Genetic Diversity Assessment in Several Barley \textit{(Hordeum vulgare L.)} Cultivars Using Microsatellite Markers

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Abstract

In the present study, genetic diversity in seven cultivars of cultivated barley \textit{(Hordeum vulgare)} populations was evaluated using 10 microsatellite markers. Genomic DNA was extracted from fresh leaves and amplification reactions were done by PCR. The amplification products were separated on 6\% denaturing polyacrylamide gels containing 7M urea and visualized via silver staining method. High level of polymorphism was observed among populations. Polymorphic bands ranged from 100 to 300 bp. Altogether 65 alleles were observed among all genotypes, with an average of 9.2 alleles per locus for all loci. Polymorphic information content (PIC) ranged from 0.80 to 0.88 with an average of 0.84. 'Sahand' populations showed the lowest mean of gene diversity whereas the highest mean of heterozygosity observed in Rayhan populations that can prepare a powerful resource of genetic diversity for breeding programs. The genotypes were clustered using unweight pair-group method on arithmetic average by POPGEN32 software. The dendrogram discriminated all the genotypes in several groups. The results showed that SSR markers have a high ability to reveal most of the information in a single locus and can be used for genetic analysis in molecular levels determination of genetic similarity and clustering barley cultivars.

Keywords: barley, genetic diversity, microsatellite markers, SSR

Introduction

Utilization of germplasm resources and efficient conservation need ample knowledge regarding the amount of genetic variation in germplasm arrays and genetic relationships between genotypes. Selection of parental combinations that will maximize gain from selection and maintain genetic diversity depends on the information about the amount of genetic variation present, and the location of the genetic determinants of diversity (Matus and Hayes, 2002). Barley, \textit{Hordeum vulgare} L., is one of the major crops in the world that is cultivated in all temperate areas and is an economically important cereal ranking fourth in world crop production (Hayes \textit{et al.}, 2003). Barley is considered a model species for genetic analysis. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. Many types of molecular markers have been used to characterize germplasm, with each method differing in principle, application, type and amount of polymorphism detected, cost and time requirement. Microsatellite markers are tandem, 1 to 5, nucleotide repeats found in eukaryotic genomes. SSRs are co-dominant, abundant, and informative and their detection can be automated. This makes them excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys, and phylogenetic studies (Liu \textit{et al.}, 1996). Most SSRs are highly reproducible and demonstrate a high degree of allelic variation. Nowadays the effectiveness and informative value of microsatellite markers in genetic studies has been demonstrated for all the major cereals (Sjakste \textit{et al.}, 2003). Microsatellites in barley were also used to study genetic diversity and trace the development of germplasm (Macaulay \textit{et al.}, 2001; Struss and Pliakse, 1998). Microsatellite maps for all seven barley chromosomes are now available for the public (Saghai-Maroof \textit{et al.}, 1994; Ramsay \textit{et al.}, 2000).

The objectives of this study were to investigate SSR polymorphism and genetic diversity of 10 microsatellite loci in a set of 7 barley cultivars and to determine the amount of genetic distance and similarity among populations.

Materials and methods

Seven cultivars of Iranian cultivated barely (`Karoon', `Valfajr', `Makooee', `Reyhan', `Cb74-2', `Sahand' and `Zarjo') were selected for this study. All of these samples were obtained from Research Station of Agricultural and Natural Resources, Khorasan Razavi, Iran.

DNA extraction

Genomic DNA was extracted from a bulk sampling of a minimum of 20 individuals for each variety using McPherson and Moller (2001) method. Equivalent amounts of DNA from 20 individual plants were pooled as 3 bulked DNA samples for PCR analyses.
SSR assay

Ten primer pairs (from Metabion International Inc, Germany) were either derived from sequences published previously or designed based on sequences containing tandem repeats from the EMBL and GenBank databases (Tab. 1). PCR amplifications were performed in a total volume of 20 µl, containing 25 ng of barley genomic DNA, 1x PCR buffer, 0.5 mM both primers, 0.2 mM dNTPs, 2.25 to 3 mM MgCl$_2$ (based on Tab. 1) and 1 unit of Taq polymerase. Amplifications were performed in an Eppendorf thermocycler. PCR amplifications were hot-started at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 50 s, annealing according to Tab. 1 for 45 s and extension at 72°C for 45 s. The final extension was done at 72°C for 5 min.

Gel and data analysis

The amplification products were separated on 1.7% agarose gel for initial analysis. Three primers (Bmac0032, Bmac0223 and HVM27) which could not produce the product of interest, removed and experiments were continued using other 7 primers. The amplification products were separated on 6% denaturing polyacrylamide gels and visualized via the silver staining method (Bassam et al., 1991). The resulting gels were scored manually. A similarity matrix using Nei coefficient was generated and the genotypes were clustered using the unweighted pair-group method on arithmetic average (UPGMA) by POPGEN32 software. To measure the informativeness of each SSR, the polymorphism information content (PIC), was calculated using the following formula: PIC = 1- $\sum_i^n p_i^2$ where $p_i$ is the frequency of the i SSR allele (Smith et al., 2000). Shannon information index were calculated for different loci for each population using the following formula:

$$H' = - \sum_{i=1}^{S} p_i \ln p_i$$

Where $S$ is the number of alleles and $p_i$ is the relative abundance of each allele (Hedrick, 1999a).

Results and discussion

Polymerase chain reaction products were electrophoresed on 1.7% agarose gel for primary evaluation. The whole primers except Bmac0032, Bmac0223 and HVM27 conducted to products of interest; therefore, the experiments were continued using 7 primers. High levels of polymorphism using these 7 primers were observed after separating PCR products on 6% polyacrylamide gel. The length of amplified DNA fragments, including microsatellites, ranged from 100 to 300 base pairs. Altogether 65 polymorphic alleles were observed among all genotypes, with an average of 9.2 allele per locus for all loci. At least one allele was observed per population. The number of alleles varied from 7 to 13 alleles per locus with an average of 9.28 alleles per locus. Maximum effective alleles (8 alleles) were observed in ‘Reyhan’ and ‘Cb74-2’ populations. The rest of the populations except ‘Zarjo’ and ‘Valfajr’ had one effective allele. The number of effective alleles among population at different loci ranged from 8.5 (Bmac0134) to 5.12 (Bmac0173) with an average of 6.65 effective allele per locus (Fig. 1).

In this study null alleles were detected at Bmac0173 and HVM7 loci in ‘Reyhan’ and ‘Sahand’ cultivars, respectively. The level of polymorphism observed in number of alleles per locus was high and comparable for all polymorphic loci. ‘Zarjo’ and ‘Valfajr’ showed the highest (100%) level of polymorphism, but ‘Sahand’ populations had a polymorphism level of 71.43% (Tab. 2). PIC values in this study were quite high and ranged from 0.80 (Bmac0173) to 0.88 (Bmac0134) with an average of 0.84 for SSR markers (Tab. 2). Maximum (0.8006) and minimum (0.5998) mean heterozygosity were observed at ‘Reyhan’ and ‘Sahand’ population respectively (Tab. 3). Thus, concerning the polymorphic information content and heterozygosity, ‘Sahand’ and ‘Reyhan’ cultivars were the most uniform and the most diverse populations respectively. ‘Reyhan’ population also showed the highest mean of gene (allele) diversity that indicated high level of heterozygosity in this cultivar.

Tab. 1. Polymerase chain reaction primers and their characteristics

| Primer    | Sequence 5’→3’ | MW (g/mol) | $T_m$ (°C) | $T_a$ (°C) | $\text{MgCl}_2$ (MM) |
|-----------|----------------|------------|------------|------------|-------------------|
| HVCMA     | F G C C T C G G T T G G A C A T A T A A G | 6774       | 58.4       | 55         | 3                 |
|           | R G T A A A G C A A T G T G A G C A C G  | 6816       | 56.5       | 55         |                   |
| Bmac0134  | F C C A A C C T C G T C A T C C G C G | 5460       | 56.0       | 53.1       | 2.5               |
|           | R C T C T G T G T C T C C T C T C C T | 5976       | 55.3       | 53.1       |                   |
| Bmac0173  | F C A T T T T T T T G T G T G A C C G G | 5552       | 51.4       | 50         |                   |
|           | R A T A A G C G G G A G G A C A C G | 5622       | 53.7       | 50         |                   |
| HVM9      | F C A T C A C A C A C A C A C C A C G | 5678       | 58.8       | 52.5       | 2.3               |
|           | R A C C A A A T C C A T C G A A C A C | 6063       | 53.2       | 52.5       |                   |
| HVM7      | F A T G T A G C G G A A A A A A T A C C A T | 7280       | 64.4       | 58.3       | 2.25              |
|           | R C T A G T A G C G G A A C C T C C T C C T | 7378       | 55.9       | 58.3       |                   |
| HVM4      | F A G A C G A A C C A T C C C A A T G G C | 7340       | 62.7       | 67.7       |                   |
|           | R G T C A A G A A G G A A G G C C C T G G T A | 7797       | 69.5       | 67.2       |                   |
| HVM3      | F A C A C T T T T C C A G G G A A C A T C C C T G | 7242       | 62.7       | 61.2       | 3                 |
|           | R A G C A G C G A C G A A A A A A G T C | 7374       | 64.4       | 61.2       |                   |
The genetic distance \( (D) \) among genotypes was estimated based on the proportion of shared alleles. A dendrogram showing the genetic relationships between genotypes was constructed to express the results of cluster analysis based on data obtained by SSR amplification products. The dendrogram discriminated all the cultivars and clustered them separately in 7 groups (Fig. 2). The smallest genetic distance was observed between ‘Zarjo’ and ‘Valfajr’ cultivars. The maximum genetic distance observed between ‘Sahand’ and ‘Makooee’ populations. Shannon information index and Polymorphic information data (PIC) were calculated and ‘Makooee’ populations. Shannon information index was observed between ‘Zarjo’ and ‘V alfajr’ cultivars. The rest of populations except ‘Zarjo’ and ‘V alfajr’ had one effective allele. In most cases, each primer pair amplified only one microsatellite allele. This indicates homozygous states in the microsatellite locus but the presence of two or more alleles in some genotypes were observed. These genotypes were regarded as heterozygous at a given locus. Other explanations for this are the presence of two or more sister lines (microsatellite phenotypes), or the presence of an impurity, i.e. non-related genotype(s) in the bulked DNA sample (Kraic et al., 2002). The number of effective alleles among population at different loci ranged from 8.5 (Bmac0134) to 5.12 (Bmac0173) with an average of 6.65 effective allele per locus. When the whole alleles have equal frequency, the number of effective alleles will be equal to \( F \) where \( F \) is the proportion of homozygotes, therefore, the proportion of homozygotes will be in inverse ratio to the number of effective alleles per locus in population. Since the alleles do not have equal frequency, the numbers of effective alleles are smaller than real alleles (Valdes et al., 1993).

In this study null alleles were detected at Bmac0173 and HVM7 loci in ‘Reyhan’ and ‘Sahand’ cultivars respectively. We also note that from the perspective of the laboratory investigation the pseudo-death of an allele or locus can occur at any stage in the life cycle due to single (or multiple) nucleotide substitutions, insertions or deletions which occur in flanking regions and prevent primer binding. This process results in null alleles (Callan et al., 1993), which may in time become fixed in the population (Chambers and MacAvoy, 2000). It is now well established that the predominant mutation mechanism in microsatellite tracts is ‘slipped-strand mispairing’ (Sia et al., 1991).
The dendrogram discriminated all the cultivars and clustered them separately in 7 groups (Fig. 2). The smallest genetic distance was observed between ‘Zarjo’ and ‘Valfajr’ cultivars that indicate their genetical kinship. The minimum genetic similarity observed between ‘Sahand’ and ‘Makooee’ populations indicates their genetical differences that might be due to mutation and/or selection. It is obvious that the measured genetic distance is a proportional distance and if the number of primers changes, the values will change too.

The relationship observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais et al., 2000). The selection process leads to an accumulation of best alleles for the traits under selection (Fernandez et al., 2002). Further morphological and physiological investigations as well as genetic approaches will help to find the exact nature of these genetic variations.

Conclusions

In general, the results of this study showed that SSR markers have a high ability to reveal most of the information in a single locus and can be used for genetic analysis at molecular level determination of genetic similarity and clustering barley cultivars.

The amplification products were separated on 6% denaturing polyacrylamide gels containing 7M urea and visualized via silver staining method. High level of polymorphism was observed among populations. Polymorphic bands ranged from 100 to 300 bp. Altogether 65 alleles were observed among all genotypes, with an average of 9.2 alleles per locus for all loci. Polymorphic information content (PIC) ranged from 0.80 to 0.88 with an average of 0.84. ‘Sahand’ populations showed the lowest mean of gene diversity whereas the highest mean of heterozygosity observed in Rayhan populations that can prepare a powerful resource of genetic diversity for breeding programs. The genotypes were clustered using unweight pair-group
method on arithmetic average by POPGEN32 software. The dendrogram discriminated all the genotypes in several groups.

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