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Original article

Genetic diversity of barley verities by RAPD markers

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ABSTRACT

The fourteen barley verities were studied by RAPD markers to assessment of their genetic diversity. From 40 used primers, 17 primers established suitable banding patterns. Totally, 435 bands were produced between 564- 4157 bp that all bands were polymorphic in varieties. The total mean of markers for each primer was 25.59 and mean of polymorphic markers for each primer and variety were 27.9 and 22.29, respectively. “Gara Arapa” with 104 and “Gorgan” with 64 bands had the highest and lowest polymorphic bands, respectively. Primer 7 had the highest polymorphic band (35) and primer 11 had the lowest polymorphic band (13). Polymorphic information content (PIC) for primers was estimated among 0.26 (Primer 2) and 0.48 (Primer 13) with mean 0.34. Marker index (MI) varied among 2.01 to 8.97 in primers 2 and 7, respectively. Nei’s gene variation index was estimated 0.288 for RAPD markers data. These amounts showed partly high diversity among under studied varieties. The genetic distance varied from 0.2509 between “Gara Arapa” and “Dari-Friz 88-A1” to 0.7135 between “Gohar Jo” and Dari-Friz 88- A7. The Cluster analysis based on Nei's genetic distance classified genotypes to three groups. The first group contains Gorgan, Makui, Goharjo and Dari-Friz88-A1. The second group contains Zarjo, Reihan, Karoon, Garearpa, Sahand, Valfajr and Dari-Friz88-A7. The third group consists of Sina, Dari-Friz88-A3 and Dari-Friz88-A5.

1. Introduction

Barley (Hordeum vulgare L.) is one of the most important crops that because of the broad ecological adaptability and resistance to environmental conditions cultivated in many parts of the world (Behnia, 2005). Increasing of the yield and product quality is main objective of breeding programs that achieving this goal, requires sufficient variation and knowledge of how to control that genetic variation and identification of genomic regions associated with them. Breeding success depends on a genetic variation in the base population (Farshadfar, 1997).

2. Materials and methods

The plant material used in this study, including 14 barley varieties (Table 1). This study was performed at greenhouse and research laboratory of Agronomy and Plant Breeding Department, Faculty of Agriculture, University of Maragheh.

Table 1
Names of barley varieties.

Number	Variety	Number	Variety
1	Gorgan	8	Goharjo
2	Makui	9	Sina
3	Zarjo	10	Valfajr
4	Reihan	11	Dari-Friz88-A1
5	Karoon	12	Dari-Friz88-A3
6	Garearpa	13	Dari-Friz88-A5
7	Sahand	14	Dari-Friz88-A7

To determine the quality and quantity of extracted DNA was used from agarose gel electrophoresis 0.8% and a spectrophotometer. In The agarose gel 0.8%, DNA produced band, but the shared DNA a smear. To determine the amount of DNA was used spectrophotometer with 260 nm. Thermal cycles of PCR reactions in this study were as follows,

stage 1, primary denaturation 5 min at 94°C, stage 2, 40 cycles, consisting of denaturation 1 min at 94° C, primers bind to the template strands 1 min at 34° C and the extension of DNA strand by DNA Taq polymerase for 2 min at 72° C, third stage, the final extension 5 min at 72º C (Selbach and Molina 2000).

PCR amplification products were separated by agarose gel electrophoresis 1.5% and detected by staining with ethidium bromide. The banding patterns were scored as 0 and 1 (absence or presence of band). To determine the size of the amplified fragments was used ladder SM (Fermentas). The total number of polymorphic bands, the number of bands in each variety, Nei's genetic diversity index were estimated using POPGEN. Polymorphic information content (PIC) and marker index (MI) was calculated. Genetic distance between groups based on Nei's unbiased genetic distances were calculated using POPGEN. The dendrogram drawn by MEGA software. Phylogenetic tree was constructed using the nearest neighbors method, and principal coordinate analysis (PCoA) using NTSYS software was performed (Rohlf, 1998).

3. Results and discussion

From the 40 tested primers, 17 primers (42.5%) produced suitable and scoring banding patterns, and some studies reported 45%, 15%, 33%, 45%, 93% and 42% suitable patterns (Kroth et al. 2005, Shi et al., 2004, Hong, et al., 2001, Kochieva et al., 2001, Selbach and Molina,). Totally 435 bands in the range of 564 to 4157 bp were produced, all of which were polymorphic. The total average of markers per primer was 25.59, and the average

number of polymorphic markers per primer and varieties were 27.9 and 22.29, respectively. Variety 6 with 104 bands and variety 1 64 bands were allocated the highest and lowest polymorphic bands.

Among the used primers, primer 7 (Figure 1) the highest polymorphic band (35 bands) and primer 11 the lowest polymorphic band (13 bands) had. Polymorphic information content (PIC) for used primers were variable between 0.26 in primer 2 and 0.48 in primer 13 with an average of 0.34. Marker index (MI) changed between 2.01 to 8.97 with the mean 6.

Nei's gene diversity index estimated 0.288. These values indicated relatively high diversity among cultivars used in this study. The genetic distances mean was estimated 0.4788. Genetic distance between verities ranged from 0.2509 between GareArpa and Dari-Friz88-A1 to 0.7135 between Goharjo Dari-Friz88-A7.

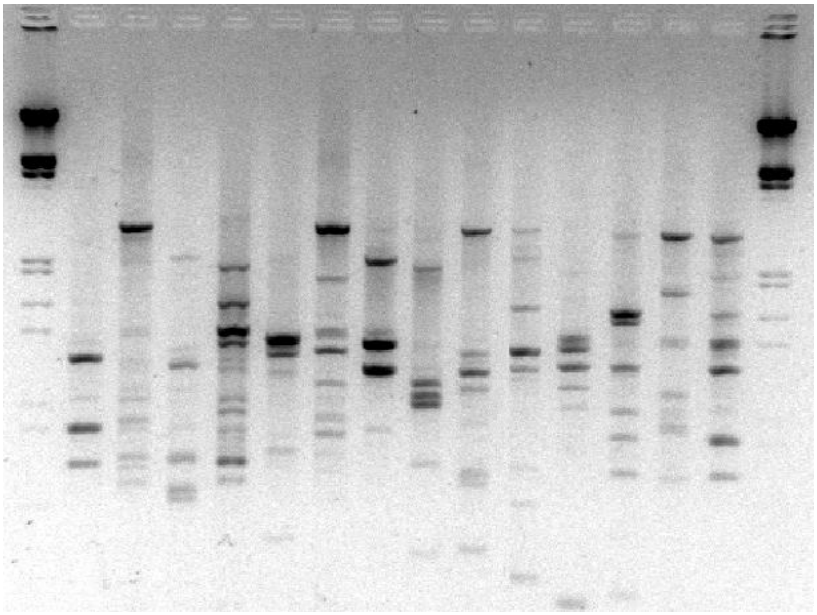

Fig. 2. Banding pattern of studied genotypes with primer 7.

Cluster analysis based on Nei's genetic distance and the nearest neighbor method is shown in Figure 2. The cophenetic correlation was 0.9 that demonstrates the suitability of this method for cluster analysis. The obtained dendrogram consists of three main groups; the first group contains Gorgan, Makui, Goharjo and Dari-Friz88-A1. The second group contains Zarjo, Reihan, Karoon, Garearpa, Sahand, Valfajr and Dari-Friz88-A7. The third group consists of Sina, Dari-Friz88-A3 and Dari-Friz88-A5.In study of 38 barley genotypes with RAPD primers by Zhang et al., (2005), cluster analysis classified genotypes into two groups and five subgroups. Hong et al., (2001) used RAPD markers to assess the genetic relationships of 42 barley cultivars. Cluster analysis using Nei & Li coefficient could be assigned genotypes to four groups.

In PCoA based on Nei's distance matrix, the first and second vectors explained 22 and 19% (totally 41%) of the variation in the data. If proportion of explained variance by the first vector was large, that means high correlation between markers. This demonstrates the relative accumulation of amplified sequences in specific regions of the genome (Mohammadi 2001). Conversely, low variance explained by early vectors was because of good distribution of primers across the genome. In this study, the low variance explained by the first and second vectors indicates the genomic proper distribution of markers. Overall, PCoA was confirmed relatively cluster analysis results.

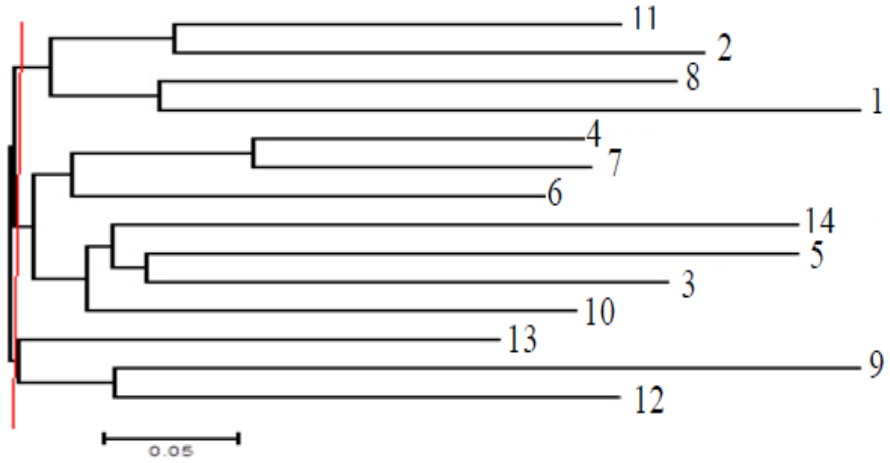

Fig. 2. Dendrogram of cluster analysis.

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