Assessment of Two Highly Polymorphic Barley Microsatellite Markers for Detecting Polymorphism in Wheat

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Abstract: Simple sequence repeat (SSR) DNA polymorphism is a PCR-based marker system, also known as the microsatellite marker system. SSRs are tandemly repeated 1 to 5 nucleotide units dispersed throughout eukaryotic genomes. They are considered one of the most powerful molecular markers for many diverse applications in genome related studies, e.g., DNA fingerprinting for discrimination of genotypes and genetic mapping. Locus specificity, co-dominant inheritance and easy detection with PCR amplifications are some of the favorable features of this marker system. On the other hand, isolation of SSR markers depends on time-and labor-consuming processes. The need to obtain sequence information for locus specific primer design is the major drawback. Therefore, the application of SSR markers in a cross-species manner would be valuable. In this study, two highly polymorphic barley SSR markers were used to test the cross transportability and polymorphism property of barley SSR primers on wheat genotypes. The results indicated that barley specific SSR primers could generate PCR amplification products on the wheat genome in the absence of polymorphism.

Key Words: SSR, microsatellite, DNA-based markers, barley and wheat.

Introduction

Simple sequence repeat (SSR) DNA polymorphism or microsatellite markers are tandem, 1 to 5, nucleotide repeats found in eukaryotic genomes. The presence of SSRs was first found in mammalian genomes (1). Later, detection of SSRs was achieved by PCR amplifications by the use of specific primers, which can anneal the flanking region of SSR repeat units (2). SSR DNA polymorphism results from the alteration of a number of repeating units in different individuals. The presence of this powerful DNA-based marker system was then demonstrated in plant genomes and used successively to detect the polymorphism in soybean cultivars (3). Following the soybean study, SSR markers have been extensively used for many genome related studies in plants, e.g., genotype differentiation, genome mapping, and marker assisted selection of agronomically important traits (4-6). Its multi-allelic nature, co-dominant inheritance, easy application with PCR and relative abundance make this marker system a very powerful tool among the other...
DNA based markers (7). On the other hand, isolation of SSR markers requires time-and labor-consuming processes in order to design locus specific primers. Therefore, the use of already available SSR markers of a species, which could amplify the same loci and detect polymorphism in other closely related species, would considerably reduce the efforts to obtain SSRs for each organism of interest. Recently, more and more data have accumulated from comparative mapping studies, demonstrating the presence of synteny within the genomes of closely related species, such as rice, maize, barley and wheat (8). Röder and co-workers (9) used 15 wheat SSR markers for PCR amplifications on rye (Secale cereale) and barley accessions (Hordeum vulgare, H. spontaneum). They observed that only one primer pair was both polymorphic for rye and barley samples. Similarly, only 26% of the barley SSR primers produced clear microsatellite bands on Avena species (10). To our knowledge, there has been no previous report of detected polymorphism levels of barley primers on wheat samples.

SSR markers with high polymorphism information content (PIC) values (13) are considered to be suitable for fingerprinting purposes. Two barley SSR markers, HVM 3 and HVM 4, were reported to be extraordinarily polymorphic markers in barley accessions, with diversity index values of 0.95 and 0.93 (11). Therefore, these two markers were selected to test the transportable use of barley specific primers on wheat. The study was performed on durum and bread wheat samples. Durum wheat is tetraploid with two sets of A and B genomes (AABB), whereas bread wheat has an additional genome, genome D, and is an hexaploid wheat (AABBDD).

Materials and Methods

Genetic Materials

Seeds were obtained from the Ministry of Agriculture, Central Research Institute for Field Crops (CRIFC), Ankara. The list of wheat and barley accessions is presented in the Table. All DNA isolations except for sample number 6 were performed on seedlings grown 11-15 days according to Incirli and co-workers (12). Isolation of DNA was followed by determination of DNA concentrations spectrophotometrically. Dilutions of genomic DNA samples were prepared for PCR amplifications.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Species</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Barley</td>
<td>‘Anadolu-86’</td>
</tr>
<tr>
<td>2</td>
<td>Bread wheat</td>
<td>‘Gerek-79’</td>
</tr>
<tr>
<td>3</td>
<td>”</td>
<td>‘Bolai 2973’</td>
</tr>
<tr>
<td>4</td>
<td>”</td>
<td>‘Kraç 66’</td>
</tr>
<tr>
<td>5</td>
<td>”</td>
<td>‘Kate-A1’</td>
</tr>
<tr>
<td>6</td>
<td>”</td>
<td>‘Gerek-79’</td>
</tr>
<tr>
<td>7</td>
<td>Durum wheat</td>
<td>‘Kunduru-1149’</td>
</tr>
<tr>
<td>8</td>
<td>”</td>
<td>‘Çakmak-79’</td>
</tr>
</tbody>
</table>

Barley SSR Primers

The sequences of primer pairs, repeat types and expected PCR product sizes of the barley SSR markers (HVM 3 and HVM 4) are available in the literature (11). These markers were originally designed after the search of GenBank by Saghai Maroof and co-workers (11). HVM 3 and HVM 4 belong to ribulose-1, 5-bisphosphate carboxylase (rubisco) activase (RcaA) and starch synthase genes (Waxy), respectively.

SSR Assay

PCR amplifications were performed in a total volume of 10 µL, containing 50 ng of wheat and barley genomic DNA, 100 pmol of each primer, 0.2 mM dNTP, 1.5 mM MgCl₂, 2 units of Taq DNA polymerase (Sigma Corp. MO) and 0.04 µL of [α³²P]-dATP (3000Ci/mmol). PCR amplifications were hot-started at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 50°C (which is 5 degrees lower than that applied for the barley genome) for 1.5 minutes and extension at 72°C for 2 minutes. The radiolabeled nucleotide incorporated PCR products were separated on 6% denaturing polyacrylamide gels. (7M Urea, 6% Acrylamide, N, N’ Methylene-bis-acrylamide (19/1 w/w), 1X TBE (90 mM Tris-base, 90 mM Boric Acid, 2 mM EDTA pH 8.0) together with the negative control (no DNA) and DNA sequence ladder (A and G sequencing products of single stranded bacteriophage M13mp18 DNA). All samples were heat denatured at 94°C just before loading. Electrophoresis was performed at 60-watt constant power (Biometra High Voltage Power Supply, Pack P30, Germany). The same 1X TBE buffer was used as the running buffer during electrophoresis. Electrophoresis was stopped depending on the expected product size of each (46 x 57 cm 3MM-chromatography paper), then covered with stretch film and dried on slabs.
gel dryer (Savant-SGD 2000) at 76°C for 30-40 minutes under constant vacuum. The dried gels were exposed to X-ray (Agfa CP-BU, 35X43 cm) film for 2-3 days at room temperature. Sequencing of ssM13mp bacteriophage DNA was performed with Sequenase version 2.0, DNA Sequencing Kit (USB, Life Science Research Products, USA), according to the manufacturers’ instructions.

Results and Discussion

PCR amplifications were performed at the annealing temperature of 50°C for all samples including barley (Table). Therefore, during PCR, annealing was kept 5°C lower than the optimized annealing temperature reported for barley (11). The observation of many PCR products for HVM 4 with barley samples might be due to lower annealing temperature in PCR, causing non-specific amplifications (data not shown).

Expected product sizes for HVM 3 and HVM 4 were reported to be 188 and 198 basepairs (bp) for barley, respectively (11). Lane 1 of Figure 1 shows that the HVM 3 primer set amplification product size of barley variety, ‘Anadolu-86’, (192 bp) is closer to the expected size for barley. Shorter but monomorphic PCR products (142 bp) were observed with the same primer set amplification on wheat samples (Figure 1A, lanes 2-8). For bread wheat, an additional smaller size locus (136 bp) appeared with the HVM 3 primer pair, which is absent in durum wheat samples. This suggests that 136 bp locus is most likely amplifying the D genome locus, present only in bread wheat.

Figure 1. Autoradiographs of PCR products separated on DNA sequencing gels. A: PCR products obtained with HVM 3 primer set. B: PCR products obtained with HVM 4 primer set. Lanes 1-8 are the same as in the Table. Lane 9 is a negative PCR control.
PCR amplifications with primer pair HVM 4 also yielded products with shorter sizes for wheat samples (Figure 1B). The HVM 4 primer set, as with HVM 3, did amplify two intense loci on bread wheat samples (202 bp and 170 bp). However, durum wheat samples are lacking the 170 bp product. Thus, we believe that 170 bp long PCR product belongs to the D genome in bread wheat. On the other hand, again a faint additional amplification product, at 232 bp length, is apparent only on lanes 3, 4, and 5 (indicated with an arrow in Figure 1B). It is anticipated that with a longer exposure time this locus could have appeared on the other lanes i.e., 6, 7 and 8, as well. As a result, higher molecular weight products observed might belong to A and B genomes of wheat (both present in bread and durum wheat), and the lower one might be the locus of the D genome (clearly absent in durum wheat). Alternatively, the explanation of the missing bands in durum wheat samples could be the presence of an additional locus to which the primers can anneal and amplify the locus in the A or B genomes of bread wheat. This would be possible only if there was a duplication event in the case of bread wheat.

Saghai Maroof and co-workers (11) used the HVM 4 primer pair on wheat template DNA. Although they reported shorter PCR products were amplified in wheat lines, they did not report the level of polymorphism. In wheat HVM 3 and HVM 4, SSR markers generated highly polymorphic alleles in barley samples (11), but no polymorphism was detected in our study for wheat samples. The absence of polymorphism suggests that these loci are most likely missing the repeat region in wheat, which can only be confirmed by sequencing of the PCR products. Further studies could aid in addressing the evolutionary processes of microsatellite repeat formation. Nevertheless, the authors would like to point out the importance of the ability of these barley SSR primers to amplify wheat loci. Although these markers cannot be used for fingerprinting in wheat, they can be used in synteny studies of the genomes.

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References