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Analysis of the Relationship between *Wx* Gene Polymorphisms and Amylose Content in Hulless Barley

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Abstract

Wu K., Yao X., Yao Y., Chi D., Feng Z. (2017): Analysis of the relationship between *Wx* gene polymorphisms and amylose content in hulless barley. Czech J. Genet. Plant Breed., 53: 144–152.

I_2-KI staining was used to phenotype 151 hull-less barley plants, which determined that five belonged to the waxy phenotype, namely 14-Z152, IG107028, Puebla, Hu Zhu Shuang Cao Ren, and APM-HC1905. Using the dual-wavelength method, the average amylose content of the 151 samples was 25.9%, ranging from 4.9 to 38.5%. The average amylose content of the five waxy varieties was 14.3%, ranging from 4.9 to 18.6%. Genomic DNA from 48 samples showing a significantly variable amylose content was used as template and PCR amplified using the primer pair P4. Statistical analysis indicated that the PCR product size positively correlated with amylose content. The *Wx* gene locus was determined to be polymorphic, and was positively correlated with amylose. Based on the electrophoresis results, the 48 samples were divided into 4 types. PCR product types I, II, III, and IV were 457, 481, 489, and 491 bp in length, respectively, with the respective amylose content ranges of 4.9–27%, 29–30%, 31–35%, and 36–38%. The primer P4 can be used as a complementary marker for the selection of hulles barley germaplasm with different amylose content.

Keywords: *Hordeum vulgare* L. var. *nudum*; molecular marker; starch property; *Waxy* gene

Hordeum vulgare L. var. *nudum* Hook. f. is a domesticated barley that belongs to the grass family and is considered as a major cereal grain. Because its lemma and palea do not adhere to the caryopsis, and the grain is hull-free at maturity, it is often called hulless or naked barley. Naked barley is extensively planted on the Qinghai-Tibet Plateau in China and is essential for the daily life of Tibetans in those regions. This species serves as a typical example of how a plant adapts to extreme environmental conditions. Starch is the main storage component in hulless barley grain, and it mainly exists in the form of starch granules. The properties of starch granules largely affect the processing and application of hulless barley. The starch in hulless barley is unique in its composition in that it contains about 74–78% of amylopectin and 22–26% of amylose.

The granule-bound starch synthase (GBSS) enzyme catalyzes one of the enzymatic steps of starch synthesis. This enzyme, also called the waxy protein, is responsible for the synthesis of amylose and is encoded by the *Waxy* gene (*Wx*). The loss or mutation in the *Wx* gene can lead to reduced GBSS enzymatic activity, and consequently a decrease in amylose content (AC) and an increase in amylopectin levels. Therefore, starch composition, flour quality, processing, and food value are affected (Chao *et al.* 1989; Denyer *et al.* 1995). Wheat and barley con-

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tain at least three GBSS isozymes, namely GBSS I, II, and III (Hylton *et al.* 1996). GBSS I is mainly responsible for the synthesis of amylose in the barley grain endosperm. AC in grain endosperm and pollen is mainly determined by the *Wx* gene (Shure *et al.* 1983). In the wheat endosperm, the loss of any one of the three waxy proteins may lead to a decrease in GBSS enzymatic activity and a reduction in AC (Clark *et al.* 1991). When all three waxy proteins are missing, the resulting barley genotype will produce amylose-free (undetectable) grain starches that are called full waxy wheat. Three waxy proteins affect the AC in wheat at different levels. Of the three, the Wx-B1 subunit has the most significant effect. In the rice *Wx* gene, (CT)n microsatellite markers are apparently associated with AC, gelatinization temperature, gel consistency, starch viscosity profile, and other starch quality traits. These microsatellite markers can be used to facilitate an improvement of starch quality traits in rice (Bao *et al.* 2002). In barley, the *Wx* gene is located on the short arm of chromosome 7H (Kramer & Blander 1961; Tabata 1961; Kleinhofs 1997) and is the key gene controlling AC in endosperm and pollen (Nakao 1950; Ono & Suzuki 1957; Rosichan *et al.* 1979). Domon *et al.* (2002) identified a single-nucleotide difference in the *Wx* gene between the amylose-free and amylose-containing barley endosperm that involves the replacement of the 580th base, T, by C. The T to C replacement is predicted to result in premature protein termination because of the generating of a stop codon, thereby consequently leading to a loss of GBSS I. The difference between near-waxy *Mochizuki D* barley and normal waxy barley involves a 418-bp deletion within the 5'-untranslated region (5'-UTR) of the *Wx* gene (Sun *et al.* 2005).

The *Wx* gene plays an essential role in controlling AC, which is a complex trait in barley (Bollieh & Webb 1973). Barley is a staple in the Tibetan plateau region, and its major raw materials have also been utilized in the wine industry. In terms of the hulless barley, its starch content and composition are one of its most important quality traits. The structure and sequences of *Waxy* genes and its encoded proteins have been reported. Some effective molecular markers have been established to facilitate the screening of barley varieties with different amylose/amylopectin compositions (Domon *et al.* 2004). The Tibetan plateau is a rich germplasm resource for hulless barley, often providing material for the selection of characteristic hulless barley varieties with unique starch quality and composition. However, no simple, fast, and efficient method for variety selection and identification has been established in hulless barley. In the present study, we investigated the relationship between starch content in hulless barley and polymorphisms in the *Wx* gene. The aim of this study was to identify molecular markers for *Wx* gene screening and to provide a genetic basis for the selection and improvement of hulless barley varieties with unique starch traits.

MATERIAL AND METHODS

Materials and *Wx* gene screening method. A population of 151 hulless barley varieties (Table 1) obtained from the field in 2009 was used in the present study. After harvesting, grains were air-dried, ground into flour using a Tornado sample grinding mill, and passed through 0.5-mm mesh sieves. For each sample, 10 ± 0.1 mg of barley grain flour was weighed and incubated in a 0.1% I_2-1% KI solution for staining. The waxy barley endosperm shows a brown-red colour, which is indicative of a very low level or absence of amylose, whereas normal barley shows a black-blue colour.

Measurement of amylose and amylopectin contents. Amylose and amylopectin contents were measured using the dual-wavelength method (Sharp *et al.* 1989). AC was measured using an absorbance of 610 nm (OD_{620}) and a reference wavelength of 490 nm. Amylopectin content was measured using an absorbance of 550 nm (OD_{550}) and a reference wavelength of 682 nm. All the measurements were repeated three times. The amylose and amylopectin standards were purchased from Sigma-Aldrich (St. Louis, USA).

Based on the regression equation obtained from the amylose standard, the amylose concentration of each sample solution was calculated using the equation: $\Delta A_{550} - \Delta A_{682}$. Based on the regression equation obtained from the amylopectin standard, the amylopectin concentration of each sample solution was calculated using the equation: $\Delta A_{550} - \Delta A_{682}$. Then, the contents of amylose and amylopectin in the samples were calculated based on the dilution factor of the samples.

Detection of simple sequence repeat (SSR) markers. The four pairs of primers used in the present study are listed in Table 2. Primer pair P1 was used to amplify the gene fragment encompassing exon 6, intron 6, and exon 7 of the barley *Wx* gene. Primer pair P3 was employed to amplify the gene fragment

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Table 1. List of 151 hulless barleys, major characteristics and origins used in this study

Code	Material	Row	Awn	Shell	Grain colour	1000-grain weight (g)	Origin
1	Kunlun 1	S	L	H	B	61.5	QH China
2	Kunlun 2	M	L	H	B	38.5	QH China
3	Kunlun 3	M	L	H	B	55.5	QH China
4	Nanfan 3	M	L	H	B	47.0	QH China
5	Beiqing 1	M	L	H	B	49.5	QH China
6	Beiqing 2	M	L	H	B	45.0	QH China
7	Beiqing 3	M	L	H	B	42.5	QH China
8	Beiqing 4	M	L	H	B	50.0	QH China
9	Mennong 1	M	L	H	B	39.5	QH China
10	Moduoji 1	M	L	H	B	44.0	QH China
11	Fu 8-4	M	L	H	B	47.5	QH China
12	Zangqingzao 1	M	L	H	B	35.5	QH China
13	Qing74s-20-5-1	S	L	H	B	42.0	QH China
14	77-114-1-2	M	L	H	B	48.0	QH China
15	78-115-8-3-1	M	L	H	B	50.5	QH China
16	76-78-9-1-4	M	L	H	B	39.4	QH China
17	137-2	M	L	H	B	45.5	QH China
18	80S-227	S	L	H	B	39.0	QH China
19	76-73-1	M	L	H	B	47.5	QH China
20	77-114-1-2	M	L	H	B	45.0	QH China
21	87F-021	S	L	H	B	40.0	QH China
22	127-2(Purple)	M	L	H	B	47.0	QH China
23	170	M	L	H	B	43.0	QH China
24	11-57	M	L	H	B	46.5	QH China
25	K5	T	L	H	B	40.8	QH China
26	Tongdexixuan 8	M	L	H	B	41.0	QH China
27	Kunlun 1/79Jian42/Beiqing 1	M	L	H	B	49.0	QH China
28	Kunlun 1/79Jian42/Beiqing 1	S	L	H	B	48.0	QH China
29	Tuppor/Fu 8-4/8026	M	L	H	B	51.5	QH China
30	Tuppor/Fu8-4/8026	S	L	H	B	42.0	QH China
31	89-828/Mennong 1	S	L	H	B	37.0	QH China
32	Kunlun10/Kangqing 3	S	L	H	B	38.0	QH China
33	89-828/Beiqing 2	S	L	H	B	44.0	QH China
34	89-828/Mennong 1	S	L	H	Y	36.0	QH China
35	89-828/Mennong 1	S	L	H	Y	43.5	QH China
36	89-828/Mennong 1	S	L	H	Y	50.5	QH China
37	Tuppor/Fu 8-4/87-5491-19-1-1-8	S	L	H	Y	41.5	QH China
38	125-4	S	L	H	Y	44.5	QH China
39	Q16(1489)	S	L	H	Y	44.0	QH China
40	125-6	S	L	H	B	44.5	QH China
41	Xining 1(Bailiuleng)	M	B	H	Y	42.0	QH China
42	Leduqingke	M	B	H	Y	40.0	QH China
43	Huangzhongliulenghuangqingke	M	L	H	B	36.0	QH China
44	Huzhushuangcaoren	S	B	H	B	41.0	QH China
45	Chahanwusubaiqingke	S	L	H	B	42.5	QH China
46	Dulanliulengqingke	S	L	H	B	45.5	QH China
47	Chakeqingke	M	L	H	B	55.5	QH China
48	Xunhualanqingke	M	L	H	B	47.0	QH China
49	Aiganqi	M	L	H	B	34.0	QH China
50	Xunhuabailiuleng	M	L	H	B	43.0	QH China
51	Xianghuabailiuleng	S	L	H	Y	26.5	QH China
52	Dulihuang	M	L	H	B	45.0	QH China
53	Zangqingke	M	L	H	B	44.5	QH China
54	Menyuanlianglan	M	L	H	B	48.0	QH China

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Table 1 to be continued

Code	Material	Row	Awn	Shell	Grain colour	1000-grain weight (g)	Origin
55	Hainanhuoshaotouluren	M	B	H	B	53.5	QH China
56	Qingguolanqingke	M	L	H	B	39.0	QH China
57	1039Sileng	M	L	H	B	45.0	QH China
58	8026	M	L	H	B	44.0	QH China
59	Huangyuantuqingke	M	B	H	Y	38.5	QH China
60	P110	M	L	H	Y	37.5	QH China
61	P111	M	L	H	Y	42.0	QH China
62	P112	M	L	H	Y	40.5	QH China
63	P113	M	L	H	Y	41.0	QH China
64	P116	M	L	H	Y	43.5	QH China
65	P117	M	L	H	Y	47.5	QH China
66	P118	M	L	H	Y	46.0	QH China
67	P119	M	L	H	Y	42.0	QH China
68	P120	M	L	H	Y	43.5	QH China
69	P121	M	L	H	Y	44.0	QH China
70	P122	M	L	H	Y	37.5	QH China
71	P123	M	L	H	Y	42.0	QH China
72	P124	M	L	H	Y	45.5	QH China
73	P125	M	L	H	Y	44.5	QH China
74	P126	M	L	H	Y	43.0	QH China
75	P127	M	L	H	Y	37.0	QH China
76	P128	M	L	H	Y	41.0	QH China
77	P129	M	L	H	Y	44.5	QH China
78	P131	M	L	H	Y	45.0	QH China
79	P133	M	L	H	Y	45.5	QH China
80	P134	T	L	H	Y	47.0	QH China
81	P135	T	L	H	Y	44.5	QH China
82	h1	M	L	H	Y	51.5	QH China
83	h2	M	L	H	B	51.0	QH China
84	h3	S	L	H	B	48.0	QH China
85	Xueduiqingke	M	L	H	B	40.0	T China
86	Bailangzong	M	L	H	B	47.0	T China
87	Dagelazaqingke	M	L	H	D	48.0	T China
88	861918	M	L	H	D	29.0	T China
89	861383	M	L	H	D	43.0	T China
90	861393	M	L	H	D	29.5	T China
91	Baidiqingke	M	L	H	Y	32.5	T China
92	Changduqingke	M	L	H	D	35.0	T China
93	Tewu	S	L	H	B	35.5	T China
94	Mali	M	L	H	Y	34.0	T China
95	Shuobanduo	S	L	H	D	37.0	T China
96	Dabaqingke	S	L	H	Y	32.0	T China
97	Yuechiheidamai	M	L	S	D	33.5	SC China
98	Mianyang 87-10	S	L	S	Y	41.0	SC China
99	Jiachuanliuleng	M	L	H	B	43.5	SC China
100	83-5319 (Kangding)	M	L	H	B	41.0	SC China
101	Duanmangdaogouliulengqingke	S	B	H	B	44.5	YH China
102	Liulengbaiqingke	S	L	H	B	46.5	YH China
103	Shuangpeiqingke	M	L	H	B	43.0	YH China
104	Guangtoulaomai	M	L	S	Y	29.0	GZ China
105	Guangtoumai	M	L	S	Y	48.0	GZ China
106	Guangtoudamai	M	L	H	B	51.0	GZ China
107	Laomai	M	L	H	Y	28.0	GZ China
108	Liulengzi	M	L	H	B	33.5	HB China

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Table 1 to be continued

Code	Material	Row	Awn	Shell	Grain colour	1000-grain weight (g)	Origin
109	Sunong 370	S	L	S	W	26.0	JS China
110	ICNBF8-582SEL.6AP	M	L	H	W	42.5	Mexico
111	ICNBF8-588SEL.1AP	M	L	H	Y	38.5	Mexico
112	ICNBF8-593SEL.3AP	M	L	H	Y	38.5	Mexico
113	ICNBF8-594SEL.2AP	M	L	H	Y	30.5	Mexico
114	ICNBF8-596SEL.3AP	M	L	H	Y	40.0	Mexico
115	ICNBF8-597SEL.1AP	M	L	H	Y	38.5	Mexico
116	ICNBF8-606SEL.5AP	M	L	H	Y	32.5	Mexico
117	ICNBF8-607SEL.5AP	M	L	H	W	37.0	Mexico
118	ViringasCMB86-0767-B-1Y-173GH-4GH-1M-OY	M	L	S	Y	39.5	Mexico
119	ViringasCMB86-0767-C-3Y-190GH-10GH-3M-OY	M	L	H	Y	42.5	Mexico
120	Hallej-513201-311AsseLJLBOB89sel.1UH-OUH	M	L	H	Y	40.5	Mexico
121	ICNBF8-609 SEL 2AP	M	L	H	B	41.0	Mexico
122	IG107028	M	L	S	Y	47.5	Mexico
123	Puebla	M	L	S	Y	43.0	Mexico
124	AMAPA „S“PC191	M	L	H	B	36.5	Mexico
125	APM-HC1905	M	L	H	B	37.0	Mexico
126	CEL-5106	M	L	H	B	51.0	Mexico
127	IB65-M66.85	M	L	H	B	37.5	Mexico
128	IC18867-M66.85	M	L	H	B	33.0	Mexico
129	JNA CBC-204	M	L	H	B	31.0	Mexico
130	JNA CBC-205	M	L	H	B	30.5	Mexico
131	SAH	M	L	H	B	31.0	Mexico
132	Ahor 443170	M	L	H	B	32.0	Mexico
133	Ahor 2194170	M	B	H	B	29.0	Mexico
134	Bang-Iu	M	L	H	B	30.0	Mexico
135	CEIBA“S“	M	L	H	B	32.0	Mexico
136	M66.85-CI12168	M	L	H	Y	33.0	Mexico
137	MANKER-OREGANO	M	L	H	Y	41.5	Mexico
138	Nepel.C.I.595	M	B	H	Y	36.5	Mexico
139	ORE“S“CBC-177	M	L	S	Y	30.0	Mexico
140	SC-31	M	L	H	B	48.5	Mexico
141	*H. vulare* ssp. *vulgare* var. *trifurcatum*	M	B	H	B	34.0	Mexico
142	4943-2398/46-Tokuji	M	L	H	B	32.0	Mexico
143	639	M	B	H	B	39.5	Canada
144	Brachvtic	M	L	H	B	21.5	Canada
145	C.I.1037 (Yonehadaka)	M	L	H	B	27.5	Canada
146	H.Vulg.L.trif	M	B	H	Y	28.5	Denmark
147	Nakate	M	L	H	Y	28.5	Denmark
148	Shikoku-Hadokaul	M	L	H	B	32.0	Denmark
149	*H. vulare* ssp. *spontaneum* var. *hongguoense*	S	B	H	B	25.5	Denmark
150	Arumir	M	L	H	B	37.0	Austria
151	14-Z152	T	L	H	Y	42.3	Austria

T – two-row; S – six-row; M – medium type; L – long awn; B – blend awn; H – barley without shell; S – barley with shell; B – brown grain; Y – yellow grain; W – white grain; D – black grain; QH China – Qinghai, China; T China – Tibet, China; SC China – Sichuan, China; YN China – Yunnan, China; GZ China – Guizhou, China; HB China – Hubei, China; JS China – Jiangsu China

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Table 2. Primer sequences and detected loci

Primer	Type	Primer sequence (5'–3')	Target locus	Reference
P1	STS	F: CTggCCTgCTACCTCAAgAgCAACT R: CTgACgTCCATgCCgTTgACgA	*Wx-B1*	Zhu and Zhang (2010)
P2	STS	F: CTggCCTgCTACCTCAAgAgCAACT R: CTgTTTCACCATgATCgCTCCCCTT	*Wx-D1*	Vrinten *et al.* (1999)
P3	STS	F: TCgTgTTCgTCggCgCCgAgATgg R: CCgCgCTTgTAgCAgTggAAgTACC	*Wx-A1*	Zhu and Zhang (2010)
P4	SSR	F: AgTATCgCAgACgCTCAC R: gTTATgTACTCgCTCgCTC	*HVWaxy4*	http://www.ukcrop.net

comprising exon 2, intron 2, exon 3, intron 3, and part of exon 4 of the barley *Wx* gene (Zhu & Zhang 2010). Primer pair P1 also amplified three gene fragments with corresponding sizes of 455, 425 and 497 bp of the *Wx* gene of wild-type wheat. Primer pair P3 also amplified three gene fragments with corresponding sizes of 389, 410, and 408 bp of the *Wx* gene in wild-type wheat (Nakamura *et al.* 1993). Primer pair P2 amplified a 2,300-bp specific fragment of the wheat genome (Vrinten *et al.* 1999). P4 primer pair was designed to amplify the *Wx* gene, which is located on barley chromosome 7H.

Three to five leaves from seedlings cultured in water were collected. Genomic DNA was extracted using the SDS universal protocol (Wang *et al.* 2007).

The volume of each PCR reaction was 20 µl, which contained 2.0 µl of a 10× buffer (20 mmol/l Mg^{2+}), 0.5 µl of 10 mmol/l of each dNTP, 0.5 µl of *Taq* DNA polymerase (final concentration 2 U), 2.5 µl of 2.0 µmol/l primers, 2.0 µl of 30 ng/µl DNA template, and 12.5 µl of ddH_2O. The reaction mixture was first denatured at 95°C for 5 min. The thermal cycles were as follows: 94°C for 1 min; 58°C (P1, P2, and P3)/66°C (P4) for 1 min; 72°C for 1 min of 36 cycles; and a final extension at 72°C for 10 min. The reaction mixture was cooled down at 4°C for at least 1 min and then kept at 4°C. The PCR products were analysed on a 6% denaturing polyacrylamide gel and visualized using silver staining. Six microliters of the PCR product were loaded after 20–30 min of pre- electrophoresis. The electrophoresis was run at a constant power of 80 W.

Fifty-one samples with different ACs were analysed by electrophoresis. The samples were arranged from left to right according to increasing AC. After electrophoresis, the SSR amplification products showing the same mobility were grouped and designated as Types I, II, III and IV, respectively. All the tested samples were grouped based on band mobility. The relationship between mobility and AC was then analysed.

RESULTS

Among the 151 samples tested, 5 of the samples (14-Z152, IG107028, Puebla, Hu Zhu Shuang Cao Ren, and APM-HC1905) showed a brown-red colour after I_2-KI staining, thereby indicating that these were waxy varieties, accounting for 3.3% of all the samples. The other 146 samples showed a black-blue colour after I_2-KI staining, indicating that these were non-waxy normal varieties, accounting for 96.7% of all the samples.

The average total starch content of the 151 samples was 60.6%, ranging from 43.4% to 73.5%. The average AC was 25.9%, ranging from 4.9% to 38.5%. The AC of the five waxy varieties was 4.9%, 12.4%, 17.3%, 18.4%, and 18.6%, respectively; with an average of

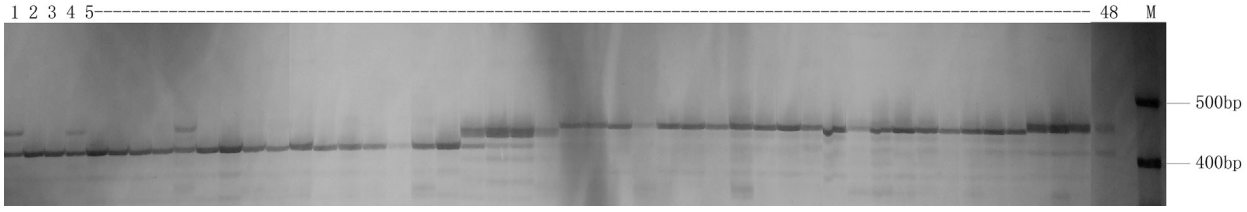

Figure 1. PCR products using primer P4 in 48 tested hulless barleys
1–48 – DNA products from templates of the 48 genotypes tested, which were coded as given in Table 3; M – marker

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Table 3. Amylose contents and amplified banding types using primer P4 in 48 tested hulless barleys

Code	Amylose content (%)	Banding type	Code	Amylose content (%)	Banding type	Code	Amylose content (%)	Banding type
122	12.4	I	104	25.0	I	113	32.6	III
144	17.3	I	89	26.7	I	93	32.7	III
2	18.4	I	95	27.7	I	24	32.9	III
44	18.6	I	88	29.5	I	18	33.1	III
123	20.0	I	146	30.1	II	127	33.1	III
133	20.6	I	94	30.1	II	139	33.3	III
141	21.0	I	87	30.2	II	147	33.5	III
142	21.2	I	105	30.9	II	150	34.2	III
131	21.6	I	149	31.1	III	38	34.2	III
148	21.7	I	56	31.4	III	85	34.3	III
124	21.8	I	135	31.7	III	11	34.8	III
125	22.1	I	136	31.8	III	6	36.5	III
96	22.6	I	130	32.2	III	40	36.6	IV
41	22.8	I	98	32.3	III	19	36.9	IV
53	23.9	I	92	32.3	III	46	37.0	IV
97	24.5	I	126	32.5	III	13	37.4	IV

Codes of materials correspond with the codes for material names given in Table 1; fragment sizes are 457 bp for type I, 481 bp for type II, 489 bp for type III, and 491 bp for type IV

14.3%. AC significantly differed between the waxy and non-waxy varieties, which was consistent with the results of I_2-KI staining. Among the five waxy varieties, only one was amylose-free, whereas the rest were low-amylose near-waxy varieties.

Based on AC, the 151 samples were divided into three types. Type I produced low AC (< 20%), Type II normal (20% ≤ AC < 30%), and Type III increased AC (≥ 30%) in the form of amylose grain starches. The AC range in Types I, II, and III was 4.9–18.6%, 20.0–29.9%, and 30.1–38.5%, respectively. The number/percentage of Types I, II, and III were 5/3.3, 108/71.5, and 38/25.2%, respectively.

Of the 151 hulless barley samples, 48 samples with ACs that were ranked from low to high were selected for further analysis. These 48 samples included all varieties in Type I (except for 14-Z152) and Type III, as well as samples from Type II, at 0.5% increments in AC. The PCR analysis was then performed using these 48 samples and four pairs of primers. The results showed that primer pairs P1 and P3 amplified 900 bp and 400 bp fragments, respectively. However, no polymorphisms were observed in any sample. Primer pair P2 failed to amplify any DNA fragment. PCR amplification using primer pair P4 was successful using all 48 samples, and the length of the PCR products increased with higher ACs (Figure 1 and Table 3). These findings suggest that the length of the PCR product generated by using primer pair P4 was positively associated with AC in hulless barley samples.

To validate the relationship between primer pair P4 PCR products and the AC, seven samples with comparable ACs (ranging from 21 to 24.5%) were used as templates for PCR amplification. Figure 2 shows that the sizes of PCR products were similar. In contrast, when three samples with significantly different ACs (23.8% DuLiHuang, 17.3% Brachvtic, and 4.9%

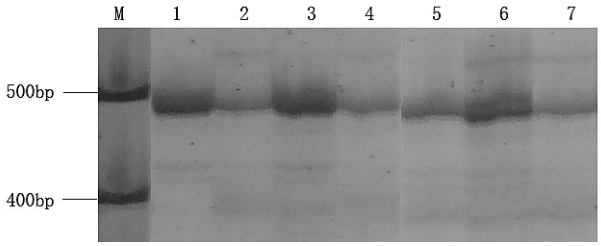

Figure 2. PCR products using primer P4 in seven tested hulless barleys with similar amylose content
M – marker; 1 – Yuechiheidamai; 2 – AMPA"S"PC191; 3 – APM-HC1905; 4 – 4943-2398/46-Tokuji; 5 – 639; 6 – Dulihuang; 7 – *H. vulgare* ssp. *vulgare* var. *trifurcatum*

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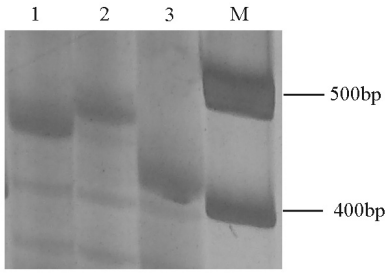

Figure 3. PCR products using primer P4 in three tested hulless barleys with different amylose contents
1 – Brachvtic; 2 – Dulihuang; 3 – 14-Z152; M – marker

14-Z152) were used as templates, a positive correlation between the size of the PCR products generated using primer pair P4 and AC was observed (Figure 3).

DISCUSSION

AC is an important quality trait of barley and an important parameter for the evaluation of waxy barley varieties. However, during the development of waxy barley varieties, it is critical to screen and identify the germplasm of the newly cultivated varieties. It was the wide scope on AC what had a different application in food processing. Baik and Steven (2008) reported that food products containing or prepared from barley grain or flour with different amylose contents exhibit a wide variation in processing properties and product qualities. They also reported that hardness or bite and chewiness of white salted noodles containing waxy and zero amylose barley flours were reduced, but there was no changes in noodles containing normal amylose barley. In the present study, we identified five waxy hulless barley varieties, which accounted for 3.3% of the total samples. The AC of these five varieties was all < 20%, which was significantly different from that of other varieties. The AC of the 151 hulless barley samples ranged from 4.9 to 38.5%, indicating a great potential to screen low-AC varieties in different hulless barley germplasms. In all the samples tested, only one showed an amylose-free phenotype, which indicated that it was a homozygous waxy variety. Most of the samples showed ACs of > 10%, which was consistent with the findings of Zhu and Zhang (2010). These findings suggest that most of the hulless barley varieties are low-amylose near-waxy varieties. To obtain the amylose-free waxy variety, wider-range screening is necessary, which may even include measuring ACs in single hulless barley plants.

Specific genes are known to be involved in regulating amylose biosynthesis, although traditional crossings apparently indicate that other genetic factors and environmental factors also influence this particular biochemical activity (Juliano & Pascual 1980; McKenzie & Rutger 1983). It has been difficult to effectively select varieties with desirable amylose traits using traditional methods. However, the use of molecular marker-assisted selection has now overcome the shortcomings of conventional crosses by avoiding the interferences from the environment, and thereby facilitating in the direct selection of the desirable phenotype based on the established genotype. This technique has significantly improved the efficiency and accuracy of producing plants with altered traits.

In the present study, one amylose-free hulless barley, which can be used as the parent plant for trait improvement, was identified from 151 hulless barley plants. Four molecular markers were used in the analysis. Primer pairs P1, P2, and P3 successfully amplified STS markers within wheat *Wx-B1*, *Wx-D1*, and *Wx-A1* gene loci. These primers were based on the characteristic mutation involving the *Wx-B1*, *Wx-D1*, and *Wx-A1* genes in wheat (Briney *et al.* 1998). The analysis of 48 samples with significantly different AC did not detect any polymorphisms in the P1 and P3 PCR products; in addition, the lengths of the PCR products were not associated with AC. Furthermore, no PCR product was amplified using primer pair P2. These results suggest that no mutant alleles exist in the *Wx* gene of hulless barley and its homologous 4H chromosome loci in wheat. These findings also suggest that none of the tested samples harboured the deletion mutation involving the *Wx* gene. This phenomenon may be attributable to the fact that *Wx* genes in wheat and hulless barley are not exactly the same. Primer pair P4 was designed based on the *Wx* gene on chromosome 4H loci in barley. P4 PCR products generated four types of bands in the 48 tested samples, and the lengths of the PCR products were positively correlated with AC. These results demonstrated that the *Wx* molecular marker in wheat may not be suitable to hulless barley. However, the molecular markers of barley effectively differentiate the AC in hulless barley, and therefore can be used in hulless barley germplasm selection.

CONCLUSIONS

Our study demonstrated that SSR markers amplified by primer pair P4 from the *Wx* gene were positively correlated with AC in hulless barley. However, these mechanisms require further investigations. Wang *et al.* (2005) described polymorphisms involving P3-amplified products in wheat, as well as the posi-

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tive correlation between the length of the P3 PCR products and AC. However, this phenomenon was not observed in our study. In future studies, more germplasms should to be included in the screening to reflect different amylose/amylopectin contents. In addition, multiple identifications at different field sites and years are necessary to further elucidate the genetic mechanism controlling starch content. Meanwhile, only a few useful *Wx* molecular markers have been established and it would be desirable to identify and validate additional molecular markers to differentiate various hulless barley germplasms.

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