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**ORIGINAL ARTICLE**

# Genetic Diversity among Wild and cultivated barley by ISSR Marker

**Monireh Rahimi[1], Islam Majidi Hervan[*1],Mostafa Valizadeh[2],FarokhDarvish Kajori[1],Farshad Ebrahimpour[3]**
1 - Department of Agronomy and Plant Breeding, College of Agriculture, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
2- Department of Agronomy and Plant Breeding, Tabriz University,Tabriz,Iran.
3- Department of Agriculture, Payame Noor University, Tehran, Iran
**Corresponding author email:** breeding60@yahoo.com

**ABSTRACT**
*Genetic diversity assessment with different methods and their comparison could provide complementary information for improvement and conservation programs. In this study, Intersimple sequence repeat (ISSR) was assayed to determine the genetic diversity of 266 wild-type, H. Spontaneum, from areas located in or out of Fertile Crescent and also 44 samples of cultivated barley from different world areas. The percentage of polymorphism is more than 78% and the average of number of polymorphic band was concluded 12.95 for each primer. A high level of polymorphism was found with ISSR marker, and the mean polymorphism information content (PIC) value was 0.85 for ISSR marker. The result study of genetic diversity showed that variation observed in populations of the Fertile Crescent was more than populations in North Africa and the Middle East (89% vs 78% and 79%).ISSR-based dendrogram in the present study revealed a powerful tool to quantify genetic diversity in barley, indicating very clear pattern of clustering according to the regions in which they are growing. The information on the genetic diversity relationship from this study is propitious to develop novel barley cultivars with desired economic traits.*
***Keywords***: *barley, Fertile Crescent, genetic diversity, ISSR, polymorphism information content.*

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## INTRODUCTION
Barley (*Hordeum vulgare* L.) is one of the five major crop species of the world which widely use for stock feed, human food and malting. In the year 2011, the global barley production was estimated over 150 million tons harvested from 54.13 million hectares [1]. Barley also is one of the oldest cultivated crops in the world. It is now generally accepted that domestication of barley is assumed to have taken place from two-rowed wild barley *Hordeumvulgare* L. subsp. *spontaneum*, however, it is not clear whether cultivated barley is of monophyletic origin or multiple origins.
Determining the level of variation within, and among, barley populations is an essential step towards analysing genetic variability of cultivars [2], select parental materials for hybridization for making new genetic recombination, select inbred parents or tester for maximizing heterotic response and identify materials that should be maintained to preserve maximum genetic diversity in germplasm sources [3]. Several studies have been conducted to reveal the substantial level of genetic diversity within barley populations collected from farmers' fields [4]. Within the framework of these analyses, morphological markers (Lasa*et al*., 2001) as well as DNA markers (Tanyolac, 2003) have been employed. While morphological markers are inexpensive and easily implemented, DNA markers are not commonly affected by the environment and selection, and are also available in almost unlimited numbers (Ghebru*et al*., 2002). Among the various molecular marker techniques inter simple sequence repeat polymorphic DNA (ISSR) has been widely used for genetic diversity studies in barley, maize and wheat (Tanyolac, 2003). In this study, we evaluate the level and organization of the genetic diversity and relationship in barely specimens Wild and cultivated in the wold using ISSR marker, in order to breeding programmes of this species. Also we aim to report the usefulness ISSR for the assessment of genetic diversity and relationships among barley specimens.

## MATERIALS AND METHODS

Plant Material

In this study, 266 wild-type Barley genotype including 22 samples from South-West of Iran , 20 samples from South- East of Turkey, 20 samples from north of Iraq, 22 samples from Syria, 22 samples from Jordan, 21 samples from Lebanon, 19 samples from Occupied Palestine, 17 samples from Morocco, 20 samples from the West of Libya, 19 samples from east of Afghanistan, 20 samples from north of Tajikistan, 22 samples from north of Kazakhstan , 22 samples of Himalaya and 44 cultivated barley (*Hordeumvulgare subsp. Vulgare L.* ) was investigated focusing on genetic diversity by ISSR marker.The specimen numbers and country of origin are listed in Table 1.

2.2. DNA Extraction

DNA was extracted from fresh leaves as described by J. J. Doyle and J. L. Doyle(1990) Total with some modifications. DNA concentration was determined by both spectrophotometry at 260nm and by 2% agarose gel electrophoresis.

Table 1:Wild and cultivated barley germplasm studied

| Country/Region | Type of material | Spike type | Number of Accessions |
|---|---|---|---|
| Iran | Wild barley | Two-row | 22 |
| Turkey | Wild barley | Two-row | 20 |
| Iraq | Wild barley | Two-row | 20 |
| Syria | Wild barley | Two-row | 22 |
| Jordan | Wild barley | Two-row | 22 |
| Lebanon | Wild barley | Two-row | 21 |
| Israel | Wild barley | Two-row | 19 |
| Morocco | Wild barley | Two-row | 17 |
| Libya | Wild barley | Two-row | 20 |
| Afghanistan | Wild barley | Two-row | 19 |
| Tajikistan | Wild barley | Two-row | 20 |
| Kazakhstan | Wild barley | Two-row | 22 |
| Himalayan | Wild barley | Two-row | 22 |
| Cultivated | Cultivated barley | Two and Six row | 44 |

2.3. ISSR-PCR Analysis:

A set of 23 ISSR primers was procured from Fermentas Company (Table 2). Foreach primer, 20 $\mu$L amplification reaction contained 2.5 $\mu$L buffer (Taq Buffer avec $(NH_4)_2SO_4$ 5x), 100 ng of genomic DNA, 2mM of $MgCl_2$ and 1U of Taq DNA polymerase. PCR amplifications were performed in Bio-Rad PCR thermal cycler system, with initial denaturation at 94◦C for 5 min followed by 35 cycles: denaturation at 94◦C for 1min, annealing at 36◦C for 1min, extension at 72◦C for 2min, with final extension at 72◦C for 7min. PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visualized on UV. The gel was photographed using Bio-Rad Gel DOC.

Table 2: ISSR primers tested in this study

| ISSR primers | Sequence of primer (5_–3_) |
|---|---|
| ISSR1 | 5'-ACACACACACACACACG-3' |
| ISSR2 | 5'-TCTCTCTCTCTCTCTCC-3' |
| ISSR3 | 5'-TGTGTGTGTGTGTGTGG-3' |
| ISSR4 | 5'-ACACACACACACACACGA-3' |
| ISSR5 | 5'-GAGAGAGAGAGAGAGAC-3' |
| ISSR6 | 5'-ACACACACACACACACC-3' |
| ISSR7 | 5'-GAGAGAGAGAGAGAGAGG-3' |
| ISSR8 | 5'-CACACACACACACACAAG-3' |
| ISSR9 | 5'-CTCTCTCTCTCTCTCTG-3' |
| ISSR10 | 5'-GAGAGAGAGAGAGAGAT-3' |
| ISSR11 | 5'-AGAGAGAGAGAGAGAGG-3' |
| ISSR12 | 5'-CTCTCTCTCTCTCTCTA-3' |
| ISSR13 | 5'-TGTGTGTGTGTGTGTGA-3' |
| ISSR14 | 5'-AGAGAGAGAGAGAGAGCTC-3' |
| ISSR15 | 5'-AGAGAGAGAGAGAGAGCTA-3' |
| ISSR16 | 5'-GAGAGAGAGAGAGAGACTT-3' |
| ISSR17 | 5'-GAGAGAGAGAGAGAGACTG-3' |
| ISSR18 | 5'-CACACACACACACACAAGC-3' |
| ISSR19 | 5'-ACACACACACACACACG-3' |
| ISSR20 | 5'-ACACACACACACACACCTG-3' |
| ISSR21 | 5'-AGTAGTAGTAGTAGTAGT-3' |
| ISSR22 | 5'-GAAGAAGAAGAAGAAGAA-3' |
| ISSR23 | 5'-AGTAGTAGTTCTCTCTCTCTCTC-3' |

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## Scoring and Data Analysis:

For each specimen, each fragment/band that was amplified using ISSR primers was treated as unit character. Molecular weight of each of the potential specific bands was calculated using the software Gel-pro analyser. Unequivocally scorable and consistently reproducible amplified DNA fragments were transformed into binary character matrices (1 for presence, 0 for absence). Moreover for each primerwas calculated percentage of polymorphism, Polymorphic Information Content(PIC) and Probably of Identity(PI). Cluster analysis for genotypes and populations based on Jaccard's coefficient was performed using the Past and Power Marker softwares and clustered with unweighted pair group method with arithmetic average(UPGMA).

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

## RESULTS AND DISCUSSION

Twenty-three ISSR primers were used to amplify the DNA of all the 310 barley genotypes and totally yielded 378 bands which contained 298 polymorphic bands. The result of ISSR amplification products and polymorphism level was shown in Table 3.Length of the ISSR amplification products ranged from 200 bp to 3000 bp. The number of polymorphic bands generated by each primer ranged from 8 to 19 with an average of 12.95(figure 1).The average PIC was 0.85, and the lowest and highest PIC values were 0.769 (ISSR1) and 0.901 (ISSR7), respectively. Four ISSR primers (i.e. ISSR7, ISSR10 and ISSR4) had the higher PIC values(Table 3).

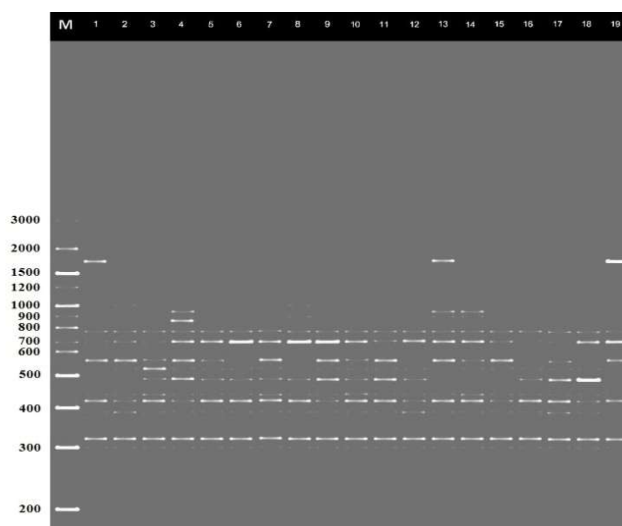

Figure 1. ISSR analysis of 19 Afghanistan's genotypes

The result study of genetic diversity showed that variation observed in populations of the Fertile Crescent was more than populations in North Africa and the Middle East (89% vs 78% and 79%).There was similar results reported by Bader *et al* (2000), Blatnr *et al* (2001) and Morrell and Clegg (2007) that have been studied barley populations from different parts of the world through DNA markers. Turkey's east genotypes was the lowest diversity in Fertile Crescent which was more diverse from populations located out of Fertile Crescent (Kazakhstan and Himalaya).Bernard *et al.* (1997) analyzed the genetic diversity in 88 genotypes from 20 populations of wild barley from Israel, Turkey, and Iran by RAPD markers. When the total genetic diversity were estimated, 75% of the variation detected was partitioned within the 88 genotypes and 25% among the populations. When variation between countries was assessed, no substantial differences were found, because most of the variation detected (97%) was partitioned within the 20 populations and the remainder among the countries. Therefore, the barely specimens were closed together independently of their geographic origin. The microsatellites or intersimple sequence repeat (ISSR) markers and randomly amplified polymorphic DNA (RAPD) markers have proved to be the most polymorphic markers in barley and hence are highly useful markers for various applications in barley (Fern´andez*et al.*2002). This research results showed The results show that, firstly, the Fertile Crescent region can be considered as the primary center of diversity of wild barley H. spontaneum , Second the possibility that for South – East of Iran and also Jordan and Lebanon collect more diverse sample and third, we can not be limited diversity of the plant to the Fertile Crescent, as well as a fairly substantial variation are also therefore this plant in farther parts of the Fertile Crescent including the Himalayas and Kazakhstan. In addition, the genetic distance between Himalaya's genotypes that is as secondary are as

for plant diversity have been proposed, Genetic distance from other populations, particularly populations in the Fertile Crescent indicate that these regions may contain genes that are not found venin the centers of diversity of barley(table 4).Dendrogram in the present study indicated very clear pattern of clustering according to the regions in which they are growing. Similar results were obtained in barley (Russell *et al*., 1997; Fernández*et al*., 2002; Bahattin, 2003). The genetic closeness among the cultivars of each group can be explained by the high degree of commonness. The higher levels of genetic variation found in this study may be due to the very diverse geographic structure in Iran and Jordan and the high degree of climatic heterogeneity of Lebanon compared to Iran. Geographically isolated population accumulates genetic differences as they adapt to different environment (Souframanien and Gopalakrishna, 2004).
This study examined populations using ISSR markers revealed cultivated samples from different parts of the world today were genetically more similar to specimens of wild Iraq andTurkey. Thus, we can conclude that the samples may have been the origin of cultivated areas of the Kurdish areas. In other words, the plant domestication processes are likely in the areas of north of Iraq and South East of Turkey has begun and then had been taken the domestic or domesticated somewhat later to other parts of the region.

Table 3: Polymorphism exhibited by ISSR and RAPD primers in barley

| Primers | Total bands | Polymorphic | (%) Polymorphism | Probably of Identity (PI) | (PIC)Polymorphic Information Content |
|---|---|---|---|---|---|
| ISSR1 | 19 | 12 | 63.16 | 0.052 | 0.769 |
| ISSR2 | 12 | 9 | 75 | 0.087 | 0.777 |
| ISSR3 | 16 | 14 | 87.5 | 0.033 | 0.863 |
| ISSR4 | 18 | 16 | 88.88 | 0.017 | 0.888 |
| ISSR5 | 18 | 15 | 83.33 | 0.029 | 0.87 |
| ISSR6 | 19 | 14 | 73.68 | 0.022 | 0.887 |
| ISSR7 | 22 | 19 | 86.36 | 0.016 | 0.901 |
| ISSR8 | 9 | 9 | 100 | 0.049 | 0.813 |
| ISSR9 | 15 | 11 | 73.33 | 0.048 | 0.823 |
| ISSR10 | 20 | 16 | 80 | 0.02 | 0.896 |
| ISSR11 | 16 | 10 | 62.5 | 0.051 | 0.815 |
| ISSR12 | 17 | 14 | 82.35 | 0.03 | 0.866 |
| ISSR13 | 20 | 13 | 65 | 0.039 | 0.844 |
| ISSR14 | 18 | 15 | 83.33 | 0.031 | 0.87 |
| ISSR15 | 21 | 11 | 52.38 | 0.039 | 0.84 |
| ISSR16 | 19 | 14 | 73.68 | 0.021 | 0.844 |
| ISSR17 | 15 | 12 | 80 | 0.043 | 0.846 |
| ISSR18 | 14 | 13 | 92.86 | 0.034 | 0.855 |
| ISSR19 | 19 | 16 | 84.21 | 0.037 | 0.86 |
| ISSR20 | 17 | 14 | 82.35 | 0.019 | 0.892 |
| ISSR21 | 10 | 8 | 80 | 0.07 | 0.786 |
| ISSR22 | 10 | 10 | 100 | 0.034 | 0.855 |
| ISSR23 | 14 | 13 | 92.86 | 0.02 | 0.895 |
| Sum | 378 | 298 | - | 0.843 | 19.564 |
| Mean | 16.43 | 12.95 | 80.12 | 0.0366 | 0.85 |

As shown in the Figure (2) displaying genetic distances in the analyzed cultivated and wild-type samples, a lot of each area samples located near each other in the same group. Also there were bands that particularly existed in the geographically neighbor areas. Based on these results it may be concluded that ISSR marker polymorphism depends on geographical diversity. This dependency may be due to a kind of compatibility between special geographical areas and DNA encoding genes or may be due to the other genes linked with.Genetic divergence among barley genotypes through cluster analysis was also reported by Bahrman*et al*., 1999; Hamza*et al*., 2004; Eshghi and Akhundova, 2010.
In conclusion, as mentioned in the figure (2) that shows genetic distances among all cultivated and wild-type barley samples, a lot of Iraq's north and Turkey's south- east samples and also cultivated samples located in the second group. Results of table (3) and figure (2) that shows genetic distance based on ISSR marker polymorphism also indicate maximum similarity between cultivated samples and wild-type samples from Iraq and Turkey. Finally, based on our result can conclude that the most possible domestication center for barley plant can be north of Iraq and Turkey's south- east areas. Furthermore, the wider phenotypic and molecular variability observed represents a good indication for the importance of barley genotypes in breeding programs.

**Table 4: Mean number of band, PI and PIC index among studied barley populations**

| Wild populations in Fertile Crescent or neighboring areas | | | |
|---|---|---|---|
| PIC | PI | Band | |
| 0.91 | 0.013 | 16.1 | Iran |
| 0.873 | 0.027 | 13.78 | Turkey |
| 0.88 | 0.022 | 13.6 | Iraq |
| 0.88 | 0.01 | 17.95 | Syria |
| 0.911 | 0.012 | 16.95 | Jordan |
| 0.9 | 0.016 | 15.43 | Lebanon |
| 0.88 | 0.025 | 12.47 | Israel |
| Wild populations in north of Africa | | | |
| PIC | PI | Band | |
| 0.764 | 0.08 | 9 | Morocco |
| 0.803 | 0.059 | 9.82 | Libya |
| Wild populations in central Asia | | | |
| PIC | PI | Band | |
| 0.81 | 0.052 | 10.82 | Afghanistan |
| 0.742 | 0.082 | 12.95 | Tajikistan |
| 0.825 | 0.043 | 12.95 | Kazakhstan |
| 0.821 | 0.049 | 11.65 | Himalaya |
| Cultivated barley's Population | | | |
| PIC | PI | Band | |
| 0.895 | 0.017 | 27.26 | Cultivated barley |

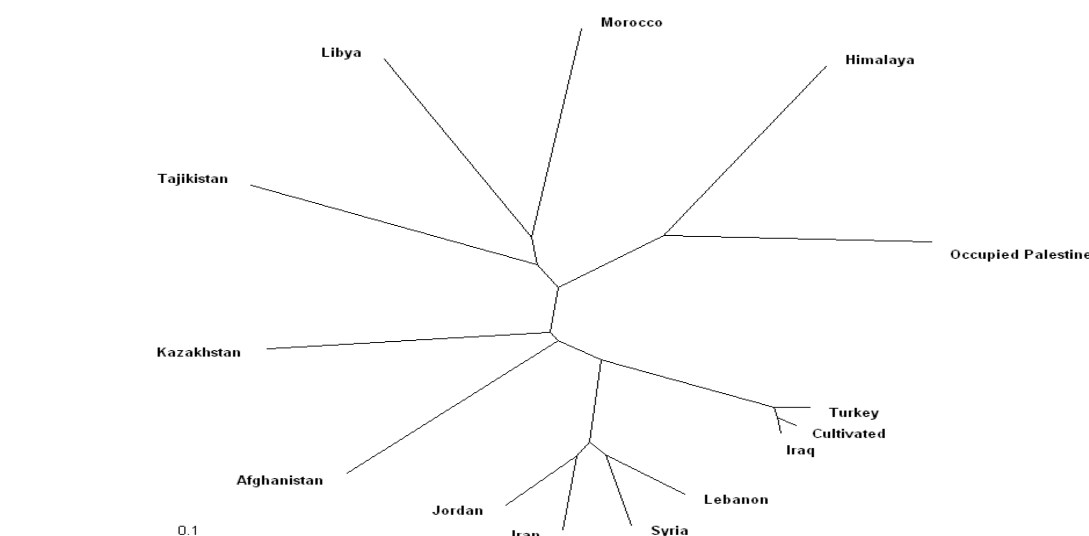


Figure2: Dendrogram showing the genetic distance among studied barley populations based on ISSR

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