Journal of Cereal Science 53 (2011) 104–111

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Waxy endosperm accompanies increased fat and saccharide contents in bread wheat (*Triticum aestivum* L.) grain

Takeshi Yasui*, Kanae Ashida

National Agricultural Research Center for Western Region, 12-1, Nishifukatsucho 6-chome, Fukuyama 721-8514, Japan

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form
23 September 2010
Accepted 12 October 2010

Keywords:
Compositional change
Pleiotropic effect
Triticum aestivum
Waxy endosperm

ABSTRACT

The contents of fat, starch, pentosan, fructan, β-glucan and several mono- and oligosaccharides in grain were evaluated to find out the possible effects of the *Wx-D1* gene of bread wheat using two sets of near-isogenic waxy and non-waxy lines and two low-amylose mutant lines with a common genetic background of Kanto 107. These materials have two non-functional *Wx-A1b* and *Wx-B1b* alleles in common. Waxy near-isogenic lines with a non-functional *Wx-D1d* allele showed consistently increased contents of fat, total fructan, β-glucan, glucose, fructose, sucrose, 1-kestose, 6-kestose, neokestose, nystose and bifurcose compared with non-waxy lines with a functional *Wx-D1a* allele throughout three growing/harvest seasons. Starch and total pentosan contents were inconsistently influenced by the allelic status of the *Wx-D1* locus, while water-soluble pentosan and raffinose contents were not affected. The compositional changes of a low-amylose mutant line with an almost non-functional *Wx-D1f* allele were closely similar to those of waxy near-isogenic lines, while significantly different changes were barely observed in another low-amylose mutant line with a partly functional *Wx-D1g* allele in two seasons. These results showed that the *Wx-D1* gene has pleiotropic effects on the fat and saccharide contents of bread wheat grain.

1. Introduction

Starch is a major component of cereal grain, and its properties affect the qualities of various cereal products. Amylose content is the most influential factor affecting starch properties and it is genetically controlled by a waxy gene(s), which encodes granule-bound starch synthase I (GBSSI) responsible for amylose biosynthesis in endosperm. Several non-functional and partly functional waxy alleles have been identified in bread wheat (*Triticum aestivum* L.) (MacGene, 2008; Yasui, 2006). Because bread wheat is a hexaploid plant, it has three waxy loci, *Wx-A1*, *Wx-B1* and *Wx-D1*, derived from three homoeologous genomes, A, B and D, respectively. Compared with the wild type in which all three waxy genes are functional, single or double null types, in which one or two waxy gene(s) are non-functional, show decreased amylose content corresponding to the number of non-functional waxy alleles. When all of the three waxy genes are non-functional, amylose is not synthesised in the endosperm, and the starch becomes a waxy type comprised only of amylopectin. Six alleles have been identified thus far on the *Wx-A1* locus, six alleles on the *Wx-B1* locus (MacGene, 2008) and seven alleles on the *Wx-D1* locus (Yasui, 2006); therefore, by producing new partly functional waxy alleles and combining appropriate alleles on each locus, amylose content would be controllable in the range of 0–30% in endosperm starch.

The waxy gene also has some effects on other properties of bread wheat grain. A crossbred waxy line showed decreased lipid content in endosperm starch (Yasui et al., 1996). The waxy mutant lines had an increased fat and β-glucan content and decreased starch content in the grain (Yasui et al., 1999). One of two near-isogenic waxy lines showed an increased arabinoxylan content in the grain (Takata et al., 2007). As expected, decreased amylose content in starch affected the gelatinisation properties of starch and flour (Takata et al., 2007; Yasui et al., 1996, 1999). Furthermore, decreased milling yield of flour was observed with the waxy genotypes (Takata et al., 2007; Yasui et al., 1999) presumably because of their increased fat and

Abbreviations: Bifurcose, β-D-Fructofranosyl-(2→1)-[β-D-fructofranosyl-(2→6)]-β-D-fructofranosyl α-D-glucopyranoside; DP, Degree of polymerisation; FFT, Fructan:fructan fructosyltransferase; β-Glucan, (1→3),(1→4)-β-D-glucan; HPAEC-PAD, High-performance anion-exchange chromatography with pulsed amperometric detection; 1-Kestose, β-D-Fructofranosyl-(2→1)-β-D-fructofranosyl α-D-glucopyranoside; 6-Kestose, β-D-Fructofranosyl-(2→6)-β-D-fructofranosyl α-D-glucopyranoside; Neokestose, β-D-Fructofranosyl β-D-fructofranosyl-(2→6)-α-D-glucopyranoside; Nystose, β-D-Fructofranosyl-(2→1)-β-D-fructofranosyl-(2→1)-β-D-fructofranosyl α-D-glucopyranoside; RRF, Relative response factor; SFT, Sucrose:fructan fructosyltransferase; SST, Sucrose:sucrose fuctosyltransferase.

* Corresponding author.
E-mail address: yas@affrc.go.jp (T. Yasui).

0733-5210/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2010.10.004

β-glucan contents (Yasui et al., 1999). On the other hand, the protein (Takata et al., 2007; Yasui et al., 1999), ash and polyphenol content of grain (Takata et al., 2007) and amylopectin properties including chain length distribution profile of endosperm starch (Yasui et al., 1996, 2009) were not influenced by the allelic status of the waxy gene.

In other cereals, a waxy mutation of the endosperm starch simultaneously induced changes in the chemical composition of their grain. In barley (*Hordeum vulgare* L.) grain, ether extract (crude fat), free sugars and β-glucan contents were significantly increased and starch content was decreased in waxy near-isogenic lines compared with non-waxy lines, while protein, arabinoxylan and ash contents were not influenced (Xue et al., 1997). Increased fat content was also observed in waxy mutants of rice (*Oryza sativa* L.) (Taira and Hiraiwa, 1982).

Fructan is a group of compounds where one or more fructosyl-fructose linkages constitute a majority of the linkages in a molecule (Lewis, 1993). *Triticeae* species, including bread wheat, contain fructan in their seeds, culms, leaves and roots (Praznik et al., 2006). Bread wheat grain contains various fructan components, including fructosyl trisaccharides, i.e., β-D-fructofranosyl-(2→1)-β-D-fructofranosyl α-D-glucopyranoside (1-kestose), β-D-fructofranosyl-(2→6)-β-D-fructofranosyl α-D-glucopyranoside (6-kestose), β-D-fructofranosyl β-D-fructofranosyl-(2→6)-α-D-glucopyranoside (neokestose), and tetrasaccharides, i.e., β-D-fructofranosyl-(2→1)-β-D-fructofranosyl-(2→1)-β-D-fructofranosyl α-D-glucopyranoside (nystose) and β-D-fructofranosyl-(2→1)-[β-D-fructofranosyl-(2→6)]-β-D-fructofranosyl α-D-glucopyranoside (bifurcose), along with other oligo- and polysaccharides (Stone and Morell, 2009). Several enzymes including sucrose:sucrose fructosyltransferase (SST), sucrose:fructan fructosyltransferase (SFT) and fructan:fructan fructosyltransferase (FFT) are involved in fructan biosynthesis (Praznik et al., 2006). It was reported that sucrose stimulates SFT and SST activities in barley leaves (Müller et al., 2000). There was a positive relationship between sucrose content and SST activity in wheat stem, suggesting that sucrose induced SST activity (Dubois et al., 1990). The increase in sucrose content and high SST and FFT activities suggested that biosynthesis of fructan oligosaccharides occurred at 12 days post-anthesis in wheat grain (Housley and Daughtry, 1987). Therefore, it is conceivable that, if sucrose content is increased in waxy wheat grain and if sucrose acts as an activation factor for fructan biosynthesis, fructan content would increase in waxy grain.

The aim of the present study was to evaluate the pleiotropic effects of the *Wx-D1* gene on the fat, starch and other saccharide contents of the grain. Near-isogenic waxy and non-waxy lines and low-amylose mutant lines with a common genetic background were used to eliminate the possible effects of other loci on the chemical composition.

2. Experimental

2.1. Materials

Two sets of near-isogenic waxy and non-waxy lines, Kanto 107*4/K107Wx1-nil and Kanto 107*4/K107Wx2-nil, were multiplied in an experimental filed at the National Agricultural Research Center for Western Region (Fukuyama, Japan) with a completely randomised design with four to six replications, each comprising a single row per line, in the 2003/2004, 2004/2005, 2005/2006 and 2006/2007 seasons under ordinary conditions. The waxy and non-waxy lines have a non-functional *Wx-D1d* and a functional *Wx-D1a* allele, respectively, and both lines have non-functional *Wx-A1b* and *Wx-B1b* alleles in common with their recurrent parent, Kanto 107. The non-waxy near-isogenic lines used in this study are genetically nearly identical to Kanto 107. Two low-amylose mutant lines, Tanikei A6599-4 with *Wx-D1f* allele and K107Afpp4 with *Wx-D1g* allele, and non-waxy Kanto 107*4/K107Wx1-nil or Kanto 107 were multiplied with two replications in the 2004/2005 and 2006/2007 seasons. All plants were grown under a transparent plastic roof after heading to prevent rain damage. Bulked grain from each row was analysed separately. Grain samples were stored at 5 °C. The representative amylose contents of endosperm starch (A-type granule) isolated from the lines were as follows: waxy near-isogenic lines, 2.6–2.8%; non-waxy near-isogenic lines, 23.6–23.9%; Tanikei A6599-4, 6.2% and K107Afpp4, 16.1% (Yasui et al., 2009).

2.2. Preparation of samples and grain property measurement

Sound grain was manually selected by naked-eye inspection and the 1000-grain weight was calculated from the number of kernels and the remaining weight after drying of crushed grain at 135 °C for 3 h. Wholemeal was prepared from the grain using a Cyclotec 1093 sample mill with a 0.5 mm screen (Tecator, Sweden). Moisture content of the wholemeal was calculated as weight loss after drying at 135 °C for 1 h. Fat content was measured using AOAC official method 920.85 (AOAC, 2010).

2.3. Starch and other polysaccharide content determination

Starch content was measured using AOAC official method 996.11 with an assay kit, K-TSTA (Megazyme International Ireland Ltd., Wicklow, Ireland), total fructan was measured using AOAC official method 999.03 with an assay kit, K-FRUC (Megazyme International Ireland Ltd.), and (1→3),(1→4)-β-D-glucan (β-glucan) was measured using AOAC official method 995.16 with an assay kit, K-BGLU (Megazyme International Ireland Ltd.) (AOAC, 2010). In total fructan determination, the extract of wholemeal was incubated with α-galactosidase from *Aspergillus niger* (E-AGLAN, Megazyme International Ireland Ltd.) as recommended (Megazyme, 2008) to exclude the possible influence of raffinose and other galactosyl-sucrose oligosaccharides on fructan quantification.

Total and water-soluble pentosan content was determined by a method reported previously (Finnie et al., 2006) using xylose as a standard with the following modifications: 100 mg of wholemeal was weighed instead of 125 mg; the reaction scale was decreased to one-half, i.e., 1 mL of solution was reacted with 5 mL of reagent. For total pentosan determination, the sample suspension was diluted (0.2 mL + Milli-Q water, 0.3 mL); for water-soluble pentosan determination, the amount of sample extract taken was increased to 1 mL and not diluted. In addition, a quadratic regression was fitted to the data for calculating the calibration curve instead of a linear regression, because the relationship between absorbance and xylose concentration was convex (Douglas, 1981). Furthermore, the colour of the reaction solutions faded appreciably with time (Douglas, 1981), so the absorbance of each sample was corrected using the difference in absorbance of the same standard solution (0.1 mg/mL) measured at the beginning and at the end of a series of measurements. Xylose (Sigma, X3877) was dried in a vacuum at 60 °C for 12 h and dissolved with Milli-Q water.

2.4. Identification of mono- and oligosaccharides

Saccharides known to be present in wheat grain (Stone and Morell, 2009), i.e., glucose, fructose, raffinose, 1-kestose and nystose, were identified by the comparison of their chromatographic retention times with those of available authentic standards described in the following section. The presence of sucrose, raffinose, 1-kestose and nystose was further confirmed by incubating the grain extract with fructanase as follows: dried 80% EtOH extract