Establishment of a multiplex-PCR system for high molecular weight glutenin subunits in wheat

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ABSTRACT

Wheat processing quality is closely correlated with composition and quantity of glutenin, in particular with the high-molecular weight glutenin subunits (HMW-GS) encoded by the *Glu-1* locus. The specific molecular markers of the subunits 1Dx5, 1Ax2*and 1Bx7^{OE} have been developed and been applied to molecular breeding for quality improvement. The purpose of this study was to establish a multiplex-PCR system to simultaneously identify 1Ax2*, 1Bx7^{OE} and 1Dx5 subunits in one reaction through exploring the influences of PCR components and cyclic parameters to the multiplex PCR results. The results showed that the primers concentration ratio and Tm value are the critical factors to the success of multiplex PCR. When the primers concentration ratio was 1Dx5/1Bx7^{OE}/ 1Ax2*=0.1/0.2/0.3 or 0.1/0.2/0.4 and Tm=60℃, the outcome of multiplex PCR was the best. The multiplex PCR system can identify more than one HMW-GS quickly and efficiently in one reaction, and can be used to carry out multiplex molecular marker assisted selection (MAS) in wheat quality breeding.

Key words: wheat, HMW-GS, multiplex PCR, molecular marker assisted selection

INTRODUCTION

High-molecular-weight glutenin subunits (HMW-GS) are directly associated with wheat processing quality[1], of which 1Dx5+1Dy10, 1Ax2*, and 1Bx7^{OE} (over-expression)are the subunits contributing to good wheat processing quality. But wheat cultivars containing 1Dx5+1Dy10, 1Ax2*, or 1Bx7^{OE} subunits are still very few in China, which is mainly because of the lack of efficient selection methods. Therefore, it is urgent to develop a set of methods with high efficiency and credibility for the wheat breeding of processing quality. The traditional method of HMW-GS identification is electrophoresis (SDS-PAGE), which can identify all the HMW-GSs of a cultivar at one time. This procedure has its limitations such as the shift distance of HMW-GS is not always linearly correlated with molecular weight, the non-expressed HMW-GS genes such as 1Axnull and 1Ay cannot be identified. With the development of PCR techniques and the cloning of HMW-GS genes, molecular marker-assisted selection (MAS) of HMW-GS has been developed and used in wheat processing quality breeding[2,3]. Multiplex PCR, an efficient method of multi-gene amplified in one reaction, is widely used in clinical fields[4], but only few reports can be found in identification of wheat HMW-GS[2,5]. Here a very useful multiplex-PCR system was established, and it may be a valuable example for MAS of other aspects of crop breeding.

MATERIALS AND METHODS

1. Materials

Seeds of wheat cultivars including Chinese spring (CS), Xiaoyan 54 (XY54), Cheyenne, Shangnong1801, Jian 11, IG4022, Yemao (YM) and N25 with different HMW-GSs (table 1) were provided by Institute of Genetics and Developmental Biology, Chinese Academy of Sciences.

Table 1 The composition of HMW-GS of different wheat cultivars.

Varieties	Glu-A1	Glu-B1	Glu-D1
Chinese Spring	N	7+8	2+12
Xiaoyan54	1	14+15	2+12
Cheyenne	2*	7+9	5+10
Wildcat	2*	7^{OE}+9	5+10
Jian11	1	7+8	5+12
SN1801	1	13+16	5+10
IG4022	2*	7+9	5+10

N25	1	17+18	5+12

2. PCR analysis

Genome DNA was extracted from leaves of wheat cultivar seedlings with the CTAB method according to Stewart and Via (1993)[6]. Primers of 1Dx5, 1Ax2* and 1Bx7^{OE} were derived from Ma *et al* (2003) and Radovanovic and Cloutier (2003)[2,3]. Optimization of multiplex-PCR conditions including concentration of Mg^{2+}, dNTP, PCR buffer, primers, the quantity of Taq and DNA template, and annealing temperature (Tm) was carried out with DNA of YM as template. Multiplex-PCR was performed according to Ma *et al* (2003)[2] with some modification. The PCR products were separated by electrophoresis in 1% agarose and visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

1 Optimization of multiplex-PCR conditions for 1Ax2*+1Bx7^{OE}+1Dx5 subunits

Specific primers of 1Dx5, 1Ax2* and 1Bx7^{OE} were used to examine the specificity of PCR markers in wheat cultivars. The results showed that only the cultivars containing 1Dx5, 1Ax2* or 1Bx7^{OE} could amplify the correct products (data not shown). PCR components affect the results of multiplex-PCR, thus the multiplex-PCR conditions including primers concentration and ratios, different amounts of PCR buffer, Mg^{2+}, dNTP, template DNA, and Taq polymerase were optimized for 1Ax2*+1Bx7^{OE}+1Dx5 subunits. Figure 1 showed that good results derived from the primer concentration (μM) and ratios of 1Ax2*/1Bx7^{OE}/ 1Dx5 = 0.3/0.2/0.1 or 0.4/0.2/0.1, 1×PCR buffer, 1.5 mM Mg^{2+}, 200 μM dNTP of each, 200 ng of template DNA, and 1 unit of Taq polymerase, of which the primers concentration and ratios are critical to the success of multiplex PCR, the PCR product with short sequence (1Dx5) could be easily amplified, while the product with long sequence (1Ax2*) was not, therefore, the concentration of 1Dx5 primer should be decreased (0.1 μM) and 1Ax2* primer increased (0.3-0.4 μM). The quantity of DNA template also affects multiplex-PCR, in the present study, 200 ng DNA template was suitable for the multiplex-PCR of 1Dx5, 1Ax2* and 1Bx7^{OE} subunits.

2. Optimization of multiplex -PCR program

PCR thermo cycle parameters also affect the success of multiplex PCR. The primers Tm is critical to the specificity of PCR amplification. Higher Tm gets fewer PCR products, while lower Tm results in more non-specific products. For 1Ax2*,1Bx7^{OE} and 1Dx5, Tm= 60□ was a good choice (figure 2A). In addition, slightly prolonged extension time (2 min) was more appropriate for the multiplex-PCR (figure 2B). Therefore, the proper parameters of multiplex- PCR program for 1Ax2*, 1Bx7^{OE} and 1Dx5 were as follows: 95℃, 15min 30sec; 94℃, 1min, 60℃, 1min, 72℃, 2min, 35 cycles; 72℃, 7 min.

Using MAS to screen target genotypes can shorten the breeding period. Multiplex-PCR, which can screen several target genes in one reaction, significantly reduces the breeding cost and improves the selection efficiency[2]. In this study, a strategy of establishment of multiplex-PCR system for wheat HMW-GS selection was provided, and may be valuable for other aspects of crop breeding.

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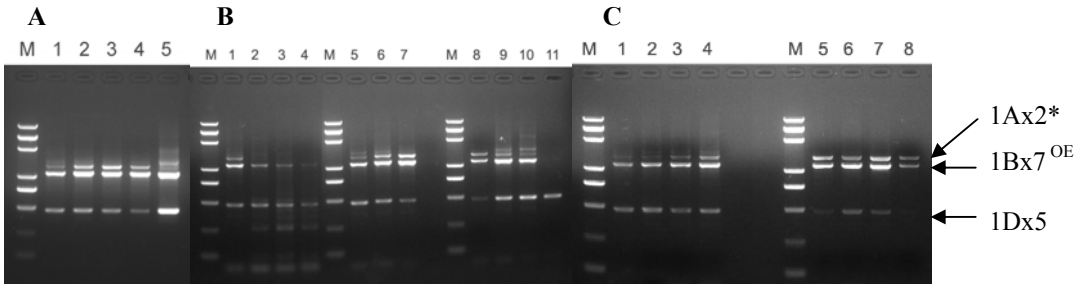

Figure 1 Optimization of multiplex-PCR conditions for 1Ax2*+1Bx7^{OE}+1Dx5 subunits. A, primer concentration and ratios, M represents DNA marker D2000plus, number 1-5 represent the different primer concentration ratios of 1Ax2*,1Bx7^{OE} and 1Dx5, 0.2/0.2/0.2, 0.3/0.2/0.1, 0.4/0.2/0.1, 0.4/0.3/0.1, and 0.4/0.3/0.2; B, different amounts of Mg^{2+}, dNTP, and PCR buffer, number 1-4 represent PCR results with different Mg^{2+} concentration (1.5, 2.0, 2.5, 3.0mM), 5-7 dNTP concentration (100, 200, 300 mM), 8-11 PCR buffer (0.8×, 1.2×, 1.6×, 2.0×); C, different amounts of template DNA and Taq polymerase, number 1-4 represent PCR results with different DNA template concentration (50, 100, 150, 200ng), 5-8 TaqPolymerase (0.5, 1, 1.5, 2U)

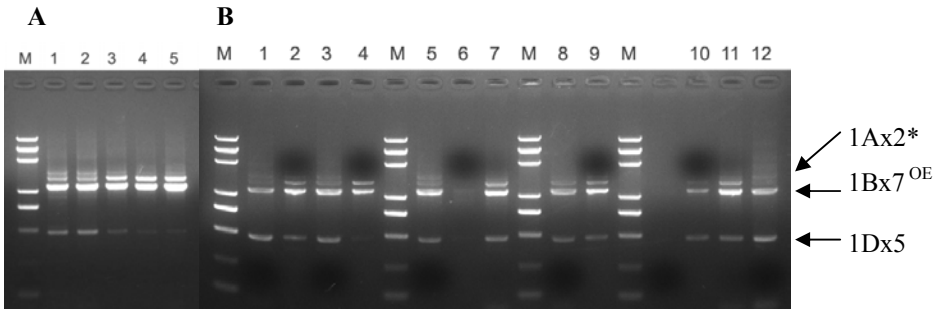

Figure 2 Optimization of multiplex-PCR program. A, different Tm values, M represents DNA marker D2000plus, number 1-5 represent PCR results with different annealing temperature (56.0，58.3，60.8，62，64□); B, different extension time, extension temperature, annealing time and cycle number, number 1-4 represent PCR results with different extension time (1，1.5，2，3min), 5-7 extension temperature (65，70，72℃), 8-9 annealing time (0.5，1min), 10-12 cycle number (25，35，40 cycles)