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Polymerase Chain Reaction Based Assays for Specific Detection of Barley

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ABSTRACT. Specific detection of barley (*Hordeum vulgare*) is needed for food and feed authentication and quality assurance.This study aimed to develop polymerase chain reaction (PCR) based assays for reliable and fast detection of barley. To this purpose new PCR primers targeting barley specific γ-hordein gene were designed. Their specificity was assayed by conventional qualitative PCRs with different plant species, namely maize *(Zea mays* L.*)*, wheat (*Triticum aestivum* L.), and soybean (*Glycine max* L.). Gel electrophoresis of the amplification products demonstrated high specificity and efficacy of three primer pairs generating amplicons of 82bp, 91bp and 150bp in size for accurate detection of barley. The developed PCR methods may be used in routine laboratory analysis of seeds, foods and feeds. © *2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *barley, Hordeum vulgare, conventional PCR, γ-hordein gene, barley detection.*

Introduction

Barley (*Hordeum vulgare*) is the fourth most important cereal grain in the world, after wheat (*Triticum aestivum* L.), maize *(Zea mays* L.*)*, and rice (*Oryza sativa*). Barley is used for both human food and animal fodder. Processed barley grains are utilized as components of many consumer products. Therefore reliable information on the presence of barley is important for food and feed authenticity and quality assessment [1].

DNA-based polymerase chain reaction (PCR) is applied as the most effective method for plant species identification, food and feed analysis, as DNA is the most stable molecule during processing and production [2]. Several authors, namely Dahinden et al., Olexova et al, and Maskova et al. developed and validated PCR based methods for the determination of barley focused on the intron of the chloroplast gene *trnL* [3-5]. Up to now, limited number of papers has been published concerning quantitative detection of barley by real-time PCR [1, 6]. These methods identify barley gene encoding a γ-hordein endosperm stor-

age polypeptide. The γ-hordein was used as endogenous reference gene for the detection of genetically modified barley [2]. In the present work, we report the development of new PCR-based methods for specific detection of barley. The choice of the primer pairs and the setting conditions are discussed.

Materials and Methods

Plant material. The locally produced grains of barley (*Hordeum vulgare)*, soybean (*Glycine max*), wheat (*Triticum aestivum),*and maize *(Zea mays)* were purchased in a supermarket in Tbilisi (Georgia). The seeds were ground by an electric grinder to obtain a fine powder.

DNA extraction. DNeasy plant mini kit (Qiagen) was applied for DNA extraction based on the results of our previous studies [7, 8]. Genomic DNAs were isolated and purified from 100 mg flour of each crop in duplicate. The yield and integrity of the extracted DNAs were assessed by gel electrophoresis.

PCR analysis. New PCR primers targeting barley γ-hordein gene were designed using bioinformatic tools. The initial primer selection was made by Primer-BLAST [9] primer-blast), Fast Pcr and Primer Quest. The final design was produced by sequence alignment tool Align_MTX [10]. The oligonucleotides were synthesized and purified by the Integrated DNA Technologies (IDT). The PCR primers used in this study are shown in Table 1.

All PCR analyses were performed with a thermal cycler Techne TC -412. All PCR reactions were carried out in a final volume of 25μl using 1.25 U Taq DNA polymerase with standard Taq Buffer (New England BioLabs), 1.5mM $MgCl_2$, 0.2mM of each dNTP (Deoxynucleotide solution mix, New England BioLabs), 1μl (60 - 70ng) of genomic DNA, and 0.5μM of each primer except of PCR with pair hordf/hordr. In this case 0.4μM of each primer was applied.

The same uniplex PCR conditions were utilized for all primers, such as: denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 60°C for 30s, 72°C for 60s; final extension step at 72^0C for 3 min except for the pair hordf/hordr. PCR with primers hordf/hordr had the following cycling profile: denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30s, 65°C for 30s, 72°C for 35s; final extension step at 72°C for 5 min.

Agarose Gel Electrophoresis. Both the genomic DNAs and PCR products were analyzed by agarose gel electrophoresis using 1.0 and 2.0% of SeaKem LE agarose (Cambrex) gels for genomic and amplified DNA, respectively. Electrophoresis was run in the 1x TBE (Tris-Borate EDTA) buffer containing 1μg/ml of Ethidium Bromide (EtBr). After electrophoresis the DNA bands were visualized and photographed by gel documentation system (PhotoDoc It, UVP).

Results and Discussion

Conventional PCR amplification is based on the hybridization of specific oligonucleotides to a target DNA and synthesis, *in vitro,* of millions of DNA copies flanked by these primers. The amplification of DNA fragments, followed by agarose gel electrophoresis for fragment size verification is the simplest PCR strategy applied to verify the presence of a species in a plant derived food products [9]. The choice of suitable species-specific genomic sequences is important for accurate and effective detection of species by PCR. In this study, the barley gene encoding a γ-hordein endosperm storage polypeptide was selected for specific identification of barley based on the previous results concerning the suitability of this gene in real-time PCR assays for barley quantification [1, 2, 6]. The γ-hordein gene consists of 1614 bp nucleotide sequences [10]. We designed 5 pairs of PCR primers (Table 1) targeting γ-hordein gene.

Assessment of the genomic DNAs by agarose gel electrophoresis exhibited high purity and integrity of the samples (data not shown). The amplificability of the genomic DNAs isolated from grains of barley, maize, soybean and wheat was checked by plant-specific PCR with primers plant1/ plant2 as previously reported [7, 11]. The results showed a high amplification quality of all the tested

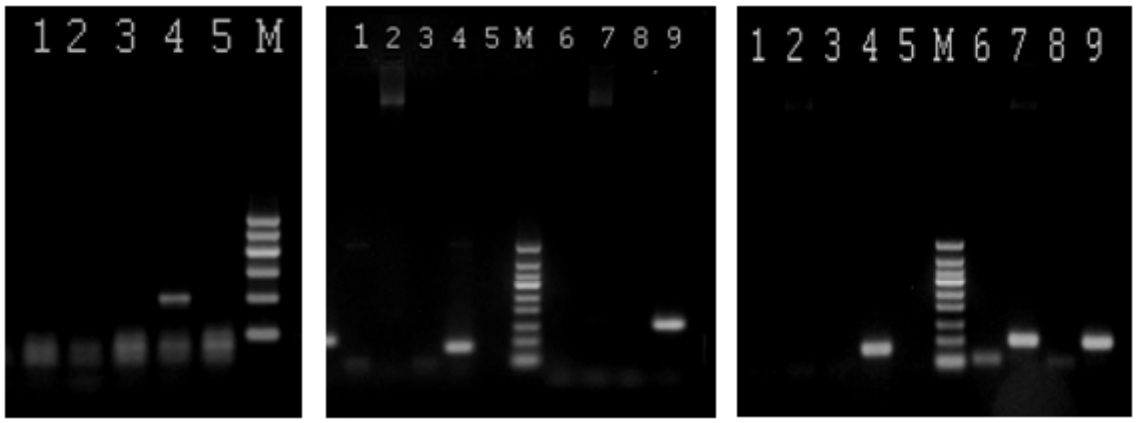

Fig. 1. Barley-specific PCR using primers hordf/hordr (**A**), hord91f/hord91r (**B**. lanes 1-5), hord150f/hord150r (**B**. lanes 6-9), hord82f/hord82r (**C**, lanes 1-5) and hord100f/hord100r (**C**, lanes 6-9).Samples: lanes 1.6. maize, lanes 2.7. wheat, lanes 3.8. soybean, lanes 4.9. barley, lane 5. water. **M (A)**GelPilot 100 bp ladder (Qiagen): 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp; **M (B, C)**GelPilot 50 bp ladder (Qiagen): 50 bp, 100 bp, 150 bp, 200 bp, 250 bp, 300 bp, 350 bp, 400 bp, 500 bp.

samples (data not shown).

All of the synthesized oligonucleotide primers relevant to barley were examined to selectively amplify their targets by qualitative end-point uniplex PCR. After optimization of the amplification conditions, PCR of barley DNA with four primer pairs, namely hordf/hordr, hord150f/hord150r, hord91f/hord91r, and hord82f/hord82r generated respectively single amplicons of 206bp, 150bp, 91bp and 82bp in size as expected (lane 4, Fig. 1A; lanes 4, 9, Fig. 1B; lane 4, Fig. 1C). However genomic DNAs from wheat, maize and soybean did not produce any PCR product (lanes 1-3, Fig.1A; lanes 1-3, 6-8, Fig.1B; lanes 1-3, Fig. 1C). No amplification signal was seen for negative water control (lane 5, Fig. 1A; 1B; 1C). The results obtained demonstrated high specificity of the tested primers, such as hordf/hordr, hord150f/hord150r, hord91f/hord91r, and hord82f/hord82r for barley detection. In addition, shorter amplicons are preferable analytes for food and feed analysis due to the DNA shearing during food/feed processing. The primer pair hordf/hordr produced amplicon of a larger size (206bp) with lower intensity compared to the 150bp, 91bp and 82bp amplicons. Correspondingly, PCR assays with primers hord150f/hord150r, hord91f/hord91r, and hord82f/hord82r are more effective tools for barley detection than PCR with the primer pair hordf/hordr. This outcome indicates that γ-hordein gene is a useful target for barley detection. Our findings are consistent with the results of Sandberg et al. [6] and Hernandez et al. [1]. They reported that real-time PCR assay targeting γ-hordein gene sequences was suitable for identification and quantification of barley. However, the present work describes development of conventional PCR methods for specific detection of barley for the first time.

An intriguing result was obtained by gel electrophoresis of amplification products generated with

H. vulgare TCGTA**AG**TCT**A**TCCGATCTAT-GATTT**A**GTTT-**A**GACAACTAGATT**A**AT**G**TT**C**CTT-**CGG**T

T. aestivum TCGTA**TT**TCT**C**TCCGATCTAT**T**GATTT-GTTTT**G**GACAACTAGATT**G**AT**T**TT**T**CTT**GTAA**T

H. vulgare G**TT**AG**TC**GTGC**A**TTGTA**GTA**GGTTCAATCT**G**GTGGT

T. aestivum G**GG**AG**AG**GTGC**T**TTGTA**AT**G**G**GTTCAATCT**T**GTGGT

Fig. 2. Sequence homology between amplicons of barley and wheat produced by primers hord100f/hord100r. The mismatches are highlighted in bold letters.

Table 1. Oligonucleotide primers used in PCR

Primers	Sequence	Amplicon (bp)
hordf	CGTGCACCATGATGTGTGATTCGAC	206
hordr	TGCACGACTAACACCGAAGGAACA	
hord150f	GTGCACCATGATGTGTGATTC	150
hord150r	CGGATAGACTTACGATTCCAGTT	
hord100f	GGAATCGTAAGTCTATCCGATCTATG	100
hord100r	GTACCACCAGATTGAACCTACTAC	
hord91f	GTACATGGATCATCGTTGCATAATC	91
hord91r	CCTGGTCGAATCACACATCA	
hord82f	GGACCAATCGATGTAATAACGATAA	82
hord82r	TTCCCACGATTGGACAAGT	

the primer pair hord100f /hord100r (Fig. 1C, lanes 6-7). As seen on the Fig. 1C, wheat (lane 7) and barley (lane 9) samples produced a single amplicon of approximately 100bp in size, whereas no PCR fragments were visible for maize and soybean samples. This result suggested some sequence similarity between the barley γ-hordein gene and the wheat genome. Therefore, bioinformatic analysis of the 100bp amplicon was performed by BLAST tool [12] to explain this interesting result of homology between barley amplicon and the wheat genome. Fig. 2 represents the most perfect match of the sequence homology study. We observed 77% identity between the 100bp amplicon of barley γ-hordein gene and the wheat chromosome 3B sequence produced by hord100f and hord100r primers. We shall also note that the amplicon and the primers show homology with more than 100 different sites of the wheat chromosome. This extensive homology could explain the appearance of the intensive band of about 100bp in the PCR reactions with the wheat genomic DNA. Our findings are in keeping with the previous reports of high homology between the barley and wheat genomes [13, 14].

In summary, new oligonucleotide primers targeting to γ-hordein gene were designed and conventional PCR methods were developed and optimized for detection of barley. These newly designed primers allow reliable identification of 82bp, 91bp and 150bp fragments of γ-hordein gene. PCR analysis of maize, soybean and wheat genomic DNA demonstrated high specificity and efficacy of the developed assays for barley detection, indicating that the methods elaborated in this study are useful for routine laboratory analysis. In addition, using bioinformatic analysis of the 100bp PCR product amplified by the hord100f /hord100r primers, we revealed sequence homology between the genomes of barley and wheat.

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მოლეკულური ბიოლოგია

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ქერის (Hordeum vulgare) სპეციფიკური დეტექცია საჭიროა ადამიანისა და ცხოველის საკვების ავთენტურობისა და ხარისხის დაცვისათვის. კვლევის მიზანი იყო პოლიმერაზული ჯაჭვური რეაქციის (პჯრ) მეთოდების შემუშავება ქერის ზუსტი და სწრაფი დეტექციისათვის. ამ მიზნით განხორციელდა ქერის სპეციფიკური γ-ჰორდეინის გენის მიმართ ახალი პჯრ-პრაიმერების დიზაინი. მათი სპეციფიკურობა შემოწმდა თვისებრივი პჯრ-ებით მცენარის სხვადასხვა სახეობით, კერძოდ, ქერით, სიმინდით (Zea mays L.), ხორბლით (Triticum aestivum L.) და სოიით (Glycine max L.). ამპლიფიკაციის პროდუქტების გელელექტროფორეზით გამოვლინდა სამი წყვილი პრაიმერის სპეციფიკურობა და ეფექტურობა ქერის ზუსტი დეტექციისათვის, ისინი იძლევიან 82bp, 91bp და 150bp პროდუქტებს. შემუშავებული პჯრ-მეთოდები შეიძლება გამოყენებულ იქნეს თესლების, კვების პროდუქტებისა და ფურაჟის ლაბორატორიული ანალიზისთვის.

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