



VARIETAL IDENTIFICATION STUDY OF SIX WHEAT VARIETIES USING ISSR MARKERS

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ABSTRACT

The identification of cereal and horticultural varieties are important for registration and agricultural systems. The traditional way to variety identification is the recording of morphological characters using descriptors. But molecular markers may serve as a modern and suitable approach to variety identification such as ISSR. The objective of the study was to identify the 17 ISSR primers, 801~849, for varietal identification of six wheat varieties, Darkhan-34, Darkhan-166 (Arvin), Darkhan-131, Darkhan-144, Khalkhgal-1 and Tsogt. As a result of ISSR marker observation on varieties, Arvin can be identified with 817, Khalkhgal-1 with 817 and 827, Tsogt with 822, Darkhan-34 with 830, Darkhan-131 with 830 and 848 and Darkhan-144 with 827, 830 and 848 primers. Among the surveyed ISSR primers, five can be used as variety-specific primers, 817, 827, 822, 830 and 848.

KEY WORDS: ISSR markers, primers, varietal identification

INTRODUCTION

The identification of cereal and horticultural varieties are important for registration and agricultural systems. At present, total of 102 cereal varieties were registered as a result of breeding program at Institute of Plant and Agricultural Sciences (IPAS) out of which 81 were wheat [1]. The traditional way to variety identification is the recording of morphological characters using descriptors. For instance, wheat morphological observation consists of 29 characters including plant height, leaf and spike shape and color etc. [2]. However, solely morphological characters are difficult to rely on and identify for large number of crop varieties. This is because of multigene of morphological characters, influenced by environment and climate, not available at all growth stages and requiring repeated observations. Furthermore, it is time consuming and less suitable when results are required rapidly for the variety confirmation. But molecular markers may serve as a modern and suitable approach to variety identification. The commonly used polymerase chain reaction (PCR)-based markers are random amplified polymorphic DNA (RAPD), amplified fragment

length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites [3]. Moreover, inter simple sequence repeats (ISSRs) are one of the PCR-based markers that have become widely used in various areas of plant research [4] such as studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology [5]. This markers overcome the low reproducibility of RAPD, high cost of AFLP and designing of specific primers and does not require genetic sequence information like for SSR markers [6]. The ISSR molecular markers are semi-arbitrary, single forward primers with 16-18 nucleotide length comprises repetitive units and anchors 2-6 arbitrary nucleotides at the 3' or 5' end. This method does not require the information about genomic sequences and therefore high level of polymorphism could be realized [7], [8]. It is successfully used for varietal identification in many crops such as potato [9], wheat [10-12] and canola [13]. The objective of the study was to detect the ISSR primers on six wheat varieties for varietal identification.

MATERIALS AND METHODS

Plant materials and DNA extraction

Total of 17 ISSR primers were tested for identifying six Mongolian wheat varieties, Darkhan-34, Arvin, Darkhan-131, Darkhan-144, Khalkhgal-1 and Tsogt,

reported in table 1. These were harvested from seed maintenance field-1 in the experimental station of IPAS in 2016. The middle two spikelet of each spike were planted in 42 x 63 pots and the second true leaf

was cut and stored at -82 freezer (ThermoScientific) for further genomic DNA extraction and usage. The genomic DNA was extracted following phenol-

chloroform method by Paul [14]. DNA concentration was analyzed (Nanodrop2000) and diluted into 50 ng/ μ l concentration for primer condition.

Table 1

Name and origin of varieties

Name of variety	Origin
Khalkhgol-1	Bo-1
Darkhan-131	Bo-1 x Skala
Darkhan-34	Buryatskaya-34 x Mironovskaya
Darkhan-166	Grekum-114 x Buryatskaya-34
Darkhan-144	416 x Grekum-114
Tsogt	HAAN229/3/SHA3/SERI /G.G.W.I/SERI

PCR condition and analysis

The reaction was performed in a total reaction volume of 25 μ l with following composition: 0.1 μ l Taq polymerase (5 U/ μ l, Takara), 2.5 μ l of PCR buffer, 2 μ l of dNTPs (2.5mmol/L, Takara), 2.5 μ l of forward and reverse primers with concentration of 5pmol/L, 5 μ l of DNA extract and 10.4 μ l of double distilled water (Milli-Q). PCR conditions include initial denaturation at 95°C for 5 min followed by 35 cycles

of 95°C for 30 sec, 50° for 30 sec, 60° for 1 min and the final extension of 72° for 5 min. 8 μ l of each PCR products amplified with primers labeled with gel loading dye of 2 μ L (SigmaAldrich) and analyzed on 1.5% agarose gel (Lonza, LSL-LE8200). Run for 65 minutes at 400W in gel electrophoresis (Biorad,PowerPac) and photo was taken by Uvitec Cambridge UVI pure camera.

RESULT AND DISCUSSION

The figure 1 shows the total number of amplified product of 7 primers with 56 bands ranging from 100 to 750 bp on six local varieties. 809 primer with maximum bp was detected on all six varieties whereas 830 primer with minimum bp was observed in Darkhan-131. The positive control was GluB3 primer with 621 bp band. **809** primer was detected on each variety with 520 and 750 bp, **817** primer was observed on only Arvin (Darkhan-166) variety with 600 bp and on Khalkh gol-1 with 450, 500 and 600 bp, **822** primer with 520 bp was recognized on Darkhan-131 and Darkhan-144 and 500 bp on Tsogt, **826** primer was detected on Darkhan-34, Arvin (Darkhan-166), Khalkhgol-1 and Tsogt varieties with 460 bp and, 580 bp on Darkhan-144 and Darkhan-131, **827** primer was identified on Darkhan-34 and Darkhan-144 with 200 and 390 bp, Darkhan-144 with

110 bp, Arvin (Darkhan-166) and Tsogt with 360 and 630 bp and Khalkhgol-1 with 700, 550 and 330 bp, **830** primer was discovered in Arvin (Darkhan-166), Khalkhgol-1 and Tsogt varieties with 665 bp, Darkhan-24 with 120 bp, Darkhan-131 with 100 bp and Darkhan-144 with 450 bp, **848** primer had 600 bp on Darkhan-34, 560 bp on Darkhan-131, 450 and 800 bp on Darkhan-144 and 450 and 650 bp on Arvin (Darkhan-166), Khalkhgol-1 and Tsogt. As a result, ISSR marker observations on six wheat varieties for varietal identification indicated that Arvin (Darkhan-166) can be identified with 817 primer, Khalkhgol-1 with 817 and 827 primers, Tsogt with 822 primer, Darkhan-34 with 830 primer, Darkhan-131 with 830 and 848 primers and Darkhan-144 with 827, 830 and 848 (Table 2).

Table 2

Primer name, sequence and amplified bands*

Number	Primer name	Primer sequence	Darkhan-34	Darkhan-131	Darkhan-144	Darkhan-166	Khalkhgol-1	Tsogt
1	UBC801	ATATATATATATATATT	-	-	-	-	-	-

2	UBC802	ATATATATATATATATG	-	-	-	-	-	-
3	UBC803	ATATATATATATATATC	-	-	-	-	-	-
4	UBC804	TATATATATATATATAA	-	-	-	-	-	-
5	UBC805	TATATATATATATATAC	-	-	-	-	-	-
6	UBC806	TATATATATATATATAG	-	-	-	-	-	-
7	UBC808	AGAGAGAGAGAGAGAGC	-	-	-	-	-	-
8	UBC809	AGAGAGAGAGAGAGAGG	+	+	+	+	+	+
9	UBC817	CACACACACACACAAA	-	-	-	+	+	-
10	UBC821	GTGTGTGTGTGTGTGTT	-	-	-	-	-	-
11	UBC822	TCTCTCTCTCTCTCA	-	+	+	-	+	+
12	UBC824	TCTCTCTCTCTCTCG	-	-	-	-	-	-
13	UBC826	ACACACACACACACACC	+	+	+	+	+	+
14	UBC827	ACACACACACACACACG	+	+	+	+	+	+
15	UBC830	TGTGTGTGTGTGTGTC	+	+	+	+	+	+
16	UBC848	CACACACACACACARG	+	+	+	+	+	+
17	UBC849	GTGTGTGTGTGTGTGYA	-	-	-	-	-	-

*The + indicates amplified bands and – is absence

Y and R are single letter abbreviation for mixed base positions. Y is presenting C or T. R is presenting A or T.

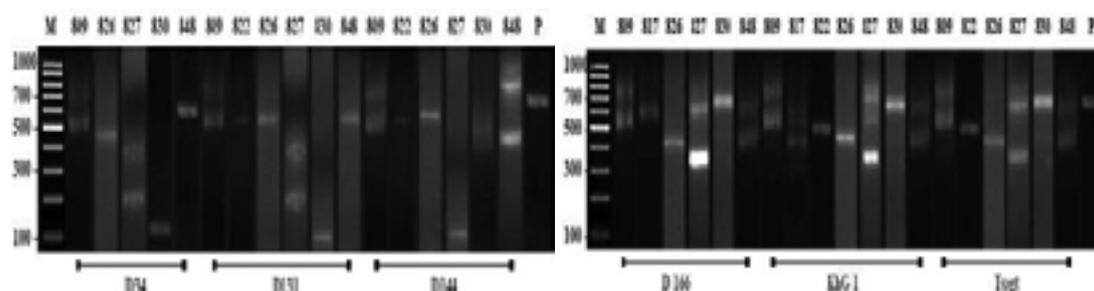


Figure 1. The result of amplification were detected by means of agarose gel electrophoresis

CONCLUSION

The ISSR markers were used on Mongolian wheat varieties and fit for the purpose of varietal identification. The marker is advantageous and proper because it does not require target sequence information. In this study, 17 ISSR markers used to identify and 5 primers, 817, 827, 822, 830 and 848, of which indicated specific bands for varieties. Used

primers were di-nucleotide but tri-, tetra-, penta- and hexanucleotide repeat sequences can be applied for precise variety identification that have higher polymorphism. Furthermore, ISSRs can be used in molecular bio-techniques for development of markers and species relationship analysis of plants.

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