GENETIC VARIATION FOR WAXY PROTEINS AND STARCH PROPERTIES IN CHINESE WINTER WHEATS

Zhonghu He [1,2*]; Zhaohua Xu[1]; Lanqin Xia[1]; Xianchun Xia[1]; Jun Yan[1]; Yan Zhang[1]; Xinmin Chen[1]

[1]Institute of Crop Sciences/National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS) No 12 Zhongguancun South Street, Beijing 100081

[2]CIMMYT-China Office, C/O CAAS, No 12 Zhongguancun South Street, Beijing 100081

*Author for correspondence (e-mail: zhhe@public3.bta.net.cn)

ABSTRACT

Starch properties contribute positively to the quality of both dry and fresh Chinese white noodle quality. Understanding the genetic variation of starch properties in Chinese wheats will therefore facilitate genetic improvement for noodle quality. The composition of waxy protein (*Wx*) subunits, amylose content, and Rapid Visco Analyzer (RVA) properties of a total of 260 wheat cultivars and advanced lines (*Triticum aestivum* L) from four Chinese winter wheat regions were characterized. Significant variation was observed for all tested parameters except for peak time. Amylose content, peak viscosity, holding strength, and breakdown values ranged 23.1 to 33.6%, 1151 to 3522cp, 1385 to 2374cp and 192 to 1711cp, respectively. Thirty-nine Chinese wheats conferred the *Wx-B1* null mutation, and significant differences in starch properties were observed between the *Wx-B1* null wheats and the normal wheats. Data on both waxy protein and starch properties are needed to characterize Chinese wheat germplasm for starch quality and for noodle products. Three cultivars (Guanfeng 2, Yumai 47, and Mianyang 26) had very good starch properties and noodle quality based on our previously report. They could be used as crossing parents in breeding programs targeting for noodle quality improvement.

Key words: *Triticum aestivum* L., common wheat, waxy protein, amylose content, pasting characteristics

Introduction

Starch, composed of 20-28% amylose and 72-80% amylopectin (Yamamori and Quynh., 2000), is the major component of wheat endosperm. Granule-bound starch synthase (GBSS) is the key enzyme involved in the production of amylose (Preiss and Sivak., 1996). In bread wheat (*Triticum aestivum* L), GBSS is encoded by the three waxy loci, i.e., *Wx-A1*, *Wx-D1*, and *Wx-B1*, located on the short arms of chromosomes 7A and 7D, and the long arm of chromosome 4A, respectively (Chao et al., 1989; Nakamura et al., 1993). However, the three waxy proteins have different effects on amylose content and pasting properties, the *Wx-B1* null mutation having the most, followed by *Wx-D1* and *Wx-A1* null mutation (Araki et al., 2000; Miura et al., 1994, 2002; Yamamori and Quynh., 2000). A correlation has been found across cultivars between the presence of GBSS null alleles (associated with lower amylose content) and higher Japanese udon noodle quality (Yamamori et al., 1992; Nakamura et al.,

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1993; Miura and Tanii.,1994). The *Wx-B1* null genotype is very common in Australian cultivars. The *Wx-B1* null mutation is closely associated with high flour swelling volume, high peak viscosity, and low final viscosity, and it can be very useful in screening for Japanese white salted noodle quality at an early breeding stage (Zhao et al., 1998). Currently, most Japanese cultivars suitable for udon noodle production also confer *Wx-B1* null mutation (H.Yoshida, pers. comm.). The Rapid Viso Analyzer (RVA) has been widely used to test noodle quality both in Australia and China since its parameters are closely associated with noodle quality (Panozzo et al., 1993; He et al., 2004).

Distribution of waxy protein differs in various countries. Therefore, it is important for a breeding program to screen for the presence of waxy protein in its elite germplasm. According to Yamamori et al. (1994), waxy proteins in bread wheat, were distributed world-wide, the *Wx-A1* null type was frequently observed in Korean, Japanese and Turkish wheats, the *Wx-B1* null type was commonly presented in Indian and Australian wheats, and the *Wx-D1* null type was only observed in a Chinese landrace Baihuomai. Mutations at the waxy loci have also been reported in wheat germplasm from USA, Canada, and Italy (Graybosch, 1998; Demeke et al., 1997; Boggini et al., 2001). Quantitative trait loci (QTLs) for peak viscosity, independent of *Wx* null mutations, have also been reported (Udall et al., 1999). This indicates that, in addition to the major gene, minor genes also contribute to the starch quality in bread wheat.

Around 40% of Chinese wheat is used to make noodles. Autumn-sown wheat, referred to as winter wheat in China, accounts for nearly 90% of all wheat production. Improvement of noodle quality has become a major breeding objectives in most breeding programs. Our previous work has clearly indicated that protein quality, starch viscosity, and color are the three most important factors in determining dry white Chinese noodle quality (Liu et al., 2003; He et al., 2004). The importance of starch parameters to Chinese fresh noodle quality have also been confirmed by other researchers (Yao et al., 2000; Zhang et al., 2005). However, the genetic variation of starch quality in Chinese wheat remains largely unclear. In this study, 260 cultivars and advanced lines form four autumn-sown wheat were used to investigate the presence of waxy protein and characterize starch pasting characteristics. The resulting information may be useful for breeders in China, and elsewhere, who are interested in utilizing this important gene pool.

Materials and methods

Plant material

In total, 260 cultivars and advanced lines collected from the major breeding programs across the autumn-sown wheat regions were used in this study. Additionally, four Australian cultivars (Eradu, Gamenya, Hartog, and Sunstate conferring the *Wx-B1* null mutation) with good Chinese dry white noodle quality (as reported by Liu et al., 2003; and He et al., 2004), were also included. These genotypes represent the major commercial cultivars covering over 85% of the crop acreage, and the most promising advanced lines in the region yield trials. The autumn-sown wheat regions include North China Plain Winter Wheat Region (Zone I), Yellow and Huai Valleys' Facultative Wheat Region (Zone II), Autumn-sown Spring Wheat in the Mid- and Lower Yangtze Valley (Zone III) and Southwestern Autumn-sown Spring Wheat Region (Zone IV). All tested genotypes were sown at Anyang Experimental station of the Chinese Academy of Agricultural Sciences in 2001-02 cropping season according to local management practices. All genotypes grow well in Anyang which is located in Zone II. After harvest, all wheat samples were cleaned before quality testing. Falling Number testing indicated that they were free of sprouting damage.

Quality testing and identification of waxy protein

Grain samples were tempered according to grain hardness, with hard, medium hard (mixed), and soft, to around 16%, 15% and 14% moisture content, respectively. Milling was performed in a Brabender Quadrumat Junior mill using a flour extraction rate of around 60%.

Starch was extracted from flour by Falling Number Glutomatic 2200 and purified as described by Batey et al. (1997). Amylose content was measured according to Kiribuchi-Otobe et al. (1997), and the ratio of amylose to amylopectin was then calculated. Flour pasting properties were measured in 3.5 g flour with Rapid Viscosity Analyzer (RVA) Super 3 (Newport Scientific Pty ltd, Warriewood, Australia) using standard 1 analysis procedure according to AACC method 76-21 (AACC, 1995) and Crosbie et al. (1999). One m*M* AgNO3 was added to inactivate alpha-amylase activity. RVA parameters recorded included peak viscosity (PV), holding strength (HS), breakdown (BD), final viscosity (FV), setback (SB), peak time (PT), and pasting temperature (PST). All tests for each genotype were performed in duplicate.

Waxy proteins were identified according to Zhao and Sharp (1996), three kernels per cultivar were used separately to avoid the mixture of samples.

Statistical Analysis

SAS (Statistics Analysis System, SAS Institute, Cary, NC) was employed to compute mean, range, and coefficients of variation (CV) for tested traits. LSD multiple comparison was used to examine the difference in wheat starch quality for different regions and *t*-test was used to compare the difference between normal and waxy protein null mutation types.

Results

Presence of waxy proteins

As presented in Table 1, among 260 cultivars and advanced lines screened, 39 (15.0%) have a null allele at the *Wx-B1* locus; null alleles at the *Wx-A1* and *Wx-D1* were not observed. However, 42.3% of tested genotypes in Zone IV carried null alleles at *Wx-B1* locus, a much higher percentage than the other three regions. This may be associated with crossing parents used in the breeding programs. Most cultivars and advanced lines in Zone IV were derived from Fan 6, of the *Wx-B1* null type, a leading cultivar from late 1960s to early 1980s. It was replaced due to the breakdown of its yellow rust resistance. It is also interesting to observe that leading cultivars such as Yumai 47, Yangmai 5, Mianayng 20, and Mianyang 26 from 1980s to late 1990s, of the *Wx-B1* null type, also shown outstanding Chinese noodle quality, according to previous reports (Liu et al., 2003; He et al., 2004; Zhang et al., 2005).

Table 1: Distribution of null *Wx-B1* genotypes in different Chinese wheat regions

Region	Genotype Number	Number of null *Wx-B1*	%	Genotypes with null *Wx-B1*
Zone I	69	8	11.6	Fengkang 8, Yuandong 971, Yuandong 8585, Jingnong 8318, Jingdong 6, Jingdong 8, Jinmai 215, Jinmai 218
Zone II	131	15	11.5	Ji 5219, Ji 95-6023, Sanghe 030, Zhongyu 5, 85 Zhong 33, Guanfeng 2, Yumai 47, Lu 94 (6) 006, Lu 9436, Yan 239, Yannong 18, Jining 936898, Shaan 160, Shaan 93302, Xinong 8925-13
Zone III	34	5	14.7	Ning 98084, Yang 96-152, Yang 97-65, Yangmai 5, Yangmai 9
Zone IV	26	11	42.3	Chuanmai 107, Chuan 89-114, Chuan 96003, Chuanmai 24, Mianyang 11, Mianyang 20, Mianyang 26, Mianyang 940112, Mianyang 98-17, Yunmai 42, Y10-8
Total	260	39	15.0	

Zone I = North China Plain Winter Wheat Region, Zone II = Yellow and Huai Valleys' Facultative Wheat Region, Zone III = Autumn-sown Spring Wheat in the Mid- and Lower Yangtze Valley, Zone IV = Southwestern Autumn-sown Spring Wheat Region.

Genetic variation for amylose content and RVA parameters

The starch properties of tested genotypes are presented in Table 2. Although significant variation for amylose content and RVA pasting parameters was presented in Chinese winter wheat germplasm, the coefficients of variation for amylose content and RVA peak time was much lower than other traits. There are 23 cultivars and lines including Yumai 21, Fengyou 6, Zhongyou 5 and Mianyang 26 with amylose content <25.0%, 32 lines including Ning 9548 and Mianyang 9401 with RVA peak viscosity >3000cp, 13 lines including Yumai 47 and Mianyang 26 with RVA breakdown >1300cp.

Table 2: Starch properties of 260 Chinese wheat cultivars and advanced lines

Parameter	Mean	Range	CV (%)
Amylose content (%)	27.6	23.1-33.6	7.3
Peak viscosity (cp)	2543.0	1151-3522	15.9
Holding strength (cp)	1755.0	1385-2374	18.5
Breakdown (cp)	788.3	192-1711	31.3
Final viscosity (cp)	2666.0	849-3456	14.9
Setback (cp)	911.4	464-1192	12.2
Peak time (min)	6.3	5.3-6.7	3.
Pasting temperature (°C)	78.8	64.4-89.0	11.6

Significant difference was observed in wheat genotypes from different regions (Table 3). For example, amylose content in wheats from Southwestern China is significant lower than in wheats of other regions. RVA parameters of wheats from south China (Zone III and IV) are more desirable for noodle quality in comparison with wheats from North China (Zone I and II).

Table 3: Comparison of starch properties among wheat cultivars and lines from different regions

Region	Number	AC (%)	PV (cp)	HS (cp)	BD (cp)	FV (cp)	SB (cp)
Zone I	69	28.7a	2382.4c	1736.3b	646.1c	2633.9b	897.6b
Zone II	131	27.3b	2539.7b	1763.8b	775.8b	2705.1b	941.3a
Zone III	34	27.2b	2827.7a	1894.8a	932.9a	2802.4a	907.6ab
Zone IV	26	26.0c	2617.5b	1578.2c	1039.3a	2380.4c	802.2c

Different letters indicated significantly different at 5% probability level.
AC = amylose content, PV = peak viscosity, HS = holding strength , BD = breakdown, FV = final viscosity, SB = set back, cp = centipoises
Zone I = North China Plain Winter Wheat Region, Zone II = Yellow and Huai Valleys' Facultative Wheat Region, Zone III = Autumn-sown Spring Wheat in the Mid- and Lower Yangtze Valley, Zone IV = Southwestern Autumn-sown Spring Wheat Region.

Effect of Wx-B1 null type on amylose content and RVA parameters

Table 4 indicates that *Wx-B1* null type has a significant effect on amylose content and RVA parameters in comparison with normal wheat. This has confirmed the previous report (Zhao et al.,1998). However, notable variation was also observed among the *Wx-B1* null and normal wheats. For example, among the *Wx-B1* null type, amylose content ranges from 23.6% (Zhongyu 5) to 31.2% (Yuandong 971), and breakdown ranges from 192 cp (Jinnong 215) to 1711 cp (Chuan 96003). Amylose content among normal genotypes ranges from 23.1% (Yumai 21) to 33.6% (Nongda 116), and breakdown varies from 324 cp (Fengkang 2) to 1215cp (Mianyang 98-32). For normal cultivars with high peak viscosity and breakdown, and *Wx-B1* null wheats with low peak viscosity and breakdown, an additional three seeds per entry were used to check the uniformity of genotype and no mixture in waxy protein was found.

Table 4: Comparison of starch properties between normal and *Wx-B1* null wheats

Type	Number	Parameter	AC (%)	PV (cp)	HS (cp)	BD (cp)	FV (cp)	SB (cp)
Normal	223	Mean	27.7a	2516.2a	1754.4a	761.8a	2674.6a	920.3a
		Range	23.1-33.6	1151-3463	1385-2374	324-1215	849-3456	464-1192
Wx-B1 null	39	Mean	26.9b	2707.1b	1759.4a	947.7b	2617.3a	857.9b
		Range	23.6-31.2	1752-3522	1052-2150	192-1711	1678-3165	626-1093

Different letters indicated significantly different at 5% probability level.
AC = amylose content, PV = peak viscosity, HT = holding strength, BD = breakdown, FV = final viscosity, SB = set back, cp= centipoises.

Discussions

Starch viscosity associated with the presence of waxy protein, is an important parameter for improving Chinese noodle quality. Compared to Japanese and Australian wheats, the frequency of *Wx-B1* null genotypes in Chinese wheat is very low (Zhao et al., 1998; H. Yoshida, pers. comm.). The low frequency of *Wx-B1* in Chinese wheat may be partially responsible for poor starch quality. The *Wx-B1* null genotypes reported here could thus serve as a source for improving the starch and noodle quality of Chinese wheat. Three cultivars (i.e., Guanfeng 2, Yumai 47, and Mianyang 26) with outstanding starch parameters in this study, also shown excellent noodle quality, as informed in our previous reports (He et al. 2004; Zhang et al. 2005).The large variation could be due to the fact that this set of germplasm are from more than 50 breeding programs and no selection for quality traits, particularly for starch trait, has been practiced in the past. This may suggest that other minor genes besides the waxy protein gene make important contributions to variation of starch property and that further research is needed to understand their effects on starch quality. Data on both waxy protein and starch properties are needed to characterize germplasm for starch quality in Chinese wheat for noodle products.

In addition to improving gluten quality and flour color, incorporating the *Wx-B1* null type into leading cultivars could help improving starch and Chinese noodle quality. RVA testing is recommended for assessing quality in early generations.

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