Journal of Integrative Agriculture 2016, 15(12): 2712–2718

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Molecular identification of ω-secalin gene expression activity in a wheat 1B/1R translocation cultivar

CHAI Jian-fang, ZHANG Cui-mian, MA Xiu-ying, WANG Hai-bo

Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences/Plant Genetic Engineering Center of Hebei Province, Shijiazhuang 050051, P.R.China

Abstract

ω-Secalin was an important factor influencing processing quality of wheat 1BL/1RS translocations. On the basis of four ω-secalin gene sequences cloned from Lankao 906 (a wheat cultivar with 1BL/1RS translocation) with putative transcription activity, a pair of primers with suitable restriction endonucleases added at their 5′ ends were designed to amplify the mature protein-coding regions of the four genes. After digestion with restriction endonucleases, the amplified products were ligated into the prokaryotic expression vector pET30a(+). The prokaryotically expressed recombinant proteins and gliadin isolated from the Lankao 906 seed were analyzed on the same acid polyacrylamide gel electrophoresis. All four prokaryotically expressed secalin bands had corresponding seed-expressed gliadin bands. The four corresponding gliadin bands were confirmed to be the expression products of the four ω-secalin genes by liquid chromatography tandem mass spectrometry (LC-MS/MS). This conclusion was further confirmed because the four ω-secalin bands could be detected in all 14 wheat 1BL/1RS translocation cultivars used in the study, although there was some interference for the detection of one ω-secalin band from nearby wheat gliadin bands. The sequence information of ω-secalin genes with expression activity will be helpful for improving the processing quality of wheat with 1BL/1RS translocations by using RNA interference method to silence the expression of the ω-secalin genes.

Keywords: *Triticum aestivum*, processing quality, storage proteins, prokaryotic expression, LC-MS/MS

1. Introduction

Wheat cultivars with 1BL/1RS translocations, in which the 1RS arm is derived from ‘Petkus’ rye, have been widely used in China and other countries for their good disease resistance, yield performance and wide adaptability (Villarreal *et al.* 1991; Carver and Rayburn 1994; Schlegel and Meinel 1994; Moreno-Sevilla *et al.* 1995; McKendry *et al.* 1996; Li *et al.* 2004; Tahir *et al.* 2014; Guberac *et al.* 2015). In some major wheat growing areas of China, cultivars with 1BL/1RS translocations account for more than 50% of the crop (Zhou *et al.* 2004). Although resistance genes on this 1RS arm are no longer effective against new biotypes of the respective pathogens (Yang and Ren 2001), some new disease-resistant 1BL/1RS translocations with non-Petkus rye origin have been developed (Lei *et al.* 2012; Yang *et al.* 2014); therefore, wheat 1BL/1RS translocations may continue to play an important role in wheat breeding and production. However, most wheat

Received 20 November, 2015 Accepted 18 January, 2016
Correspondence CHAI Jian-fang, Tel: +86-311-87652130, E-mail: chai87652130@163.com; WANG Hai-bo, Tel: +86-311-87652006, E-mail: nkywanghb@163.com

doi: 10.1016/S2095-3119(15)61290-4

1BL/1RS translocations have an obvious shortcoming in that they are not suitable for making bread because the translocation leads to serious defects such as poor mixing tolerance, superficial dough stickiness, and low bread volume (Dhaliwal *et al.* 1987, 1990; Burnett *et al.* 1995; Lee *et al.* 1995). Poor quality in noodle processing has also been reported to correlate with this translocation (Liu *et al.* 2004).

The poor quality of cultivars with 1BL/1RS translocations is thought to be partially caused by the expression of ω-secalins encoded by genes on 1RS (Barbeau *et al.* 2003), which are a family of small, highly water-soluble monomeric proteins related to wheat ω-gliadin (Dhaliwal *et al.* 1990; Graybosh *et al.* 1990; Lookhart *et al.* 1991; Hussain and Lukow 1994). The ω-secalin gene family has 15 copies that are arranged in a head-to-tail fashion (Clarke *et al.* 1996; Clarke and Appels 1999; Yamamoto and Mukai 2005). To date, many DNA sequences of ω-secalin genes have been published (Hull *et al.* 1991; Clarke *et al.* 1996; Chai *et al.* 2005; Jiang *et al.* 2010; De Vita *et al.* 2012). Of these, 5, 7 and 23 genes were cloned from 1B/1R translocation lines Lankao 906, Bobwhite and Kavkaz, respectively, using a homoeologous cloning method (Chai *et al.* 2005; Jiang *et al.* 2010; De Vita *et al.* 2012). However, no consistent conclusion has been reached regarding how many ω-secalin genes are expressed in a 1B/1R translocation line. Of the published ω-secalin gene sequences, only three that we reported (Chai *et al.* 2005) possess corresponding genomic DNA and cDNA. Thus, in the present study, we further analyzed whether the three ω-secalin genes are translated into ω-secalin proteins in the seeds. Additionally, we analyzed an unpublished ω-secalin gene that also had corresponding genomic DNA and cDNA sequences that we cloned from the same 1B/1R translocation line Lankao 906.

2. Materials and methods

2.1. Plant materials

Fourteen wheat 1BL/1RS translocations were used in this study: Heng 7228, Lankao 906, Zhoumai 13, Yumai 70, Lumai 14, Laizhou 953, Zhongmai 9, Jinghua 1, Jingdong 8, Chuanmai 17, Chuanmai 12, Huaimai 18, Jinmai 45, and Predgornaia. The 1RS arms in these 1BL/1RS translocations were all derived from 'Petkus' rye. Lankao 906 was used to clone ω-secalin gene sequences for the construction of prokaryotic expression vectors and to extract the seed storage proteins for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. The other 13 lines, together with Lankao 906, were used for the detection of ω-secalin bands from the acid polyacrylamide gel electrophoresis (A-PAGE).

2.2. Construction of prokaryotic expression vector

The encoded proteins of ω-secalin genes consist of signal peptide and mature protein. PCR primers were designed using DNAStar software, based on three published ω-secalin gene sequences (Chai *et al.* 2005). Primer sequences used were as follows: ω-sec-P5 (5′-AATCATATGAGG CAGCTAAACCCTAGCGAACAAGAG-3′, sense) and ω-sec-P6 (5′-AATGAGCTCCCGATGCCTATACCACTACTA CAAATG-3′, antisense). The underlined oligonucleotides (corresponding to *Nde*I and *Sac*I sites) were added to the 5′ terminus of the primers to facilitate cloning. The ATG in *Nde*I also provided a translation start codon for the mature protein-coding sequences. We used the recombinant plasmids of four ω-secalin gene sequences with corresponding genomic DNA and cDNA (three were published in Chai *et al.* 2005) as PCR templates to amplify the mature protein-coding regions in this study. PCR was conducted using a Pfu PCR Kit KP-101 (Tianweishidai Co., China) and PTC-100TM Programmable Thermal Controller (MJ Research) with the following program: 3 min at 94°C (initial denaturation) and 30 cycles of 45 s at 94°C, 45 s at 64°C, and 1.5 min at 72°C. An additional 7-min extension at 72°C was applied after the 30 cycles. The 50-μL reaction volume contained 20 mmol L^{-1} Tris-HCl (pH 8.8), 10 mmol L^{-1} KCl, 2 mmol L^{-1} $MgSO_4$, dNTPs (0.2 mmol L^{-1}), the sense and antisense primers (0.4 μmol L^{-1} each), 3.75 U of Pfu DNA polymerase, and approximately 1 ng of plasmid DNA. Partial PCR products were subjected to electrophoresis in 1× TAE buffer (40 mmol L^{-1} Tris-acetate, 1 mmol L^{-1} EDTA (ethylene diamine tetraacetic acid)) on a 1% agarose gel and visualized by ethidium bromide staining.

Amplified products of the expected length were purified using a DP-204 PCR Product Purification Kit (Tianweishidai Co., China), digested using *Nde*I and *Sac*I restriction enzymes, and cloned into bacterial expression vector pET30a(+) at the same restriction enzyme sites. The resulting plasmids were then transformed into BL21 competent bacterial cells. Plasmids from PCR-positive clones were purified and sequenced using a 3730 DNA Sequencer (ABI, USA) and the cycle sequencing method to confirm the specificity of the cloned fragments.

2.3. Prokaryotic expression of ω-secalin genes

The positive clones were cultured in separate liquid LB media with 30 μg mL^{-1} kanamycin under agitation. When their concentration reached 0.5–1.0 OD_{600}, 100 mmol L^{-1} isopropyl β-D-1-thiogalactopyranoside was added to a final