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Original paper

Wheat varietal identification study using common quality and disease PCR markers

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Abstract

The main vital cereal crop in Mongolia is wheat (*Triticum spp*.). There are more than 10000 wheat varieties are released and registered in the world wheat atlas up to the date. Internationally, wheat varieties are identified by their genotypes. However, local wheat varieties are being identified only by its phenotypic traits in Mongolia. Not significant study was carried out on wheat varietal identification. In this study, we aim to differentiate widely planting 6 local wheat varieties Darkhan-34, Darkhan-131, Darkhan-144, Darkhan-166, Tsogt and Khalkh-gol-1 using PCR based common quality and disease 22 markers. As a result, 10 out of 22 markers were detected in all wheat varieties in distinct sizes, 8 markers detected otherwise and 4 markers was not detected at all. Thus, the ZSBy9 marker which is specific marker to distinguish Darkhan-144, ZSBy8 primer which is specific marker to distinguish Khalkh gol-1, GluA1c and GluB1 Bx642 markers those are specific markers to distinguish Tsogt variety from other studied varieties respectively.

Key words: Marker, gel electrophoresis, varietal identification**,** primer

1 Introduction

Spring wheat is the dominant stable food crop, which is cultivated 90% of agricultural land in Mongolia [1]. Development of new wheat variety takes more than decades to be released, At the moment, Institute of Plant and Agricultural science released 81 wheat varieties by national breeding programs [2]. The customary method to identify variety is the noting morphological characters using descriptors. For instant, morphological observation of wheat comprises of 29 characters covering plant height, spike and leaf shape and color etc. [3]. However, exclusively morphological characters are challenging to rely on and identify for large number of wheat 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 consuming time and less suitable when results are urgent for variety confirmation. But molecular markers are modern, rapid and appropriate method to variety identification. The widely applied 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 [4].
Moreover, inter simple sequence repeats (ISSRs) are one of the PCR-based markers that have become widely used in various areas of plant research [5] such as studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology [6].

ISSR primers worldwide successfully used for varietal identification in wheat [7-9] as well as in Mongolia [10]. Varietal Identification study in Mongolia by genotype is solitary fundamental stage in Mongolia. In this study, we aim to apply 22 common quality and disease markers on identifying 6 wheat varieties.

2 Methodology and Method

Plant materials and DNA extraction

Overall, 22 PCR based common quality and disease primers were tested for identifying six Mongolian wheat varieties, Darkhan-34, Darkhan-166, Darkhan-131, Darkhan-144, Khalkhgol-1 and Tsogt, reported in table 1. Seeds were collected from seed maintenance field-1 in the experimental field at Institute of Plant and Agricultural Sciences in 2016. The middle two spikelet of each spike were planted in 40 x 60 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 [11]. DNA concentration was analyzed (Nanodrop2000) and diluted into 50 ng/µl concentration for primer condition.

Table 1. Name and origin of varieties

Names of Variety	Origin
Khalkh gol-1	Bo-1
Tsogt	HAAN229/3/SHA3/SERI /G.G.W.I/SERI
Darkhan-34	Buryatskaya-34 x Mironovskaya
Darkhan-131	Bo-1 x Skala
Darkhan-144	416 x Grekum-114
Darkhan-160	Grekum-114 x Buryatskaya-34

PCR condition and analysis

The PCR was performed in a total reaction volume of 25 µl with following compositions: 0.1 µl Taq polymerase (5 U/µl, Takara), 2.5 µl of PCR buffer, 2 µl of dNTPs (2.5mmol/L, Takara), 2.5 µl of forward and reverse primers with concentration of 5pmol/L, 2µl of DNA extract and 13.4 µl of double distilled water (Milli-Q). The PCR includes starting temperature of denaturation at 95℃ for 5 min followed by 35 cycles of 95℃ for 30 sec, 50° for 30 sec, 60° for 1 min and the final extension of 72° for 5 min. 8 µl of each PCR products amplified with primers labeled with gel loading dye of 2 µL (SigmaAldrich) and analyzed on 1.5% agarose gel (Lonza, LSL-LE8200). The gel were run for 65 minutes at 300W in gel electrophoresis (Biorad,PowerPac) and photo was taken by Uvitec Cambridge UVI pure camera.

3 Result and Discussion

PCR quality and disease controlling 22 markers were studied. Out of 22 markers, GluAx2, Bx, Bx7, GluB1NonBy9, Lr34, Lr37, P3/P4, UMN25, UMN26, Yr36 markers on all wheat varieties. Dx5, GluA1c, GluB1 Bx642, GluB3c, GluD1d, UMN19, ZSBy8, ZSBy9 primers partially amplified on all wheat varietes whereas GluA1x1, GluD1a, Lr47, ZSBy9a markers not detected. Amplicons of 18 markers were ranged from 150-1320 bp. GluAx2 primer with maximum bp was detected on Khalkhgol-1 whereas Yr36 primer with minimum bp was observed on all six varieties [Table 2]. **GluAx2** primer was detected on Darkhan-34, Darkhan-131, Darkhan-144, Darkhan166, Tsogt with 630bp length whereas Khalkhgol-1 with 580 and 1320 bp. **Bx** primer was detected on Darkhan-34, Darkhan-144 varieties with 370 bp, on Darkhan-131 with 650 bp, on Darkhan-166 with 320 and 720 bp, on Tsogt with

420 bp, on Khalkhgol-1 with 420,530,700 and 800 respectively. **Bx7** primer was detected on Darkhan-34with 140 and 180 bp, on Darkhan-131with 175 bp, on Darkhan-144 with 140 and 700 bp, on Darkhan-166 with 140 bp, on Khalkhgol with 240 and 340 bp, on Tsogt with 340 bp respectively.

Lr 34 rust resistance marker was observed on all varieties with 300 bp whereas **Lr 37** was observed on all varieties with 380 bp. **GluP3/P4** primer was detected on all varieties with double band 750 and 400 bp. **UMN25** was amplified on all varieties with 360 bp whereas **UMN26** with 560 bp when **Yr 36** detected with 220 bp repectively. **Dx5** primer was observed on Darkhan-34 with 550 bp, on Darkhan-131 witg 490 bp, on Darkhan-144 and Darkhan-166 with 300 pb, on Khalkhgol-1 with 590 bp. **GluD1d primer** was detected on all varieties except Tsogt with 475 bp whereas **UMN19** primer was observed on all varieties except Tsogt with 485 pb. **GluB3c** primer detected on Khalkhgol-1 and Tsogt with 600 bp, **GluB1 Bx642** primer was observed on Tsogt with 570 bp. **GluA1c** was detected on Tsogt with 960 bp, **ZSBy8** was only on Khalkhgol-1 with 550 and 400 bp. **ZSBy9** was observed on Darkhan-144 with 440 bp respectively. [Figure 1-3]

Table 2. Primer name, sequence and amplified bands

*The + indicates amplified bands and – is absence

№	Name of primer	Primer sequence	Д34	Д131	Д144	Д166	ХГ-1	Цогт
1	GluAx2	ATGACTAAGCGGTTGGTTCTT ACCTTGCTCCCCTTGTCTTT	+	+	+	+	+	+
2	Bx	CGCAACAGCCAGGACAATT AGAGTTCTATCACTGCCTGGT	+	+	+	+	+	+
3	Bx7	CACTGAGATGGCTAAGCGCC GCCTTGGACGGCACCACAGG	+	+	+	+	+	+
4	Dx5	CGTCCCTATAAAAGCCTAGC AGTATGAAACCTGCTGCGGAC	+	+	+	+	+	-
5	GluA1c	ACG TTC CCC TAC AGG TAC TA TAT CAC TGG CTA GCC GAC AA	-	-	-	-	-	+
6	GluA1x1	TCACCGACAGTCCACCGA ACCAAGCGAGCTGCAGAG	-	-	-	-	-	-
7	GluB1 Bx642	GGG CAA TCG GGG TAC TTC C CCC TTG TCT TGG CTG TTG TC	-	-	-	-	-	+
8	GluB1 Non By9	TTC TCT GCA TCA GTC AGG A AGA GAA GCT GTG TAA TGC C	+	+	+	+	+	+
9	GluB3c	CAAATGTTGCAGCAGAGA CATATCCATCGACTAAACAAA	-	-	-	-	+	+
10	GluD1a	CTC GTC CCT ATA AAA GCC TAG T GAG ACA TGC AGC ACA TAC T	-	-	-	-	-	-
11	GluD1d	GCCTAGCAACCTTCACAATC GAAACCTGCTGCGGACAAG	+	+	+	+	+	-
12	Lr34	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	+	+	+	+	+	+
13	Lr37	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACA AA	+	+	+	+	+	+
14	Lr47	GCTGATGACCCTGACCGGT TCTTCATGCCCGGTCGGGT	-	-	-	-	-	-
15	P3/P4	GTTGGCCGGTCGGCTGCCATG TGGAGAAGTTGGATAGTACC	+	+	+	+	+	+
16	UMN19	CGAGACAATATGAGCAGCAAG CTGCCATGGAGAAGTTGGA	+	+	+	+	+	-
17	UMN25	GGGACAATACGAGCAGCAAA CTTGTTCCGGTTGTTGCCA	+	+	+	+	+	+
18	UMN26	CGCAAGACAATATGAGCAAACT TTGCCTTTGTCCTGTGTGC	+	+	+	+	+	+
19	Yr36	TCTCCAAGAGGGGAGAGACA TTCCTCTACCCATGAATCTAGCA	+	+	+	+	+	+
20	ZSBy8	TTAGCGCTAAGTGCCGTC TTGTCCTATTTGCTGCCCTT	-	-	-	-	+	-
21	ZSBy9	TACCCAGCTTCTCAGCAG TTGTCCCGACTGTTGTGG	-	-	+	-	-	-
22	ZSBy9a	TTCTCTGCATCAGTCAGGA AGAGAAGCTGTGTAATGCC	-	-	-	-	-	-

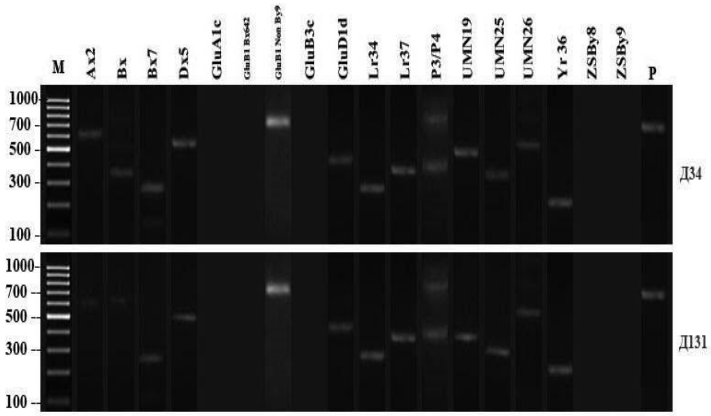

Fig. 1. The result of amplification detected on Darkhan-34, Darkhan-131

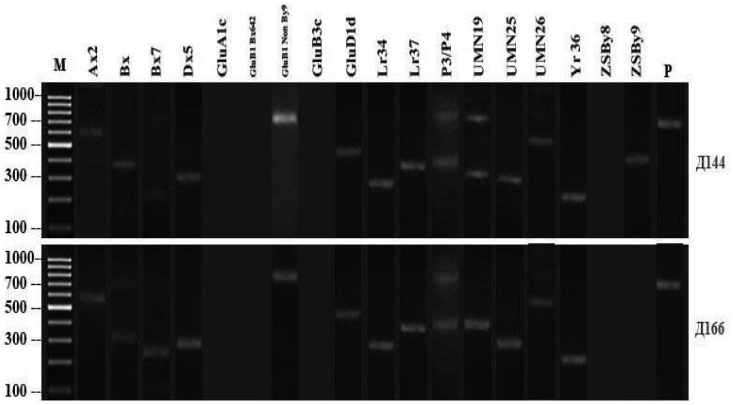

Fig. 2. The result of amplification detected on Darkhan-144 and Darkhan-166 varieties

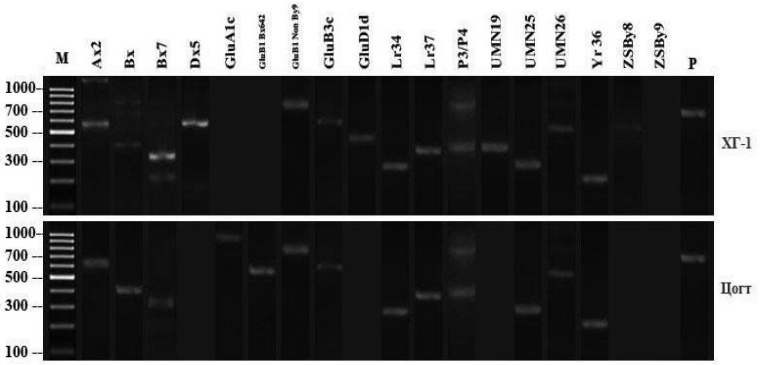

Fig. 3. The result of amplification detected on Khalkh gol-1, Tsogt varieties

4 Conclusion

The common quality and disease PCR markers can be used as markers for Mongolia wheat varieties and fit for the purpose of varietal identification. The marker is beneficial and applicable because we can use common markers for the varietal identification purposes. In this study, 22 PCR common markers used to identify possibility to differentiate Darkhan-144 variety with ZSBy9 marker, Khalkhgol variety with ZSBy8 as well as Tsogt variety with GluA1c, GluB1 Bx642 respectively. PCR type of markers may be one of opportunity to detect varietal identification where di-nucleotide but tri-, tetra-, penta- and hexanucleotide repeat sequences cannot be applied for precise variety identification.

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