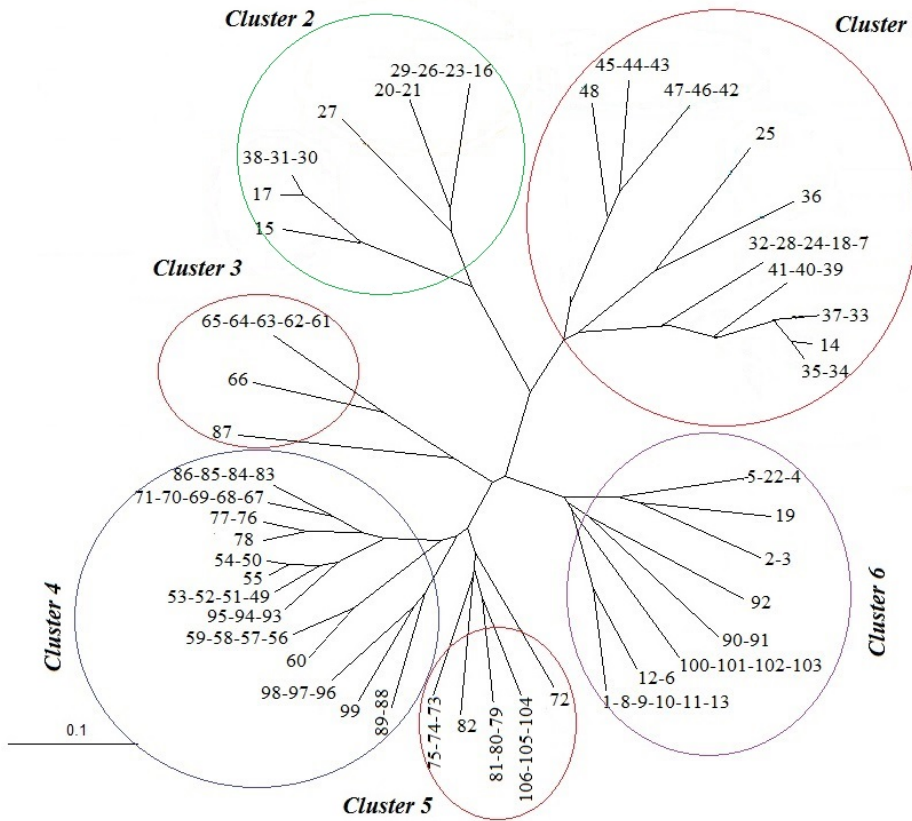


Figure 1. Dendrogram with Nei genetic distance of 106 barley accessions from different countries

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