ELSEVIER

Journal of Cereal Science 47 (2008) 153–161

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jcs

Generation of transgenic wheat lines with altered expression levels of 1Dx5 high-molecular weight glutenin subunit by RNA interference

S.J. Yue[a,d], H. Li[b], Y.W. Li[a], Y.F. Zhu[a], J.K. Guo[c], Y.J. Liu[c], Y. Chen[a,*], X. Jia[a,*]

[a]*State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[b]*Institute of Food and Oil Crops, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, Hebei Province, 050031, China*

[c]*Shijiazhuang Academy of Agricultural Sciences, Hebei Province, 050041, China*

[d]*Graduate School of Chinese Academy of Sciences, Beijing 100039, China*

Received 28 July 2006; received in revised form 11 March 2007; accepted 12 March 2007

Abstract

In recent years, high molecular weight glutenin subunit (HMW-GS) null mutants have been found to be useful for studying the contribution of HMW-GS to the flour processing quality of wheat (*Triticum aestivum* L. em. Thell.). However, few reports have dealt with the development and characterization of such variants. In the present study, the RNA interference (RNAi) method was applied to Bobwhite wheat, which has five actively expressed HMW-GS genes (namely *1Ax2**, *1Dx5*, *1Bx7*, *1By9*, *1Dy10*), with the aim of silencing the expression of *1Dx5*. Out of the six transgenic events characterized, *1Dx5* expression was completely blocked in four transgenic events (L1–L4), and partially reduced in the other two (L5, L6). In contrast, the protein levels of 1Ax2*, 1By9 and 1Dy10 were not significantly affected in any of the six transgenic events. Interestingly, 1Bx7 protein accumulation was negatively affected in all six events and their progenies. *1Dx5* transcript levels in developing seeds at 15 days after pollination (DAP) were undetectable in L1 and dramatically reduced in L5. The silencing of *1Dx5* expression caused a substantial decrease in flour processing quality based on Farinograph, gluten and Zeleny tests. Collectively, our data suggest that RNAi is useful for silencing HMW-GS genes. The resultant transgenic lines are of value for studying the contributions of specific HMW-GS to wheat flour processing quality.

Keywords: Wheat; RNAi; HMW-GS; Gene silencing

1. Introduction

The processing quality of wheat (*Triticum aestivum* L. em. Thell.) flour is closely associated with the elasticity and viscosity of the gluten complex. As the major component of gluten proteins, high molecular weight glutenin subunits (HMW-GSs) play a key role in determining the viscoelasticity of gluten (Payne et al., 1987; Shewry et al., 1995; Shewry and Halford, 2002). Genetically, HMW-GSs are encoded by *Glu-1* loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) on the long arms of the homeologous group 1 chromosomes of wheat. At each locus, one x- and one y-type subunit are encoded. Because of natural gene silencing, only three to five subunits are usually expressed in a given hexaploid wheat variety. This, plus allelic variation, results in HMW-GS compositions that differ greatly among different wheat varieties. It has been demonstrated that the variation in HMW-GS composition has a strong impact on wheat bread-making quality (Payne et al., 1987; Shewry and Halford, 2002). During the past two decades, many correlative studies have been reported with the aim of understanding the relationship between the presence or

Abbreviations: DAP, days after pollination; DsRNA, double-stranded RNA; HMW-GS, high molecular weight glutenin subunits; LMW, low molecular weight; NIL, near isogenic line; NOS, nopaline synthase; RNAi, RNA interference; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SiRNAs, small interfering RNAs; VIGS, virus induced gene silencing; WT, wild type

*Corresponding authors. Tel.: +8 61 06 487 8103; fax: +8 61 06 485 4467.

E-mail addresses: ychen@genetics.ac.cn (Y. Chen), xjia@genetics.ac.cn (X. Jia).

0733-5210/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2007.03.006

absence of specific HMW-GSs and flour processing properties. It is clear that several subunits, such as 1Dx5, 1Dy10, and 1Ax1, are generally associated with better quality, while 1Dx2 and 1Dy12 have negative impacts on this trait (Payne et al., 1987; Weegels et al., 1996). Moreover, studies using near isogenic lines (NILs) and transgenic lines with over expression or silencing of one or more HMW-GSs have partially validated the foregoing conclusion (Barro et al., 1997; MacRitchie and Lafiandra, 2001; He et al., 2005; Naeem and MacRitchie, 2005). However, it is still difficult to precisely evaluate the contribution of single HMW-GSs because of the lack of sufficient mutant lines that have the same genetic backgrounds.

Because of co-suppression, silencing of endogenous HMW-GS gene expression has been observed in some transgenic lines that contain HMW-GS transgenes designed for over expression. For example, introduction of a 1Ax1 transgene into common wheat led to silencing of the endogenous 1Ax1 (Alvarez et al., 2000). The expression levels of the endogenous subunits encoded by *Glu-B1* genes was significantly decreased after the introduction of a 1Dx5 transgene (He et al., 2005). However, these transgenic lines are not suitable for studying the function of single subunits because of undesirable changes in the expression of other untargeted subunits. HMW-GS gene knockout mutants can also be generated by chemical mutagenesis (Masci et al., 1993; Upelniek et al., 1995; Svec et al., 1999; Zhu et al., 2005). However, this approach has disadvantages stemming from the randomness of mutation, and the mutants obtained must be backcrossed to a wild type (WT) parent for several generations to eliminate background mutations. Furthermore, a low mutation frequency has limited the efficiency of this approach for generating sufficient mutant lines for functional studies (Zhu et al., 2005).

RNA interference (RNAi) has been successfully used in a number of plants as an efficient tool to decrease or knockout gene expression for analyzing gene functions (Waterhouse et al., 1998; Buhr et al., 2002; Chen et al., 2003; Segal et al., 2003; Han et al., 2004; McDonald et al., 2002). The primary advantage of RNAi is the ability to specifically silence target genes. Using this approach, it is possible to silence an individual gene family member by targeting its unique sequence regions or multiple members of a gene family by targeting the shared conserved sequence domains (Miki et al., 2005). Furthermore, variable levels of gene silencing can often be achieved in different transgenic lines, which may facilitate the study of the relationship between gene expression level and phenotype plasticity. However, successful application of RNAi in wheat by stable genetic transformation has been reported in only a few studies (Ma et al., 2004; Loukoianov et al., 2005; Regina et al., 2006). This may be due to the complexity of the wheat genome and the difficulty of wheat transformation. To circumvent this problem, virus induced gene silencing (VIGS) has been carried out to analyze gene functions in wheat (Holzberg et al., 2002; Lacomme et al., 2003). However, the VIGS approach is mainly useful for studying the genes expressed in the leaf tissues. There is so far no report on the silencing of wheat storage protein genes by VIGS.

In the present study, we report the silencing of 1Dx5 HMW-GS gene by RNAi in transgenic lines of a hexaploid wheat variety Bobwhite. The results show that the transcription of 1Dx5 was completely or partially blocked in the transgenic plants and the transgenic phenotypes were stably inherited in subsequent generations. Using the RNAi lines, we demonstrate that altered expression of 1Dx5 decreased the processing quality of flour, thus validating the usefulness of RNAi in the efficient development of HMW-GS gene expression mutants suitable for functional studies of this important family of genes.

2. Materials and methods

2.1. Plant materials

The wheat cultivar Bobwhite was used as the recipient for transformation. The HMW-GS genes actively expressed in Bobwhite are *1Ax2**, *1Dx5*, *1Bx7*, *1By9* and *1Dy10*. Wheat plants were grown in a greenhouse with a day/night temperature regime of 20–28 °C/15–18 °C to provide immature embryos for genetic transformation. The subunits expressed in the wheat variety Chinese Spring (1Dx2, 1Bx7, 1By8, 1Dy12) were used as a control in SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis) analysis of HMW-GSs.

2.2. Plasmid construction

The 210 bp fragment from the 5′-terminal coding region of *1Dx5* (from 154 to 363 bp relative to the translation start codon, GenBank accession number X12928.3) was used to design the RNAi effector construct. At the nucleotide level, this fragment is approximately 81–91% identical to the corresponding elements in *1Ax2**, *1Bx7*, *1By9*, and *1Dy10* (Fig. 1A and Table 1). Furthermore, these homeologous elements also share 22 to 32 bp stretches of perfect nucleotide identity (Table 1). The sequence identities were analyzed using the Megasoft software, which is an integrated tool for automatic and manual sequence alignment analysis, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, and testing evolutionary hypotheses (www.megasoftware.net). For preparing the RNAi cassette, the fourth intron of the wheat *waxy* gene (GenBank accession number AB019624) was amplified by PCR and used as the spacer. The forward fragment (in sense orientation), the spacer and the reverse fragment (in antisense orientation) were sequentially inserted into the pBluscript SK plasmid vector (Stratagene, La Jolla, CA, USA) to produce a 607 bp long hairpin fragment. This fragment was then inserted downstream of the Ubiquitin promoter in place of *UidA* gene in the plant transformation vector pAHC25 (Christensen and Quail, 1996), giving rise to the final transformation construct pAHC-1Dx5RNAi, which contains