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Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure

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Abstract

Starch was extracted from 10 hull-less barley (HB) genotypes [waxy (CDC Candle, CDC Alamo, SB 94912, and SB 94917), normal amylose (Phoenix, CDC Dawn, SR 93102, and SB 94860), and high amylose (SB 94893 and SB 94897)]. Starch content ranged from 56 to 65%. The purity of the isolated starches was greater than 96%. Average starch yield and extraction efficiency were 44 and 71%, respectively. The starches from all genotypes consisted of a mixture of large lenticular and small irregularly shaped granules. The granules of most starches were intact, whereas in others (SB 94917, SR 93102, and SB 94860) they were compound (clustered). The proportions of small (diameter $\leqslant 10$ μm) and large granules (diameter > 10 μm), by total number and by total weight differed among genotypes. Bound lipid content was positively correlated ($r = 0.92$, $P < 0.01$) with total amylose content. Free and bound lipid contents ranged from 0.1–0.3% and 0.3–1.7%, respectively. The apparent and total amylose contents ranged from 0–39% and 0–45%, respectively. The amounts of amylose complexed with native lipids (total amylose–apparent amylose) ranged from 0.5 to 7.8%. The proportion of small granules was correlated with total amylose content ($r = 0.59$, $P < 0.1$). However, the average granule diameter was negatively correlated ($r = -0.65$, $P < 0.05$) with total amylose content. The debranched amylopectins of all starches exhibited the highest peak in the MALDI-MS spectrum at DP 12. The average chain length (CL) and degree of branching ranged from 17.6–22.7% and 4.4–5.5%, respectively. The short (DP 5-17) and long (DP $\geqslant 35$) chains ranged from 58.2–59.1% and 3.0–12.8%, respectively. The study showed that amylose/amylopectin ratio and amylopectin branch chain length have high correlation with granule size and size distribution in this set of barley genotypes.

Keywords: Hull-less barley; Starch; Granule; Morphology; Amylopectin; Structure

1. Introduction

Barley is a grass belonging to the family Poaceae, the tribe Triticeae, and the genus Hordeum (Nilan & Ullrich, 1993). Cultivated barley (*Hordeum vulgare*) is the fourth largest cereal grain crop produced worldwide and the most under utilized cereal grain in terms of human consumption (Bhatty, 1993). About 90% of barley grain is used in alcoholic beverage production and as a livestock feed. Barley is an excellent source of complex carbohydrates, which constitute about 80% of barley grain weight (Szczodrak, Czuchajowska, & Pomeranz, 1992). Starch is the largest single component in barley grain, representing up to 65% of kernel dry weight. Hull-less or naked barley (HB) production in Canada is estimated to be between 300,000 and 350,000 ha with an estimated grain production of about 800,000 t in 1998 (Bhatty, 1999). Canada is the leading producer and the major source of published information on HB. Several two and six-rowed cultivars of HB have been registered in Canada in the last 15 years, including waxy and zero amylose types (Bhatty, 1995; Bhatty & Rossnagel, 1997). HB cultivars were developed, first for use in swine and poultry feeds and, later, for use in human foods as a source of dietary fibre. HB has been used for preparation of food malt, production of ethanol, extraction and enrichment of β-glucan, preparation of native and modified starches and preparation of bran and flour for use in bakery products (Bhatty, 1995, 1999). In Canada, the emphasis now is to extend the use of HB in food and industry, including the malting and brewing industries (Bhatty, 1999). HB contains 60–75% starch (Bhatty, 1997). Amylose content in HB starches varies from 0 to 40% (Zheng, Han, & Bhatty, 1998).

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Granule size varies from large lenticular to small spherical (Vasanthan & Bhatty, 1995). HB genotypes [normal (20–30% amylose), waxy (1–5% amylose), zero amylose and high amylose (30–45%)] have been developed via traditional breeding practices. Recently, several new HB genotypes have been developed at the Crop Development Center, University of Saskatchewan. A preliminary study showed that starch granules from these new genotypes of HB differed widely in composition, granule morphology, and physicochemical characteristics. Therefore, a study of the structure–property relationships of these starches is needed to understand their functionality in food systems. With this information and available genetic variation it should be possible to produce HB starches which are designed for specific uses in the food industry.

This study reports the granule morphology, composition, and amylopectin structure of starch from 10 genotypes of HB grown at Saskatoon, Saskatchewan in 1998. Also, the intent of this study was to compare the aforementioned barley starch properties with those of commercial maize starches. This is because maize starches are currently used in North America in a number of food and industrial applications. An accompanying paper describes the structure–property relationships for these HB starches.

2. Materials and methods

2.1. Materials

Four registered cultivars (CDC Alamo, CDC Candle, CDC Dawn, and Phoenix) and six breeding lines (SB 94912, SB 94917, SR 93102, SB 94860, SB 94893, and SB 94897) of HB grown and harvested at Saskatoon in 1998 were obtained from the Crop Development Center, University of Saskatchewan, Saskatoon, Canada. The barley grains were ground in a UDY cyclone sample mill equipped with a 0.5-mm screen. Commercial waxy and regular maize starches (A.E. Staley Manufacturing Company, Decatur IL) were used for comparison. Isoamylase (EC 3.2.1.68, debranching enzyme) and maltoheptaose (DP7, internal standard) were obtained from Sigma Chem. Co. (St. Louis, MO); Seppak C18 cartridges from Waters Corp. (Milford, MA). Macro-sep centrifuge concentrators (30 K) from Filtron Tech Corp. (Northborough, MA); Sephadex G-10 (desalting columns) from Amersham Pharmacia Biotech AB, (Uppsala, Sweden); and 2,5-dihyroxybenzoic acid (matrix) from Aldroich Chemical Co. (Milwaukee, WI).

2.2. Starch isolation

Starch was isolated from ground barley grains according to the method of Wu, Sexson, and Sanderson (1979). The small granule starch in the protein/fiber layer (the brown layer on the top of the white starch layer in the centrifuge bottle) was recovered by washing and gravity settling and added back to the main stock starch.

2.3. Chemical composition of starch

Quantitative estimation of moisture, ash and nitrogen were performed by standard AACC (1983) procedures. Lipids were determined by procedures outlined in an earlier publication (Vasanthan & Hoover, 1992). Apparent and total amylose contents were determined by the method of Chrastil (1987). Starch content was determined using a total starch Megazyme assay kit (Megazyme International Ireland Limited, Wicklow, Ireland). Quantification of β-glucan was performed using an assay kit from Megazyme.

2.4. Granule morphology

Granule morphology of native starches was studied by scanning electron microscopy. Starch samples were mounted on circular aluminium stubs with double-sided sticky tape and then coated with 12 nm gold and examined and photographed in a JEOL (JSM 6301FXV) scanning electron microscope (JEOL, LTD, Tokyo, Japan) at an accelerating voltage of 5 kV.

2.5. Granule size analysis

Granule size distribution was evaluated by a BioQuant system IV image analyzer (BioQuant R & M, Biometrics, Inc. Nashville, TN, USA), equipped with an image acquisition and computer processor. Five hundred granules were observed for each sample. Granule size was expressed in terms of the diameter of image surface. The weight of starch granules was derived from the volume of granules. The volume of granules was calculated assuming spherical particles.

2.6. Molecular characterization of amylopectin

Debranching of starches and molecular characterization of debranched amylopectins, using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), was carried out according to the method of Wang, Jiang, Vasanthan, and Sporns (1999).

2.7. Statistical analysis

All measurements are means of two independent samples analyzed by the Statistical Analysis System for Window Version8 (SAS Institute, Cary, NC). Significance levels used were at $P<0.1$, $P<0.05$, and $P<0.01$.

3. Results and discussion

3.1. Chemical composition of barley grains

The chemical compositions of 10 genotypes of HB grains are presented in Table 1. Starch, β-glucan, protein, lipids, and ash contents were in the ranges of 56.0–64.7, 3.7–7.7, 11.5–14.2, 4.7–6.8, and 1.8–2.4%, respectively. These values are within the ranges reported for registered Canadian (Bhatty & Rossnagel, 1998) and USA (Czuchajowska, Szczodrak, & Pomeranz, 1992; Oscarsson, Parkkonen, Autio, & Aman, 1997) barley cultivars. Starch content was negatively correlated with protein content ($r=-0.84$, $P<0.01$). The β-glucan content of normal starch HB grains was 3.65 and 4.44%, respectively, in CDC Dawn and Phoenix. However, in all other genotypes, the β-glucan content was higher than 5.9%. β-glucan content showed no correlation with amylose content. Bhatty (1999) reported that the β-glucan content in 10 different Canadian HB cultivars was positively correlated with total dietary fibre ($r=0.81$, $P<0.01$) and soluble fibre ($r=0.86$, $P<0.01$). Other studies have shown that the β-glucan content of barley is influenced by cultivar and growth environment (Lee, Horsley, Manthey, & Schwarz, 1997; Perez-Vendrell, Brufau, Molinocano, Francesh, & Guasch, 1996). The ash contents of the high amylose HB genotypes (SB 94893 and SB 94897) were higher than those of the other genotypes (Table 1). The HB genotypes showed a broad range of crude protein levels (11.5–14.2%). High levels of protein are due to a concentration effect caused by the lack of hulls and/or to the result of breeding for increased protein content in feed barley (Edney, Tkachuk, & MacGregor, 1992). The genotypes used in this study were grown at the same location in Saskatchewan, therefore, the observed variation in protein content (Table 1) cannot be attributed to soil type, climatic conditions or applied nitrogen fertilizer. Lipids contents of HB were in the ranges of 4.7–6.8% and in the order of waxy > normal > high amylose starch types.

3.2. Isolation and chemical composition of HB starches

Average yield and extraction efficiency of isolated starches were 44.4% of the total grain weight (dry basis) and 70.9%, respectively. Waxy HB genotypes with high β-glucan content (6.4–7.4%) gave lower starch yields (42.1%) and extraction efficiencies (68.0%). The low values for protein (N×6.25, 0.2–0.4%) and ash (0.06–0.4%) contents showed that the starches were of high purity. Isolated starches contained 95–98% starch. β-glucan levels were very low (0.01–0.06%; Table 2). Free lipids [obtained by extraction with $CHCl_3$-CH_3OH (CM)] ranged from 0.1 to 0.3%. However, variations in bound lipid content [obtained by extraction of CM residues with hot n-propanol–water (PW)] were higher (0.3–1.7%). Bound lipid content was positively correlated ($r=0.92$, $P<0.01$) with total amylose content. It is plausible, that both free and bound lipids may be present on the granule surface, as well as within the granule interior. The bound lipids probably represent: (1) lipids that are present in the form of V-amylose–lipid inclusion complexes (Morrison, 1981); and (2) lipids that are trapped between starch chains (Morrison, 1981).

The apparent and total amylose contents of HB starches ranged from 0–39% and 0–45%, respectively (Table 2) and the amounts of amylose complexed with native lipids (total amylose–apparent amylose) ranged from 0.5 to 7.8%.

Table 1
Chemical composition of hull-less barley grain

Line analyzed	Starch type[a]	Starch (%db)	β-glucan (%db)	Protein (%db)	Lipids (%db)	Ash (%db)	Other[b] (%db)
CDC Alamo	Zero amylose waxy	58.47	7.27	13.8	6.57	1.94	9.87
CDC Candle	Waxy	64.73	6.38	11.8	6.13	1.86	6.77
SB 94912	Waxy	59.66	7.14	13.0	6.06	1.77	10.3
SB 94917	Waxy (Compound granules)[c]	58.19	7.41	12.9	6.81	2.10	10.7
SR 93102	Normal amylose (Compound granules)[c]	59.87	5.94	12.8	5.72	1.78	11.9
SB 94860	Normal amylose (Compound granules)[c]	61.02	6.25	13.2	5.34	1.90	10.3
Phoenix	Normal amylose	63.73	4.44	12.6	5.60	1.82	9.40
CDC Dawn	Normal amylose	64.61	3.65	11.5	5.19	1.78	10.6
SB 94893	High amylose	55.95	7.04	14.2	4.81	2.36	13.3
SB 94897	High amylose	56.58	7.67	14.0	4.69	2.33	12.6
LSD[d]		1.30	0.45	0.23	0.32	0.18	

[a] Based on amylose content.
[b] Mainly dietary fiber components, including pentosans, cellulose, lignin, uronic acid, and low molecular weight carbohydrates (Bhatty, 1997).
[c] Some of the starch granules appeared to be a cluster of a few irregular compound starch granules under scanning electron microscopy (Figs. 1 and 3).
[d] Least significant difference at $P<0.05$.

3.3. Granule morphology

HB starch granules consist mostly of a mixture of large lenticular granules and smaller, irregularly shaped granules (Fig. 1). Maize starch granules are angular and more uniform in size with polyhedral faces (Fig. 1). "Pin holes" and equatorial grooves or furrows were present on large granules of HB and maize starches (Fig. 2). Starches of SB 94917, SR 93102 and SB 94860 consisted of small irregularly shaped granules (Fig. 1), large oval shaped granules (Figs. 1 and 2), and very large "dumbbell" shaped and compound granules (clusters of a few granules which looked like single granules; Fig. 3). Compound granules were more predominant in SB 94917 and SR 93102 than in SB 94860 (Fig. 1).

3.4. Granule size distribution

Granule size distributions of the starches are presented in Fig. 4 and size variations of small ($\leqslant 10$ μm) and large (>10 μm) starch granules are presented in Table 3. HB starches showed a bimodal size distribution

Table 2
Chemical composition of hull-less barley and maize starches[a]

Line analyzed	Starch (%)	Amylose (%)[b]		Lipid (%)		Protein (%)	Ash (%)	β-glucan (%)
		Apparent	Total	CM[c]	PW[d]			
CDC Alamo	97.80	0	0	0.22	0.34	0.26	0.06	0.06
CDC Candle	96.88	3.81	4.31	0.18	0.75	0.25	0.19	0.06
SB 94912	98.08	4.42	6.44	0.14	0.65	0.26	0.31	0.02
SB 94917	97.57	4.03	4.60	0.15	0.48	0.38	0.24	0.04
SR 93102	96.94	24.6	27.6	0.16	1.11	0.42	0.30	0.04
SB 94860	96.09	23.0	29.0	0.20	1.32	0.43	0.31	0.06
Phoenix	96.50	23.8	25.8	0.18	0.90	0.30	0.20	0.03
CDC Dawn	96.67	22.5	23.6	0.11	0.80	0.36	0.20	0.03
SB 94893	96.63	38.6	44.5	0.17	1.69	0.43	0.42	0.01
SB 94897	96.48	33.9	41.7	0.12	1.22	0.41	0.36	0.02
Waxy maize	97.07	0	0.62	0.23	0.27	0.21	0.09	–
Normal maize	98.21	21.6	24.5	0.29	0.93	0.33	0.07	–
LSD[e]	1.13	0.64	1.11	0.05	0.09	0.03	0.06	0.02

[a] All data reported on dry basis and represent the mean of two determinations.
[b] Apparent and total amylose determined by I_2 binding before and after removal of bound lipids by hot 1-propanaol–water 3:1 v/v.
[c] Lipids extracted from native starch by chloroform–methanol (CM) 2:1 v/v at 25 °C (mainly free lipids).
[d] Lipids extracted by hot 1-propanol–water 3:1 (v/v) from the residue left after CM extraction (mainly bound lipids).
[e] Least significant difference at $P<0.05$.

Table 3
Granule size and size distribution of hull-less barley and maize starches

Line analyzed	Average diameter (μm)			Number (%)		Weight (%)	
	Mean	Small[a]	Large	Small	Large	Small	Large
CDC Alamo	9.8	4.5	15.6	52.1	47.9	4.6	95.4
CDC Candle	9.1	4.0	15.4	55.9	44.1	4.5	95.5
SB 94912	8.8	5.0	16.2	66.3	33.7	7.9	92.1
SB 94917	6.4	6.0	12.2	92.9	7.1	66.9	33.1
SR 93102	6.9	5.9	12.7	84.8	15.2	41.1	58.9
SB 94860	8.4	6.5	13.5	71.7	28.3	24.7	75.3
Phoenix	7.1	4.2	18.8	80.6	19.5	7.8	92.2
CDC Dawn	6.7	4.2	15.6	77.3	22.7	11.0	89.0
SB 94893	6.8	5.5	12.6	82.8	17.2	37.8	62.2
SB 94897	6.2	5.2	13.2	87.5	12.5	39.3	60.7
Waxy maize	10.6	6.3	13.9	46.8	53.2	9.9	90.1
Normal maize	10.3	7.7	13.2	46.7	53.3	16.0	84.0

[a] Granule diameter $\leqslant 10$ μm.

in the diameter range of 2–30 μm, whereas the maize starches showed a relatively narrow and uniform size distribution. The average granule size of HB starches (6.2–9.8 μm) was smaller than that for the maize (10.3–10.6 μm; Table 3) and wheat (Raeker, Gaines, Finney, & Donelson, 1998; Stoddard, 1999) starches. The proportions of small and large granules, by total number and by total weight, differed among genotypes. In compound waxy and compound normal HB starches (SB 94917, SB 94860, and SR 93102), small granules of SB 94917 (waxy) constituted the largest proportion of the total number of starch granules (93%) and total weight (67%). The proportion of small granules by weight in compound normal (SB 94860 and SR 93102), high amylose (SB 94893 and SB 94897) and normal (Phoenix and CDC Dawn) HB starches were 25–41, 38–39 and 8–11%, respectively. Large granules from waxy (CDC Alamo, CDC Candle, and SB 94912) and normal (Phoenix and CDC Dawn) HB starches constituted 34–48 and 20–23% of the total number of starch granules, respectively, and made up 89–96% of the total starch weight. Small granules from high amylose HB starches

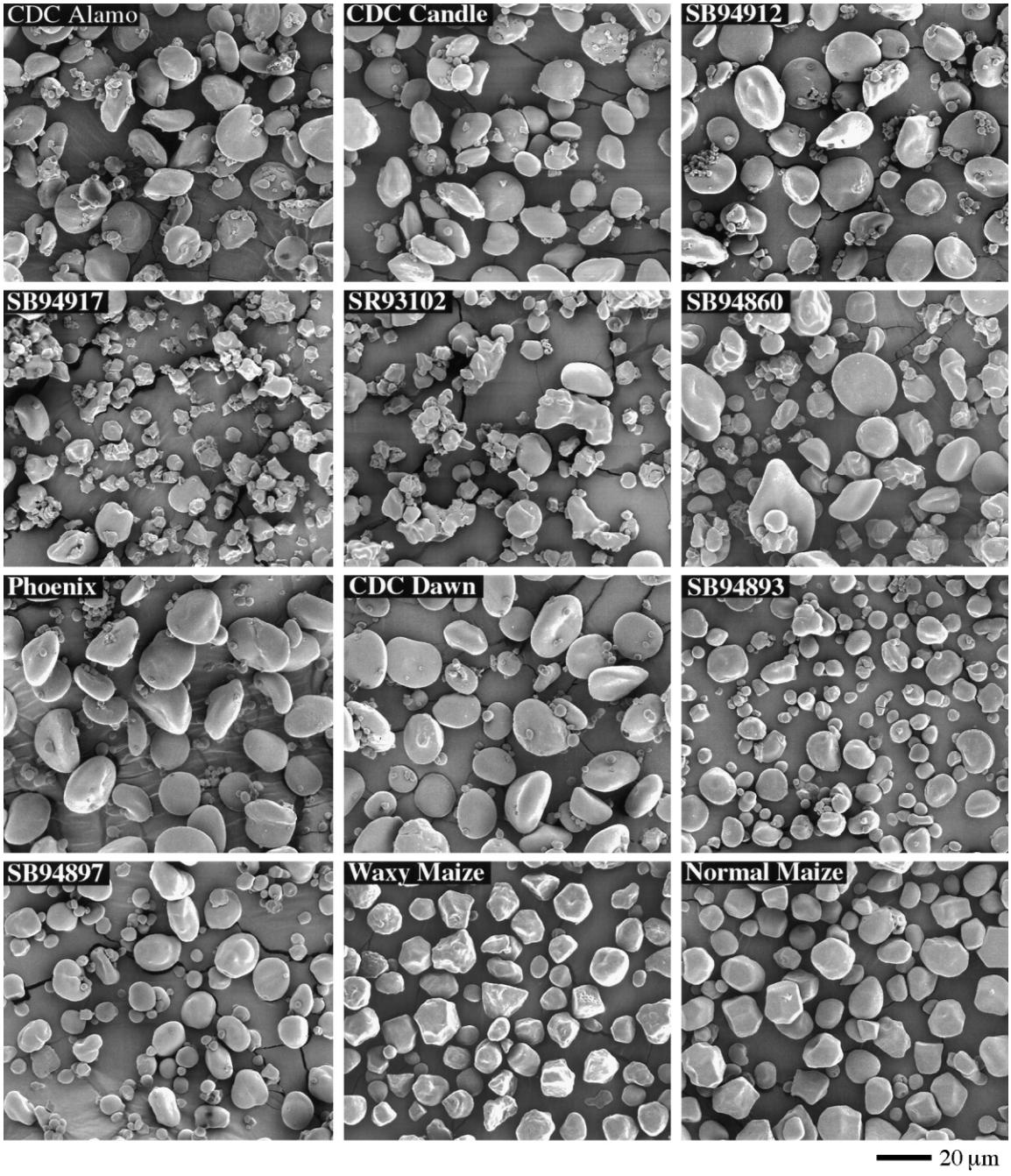

Fig. 1. Scanning electron micrographs of hull-less barley and maize starches (Magnification ×450).

constituted 83–88% of the total number of starch granules, but accounted for only 38–39% of the total starch weight (Table 3).

The proportion of small granules by number was correlated with total amylose content ($r = 0.59$, $P < 0.1$). The average granule diameter was negatively correlated with total amylose content ($r = -0.65$, $P < 0.05$). The correlations mentioned above, have also been reported for other barley starches (Czuchajowska et al., 1992; Morrison, Scott, & Karkalas, 1986; Szczodrak & Pomeranz, 1991).

3.5. Amylopectin structure

The MALDI-MS spectrum and chain length distribution of debranched amylopectin from high amylose (SB 94893) HB starch are presented in Fig. 5. Debranched amylopectins of all HB starches had nearly

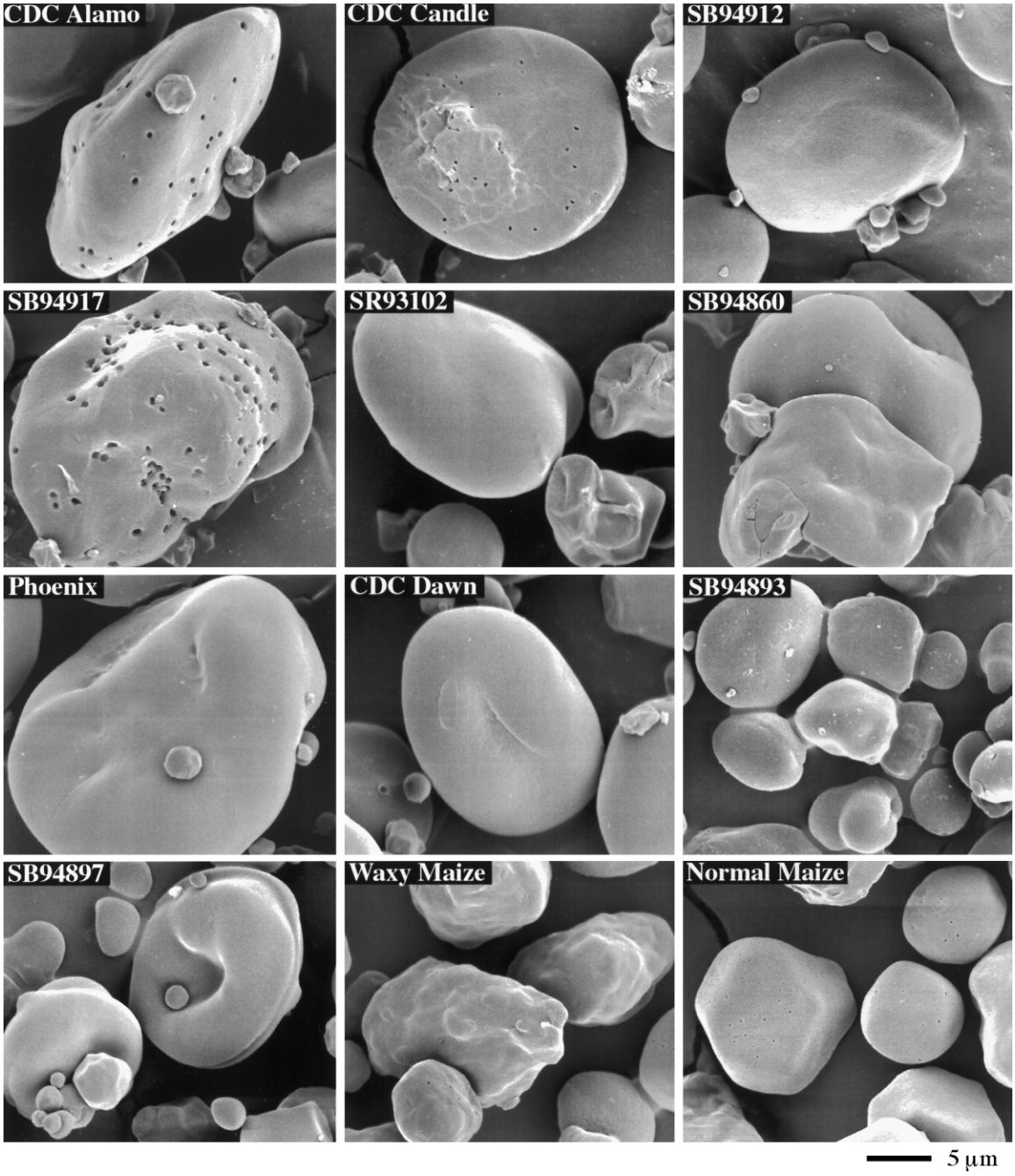

Fig. 2. Scanning electron micrographs of hull-less barley and maize starches (Magnification ×2200).

similar chain length distributions, with the highest peak at degree of polymerization (DP) 12 (Table 4; Fig. 5). The corresponding DP values for normal and waxy maize starches were 13 and 14, respectively. The average chain length (CL) and degree of branching ranged from 17.6–22.7% and 4.4–5.7%, respectively, for the HB starches. The corresponding values for the maize starches were 18.5 and 5.4% (waxy maize) and 19.5 and 5.2% (normal maize). The short (DP 5-17) and long (DP $\geqslant$ 35) chains ranged from 48.2–59.2% and 3.0–16.1%, respectively, for the HB starches. The corresponding values for the maize starches were 59.2 and 6.8% (waxy maize) and 57.2 and 9.4% (normal maize). Average branch chain length was correlated with small granule size ($r = 0.81$, $P < 0.01$), the proportion of small granules by number ($r = 0.71$, $P < 0.05$), the proportion of small granules by weight ($r = 0.78$, $P < 0.01$), the short chains ($r = -0.92$, $P < 0.01$), and long chains ($r = 0.99$,

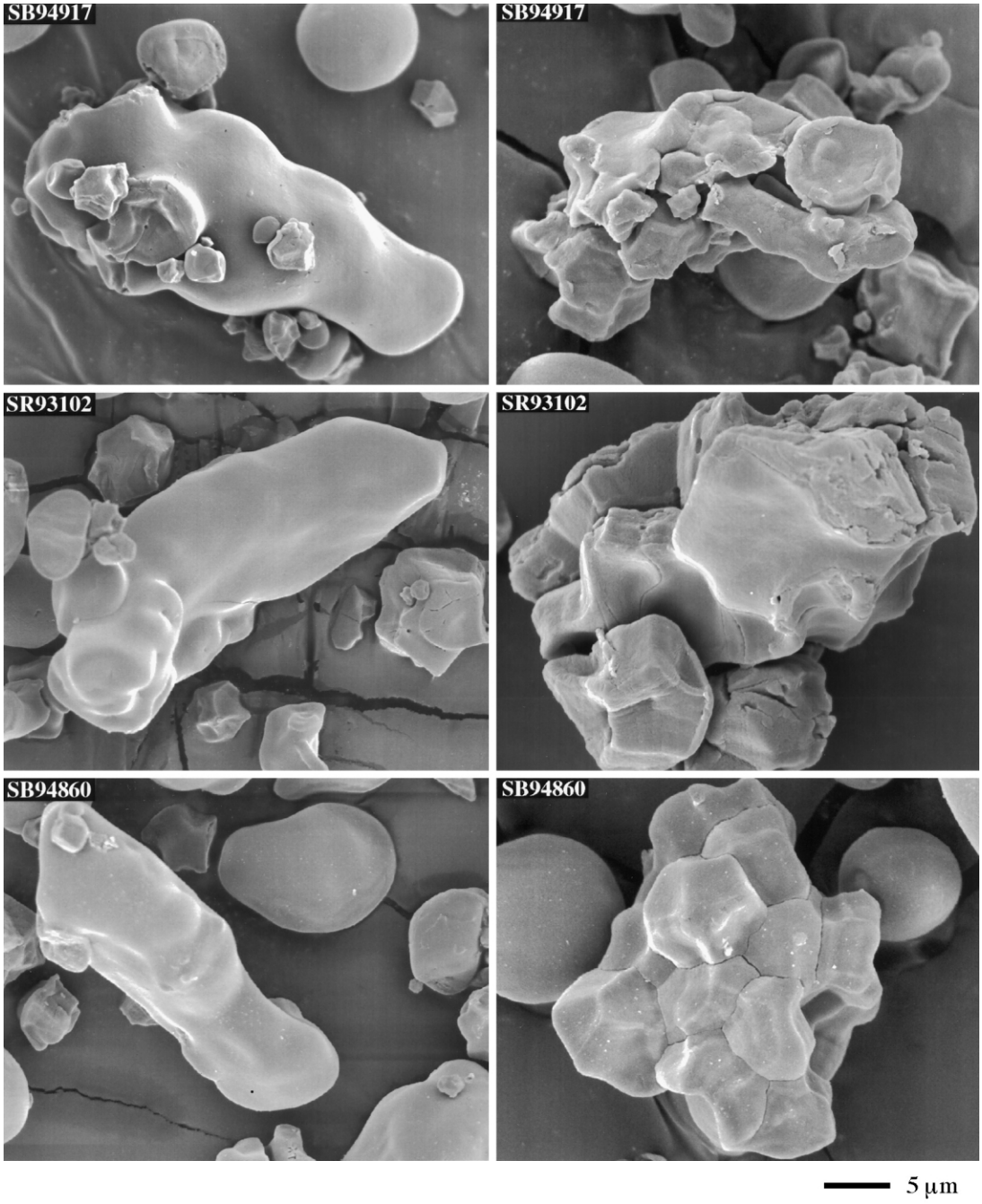

Fig. 3. Scanning electron micrographs of starches from SB 94917, SR 93103 and SB 94860 (Magnification ×2000).

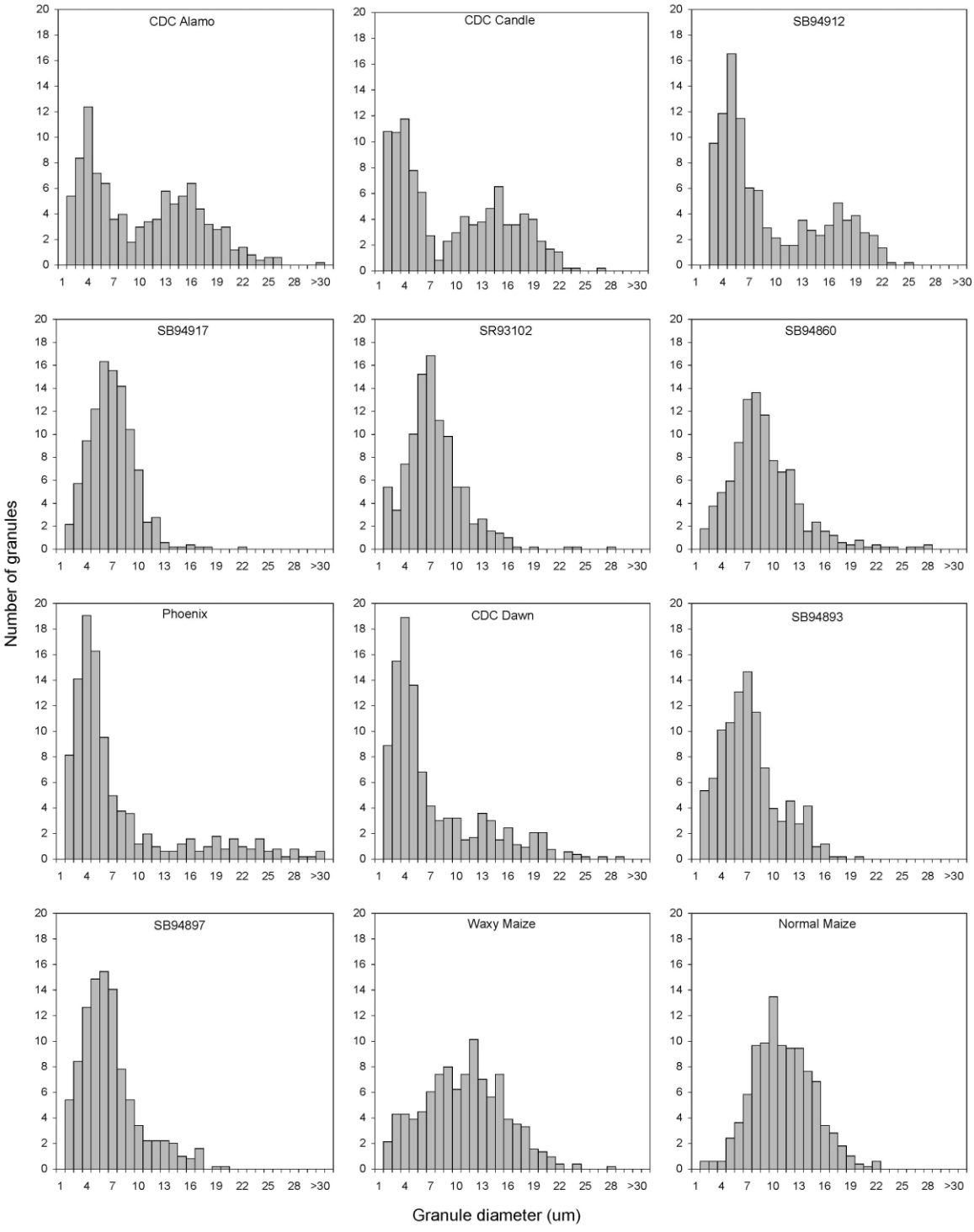

Fig. 4. Granule size distribution of hull-less barley and maize starches.

$P < 0.01$) indicating that amylopectins of small granule high amylose and compound HB starches contained a larger proportion of long chains. Salomonson and Sundberg (1994), Cheetham and Tao (1997) also reported relationships between amylose content and amylopectin branch chain length for barley and maize starches, respectively. This study has shown that the amylose/amylopectin ratio and branch chain length have high correlation with granule size and size distribution in HB starches.

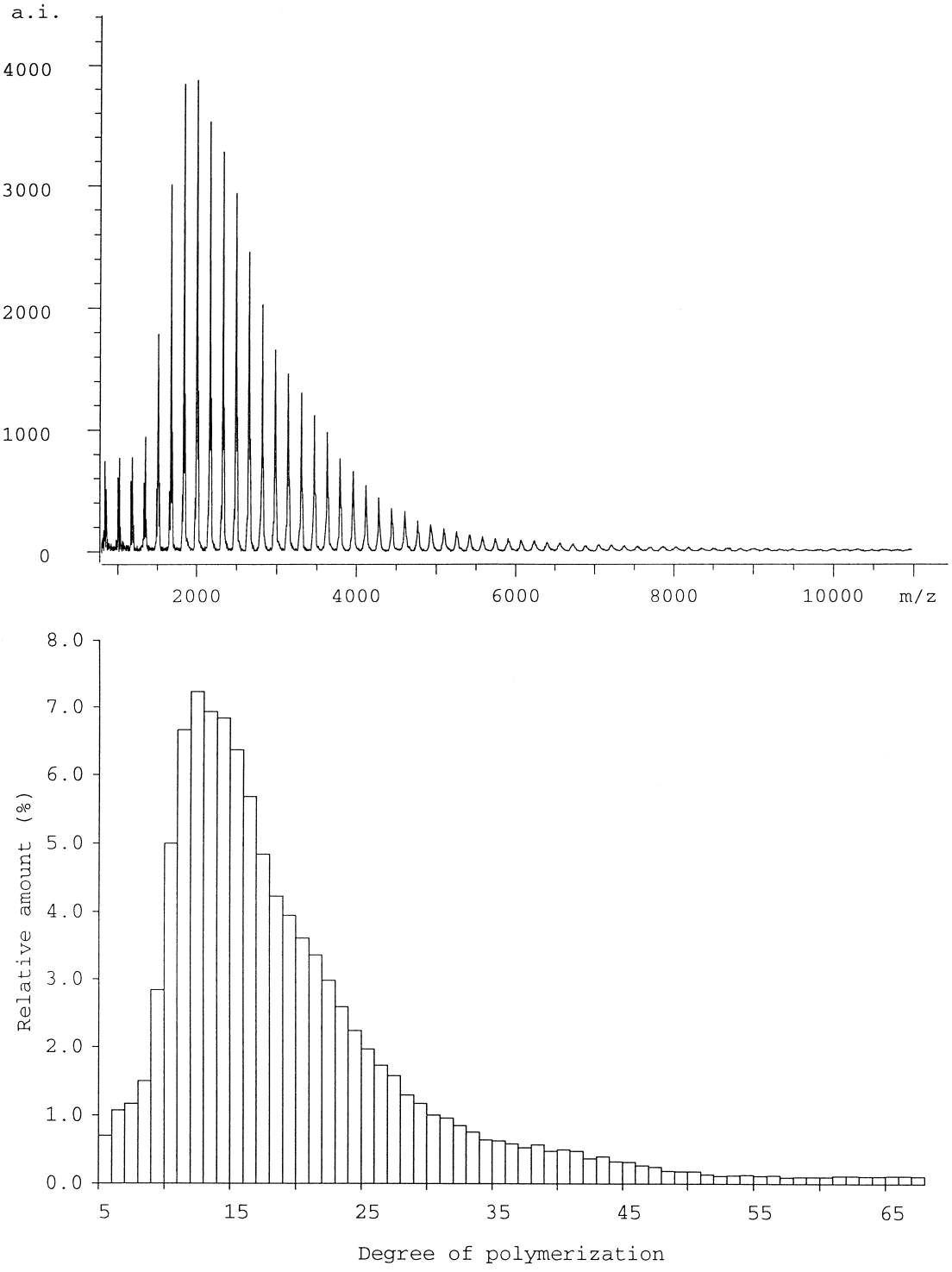

Fig. 5. MALDI-MS spectrum of debranched starch (SB 94893) passed through the Macro-sep and desalting procedure (Top figure) and chain length distribution (Bottom figure) of debranched starch (SB 94893). 2, 5-dihydroxybenzoic acid (DHB) was used as the matrix.

4. Conclusion

This study showed that the starches, from the 10 HB genotypes studied, differed in chemical composition, morphology, granule size, size distribution, and amylopectin branch chain length distribution. Further work is in progress to obtain structure–property relationships for these starches.

Table 4
Chain length distribution of debranched amylopectins of hull-less barley and maize starches

Line analyzed	Peak DP[a]	CL[b]	BP[c] (%)	Chain length distribution (%)		
				DP 5-17	DP 18-34	DP 35-67
CDC Alamo	12	18.1	5.5	57.4	38.0	4.5
CDC Candle	12	17.6	5.7	58.8	36.3	4.9
SB 94912	12	19.5	5.1	57.1	34.2	8.7
SB 94917	12	22.7	4.4	48.2	35.8	16.1
SR 93102	12	20.4	4.9	53.8	34.2	12.0
SB 94860	12	21.6	4.6	50.1	36.0	13.8
Phoenix	12	19.3	5.2	59.1	31.4	9.5
CDC Dawn	12	19.4	5.2	57.4	33.8	8.8
SB 94893	12	19.1	5.3	56.9	35.0	8.2
SB 94897	12	20.2	5.0	52.6	36.1	10.6
Waxy maize	14	18.5	5.4	59.2	34.0	6.8
Normal maize	13	19.5	5.2	57.2	33.4	9.4
LSD[d] ($P<0.05$)		1.4	0.4	7.0	5.4	2.1

[a] Degree of polymerisation.
[b] Average chain length.
[c] Branch points.
[d] Least significant difference.

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