Waxy Starch Barley Genotypes with Reduced β-Glucan Contents

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ABSTRACT

Random inbred lines were produced from a cross between the genotypes Chalky Glenn and Waxy Hector, and two-row lines were classified as waxy or nonwaxy by an iodine staining test. Mean nitrogen and β-glucan contents of the waxy types were higher than those of the nonwaxy types but, in contrast to previous data, mean milling energies of the two groups were not significantly different. Waxy lines with low milling energy had much lower β-glucan levels than those with high milling energy, and they also demonstrated much more extensive cell wall modification during malting. From a trial grown the following season, the waxy types with low milling energy were again identified and had levels of β-glucan content similar to those of nonwaxy types. β-Glucan contents and, particularly, milling energies showed good agreement between seasons. It is suggested that, although waxy starch is usually associated with high β-glucan content, a genetic factor from Chalky Glenn that confers low levels of β-glucan can express in a waxy background.

The waxy (*wx*) allele is associated with an absence of one of the enzymes of starch synthesis, granule-bound starch synthase (Shure et al 1983). Waxy starches consist mainly of amylopectin, with amylose typically <10%, and they occur in a range of species. The waxy allele has been introduced into many barley cultivars, either by back-crossing (Goering et al 1973) or by directed mutation (Eslick 1981).

Initial interest in waxy barley centered on the perceived advantages of the starch, especially with regard to its lower pasting temperature (Eslick 1981). However, studies of waxy and nonwaxy isogenic lines revealed a number of disadvantages associated with the waxy allele. Waxy barley grain was smaller and contained slightly less starch than nonwaxy grain (Tester and Morrison 1993). It also had higher levels of β-glucan in the endosperm cell walls (Ullrich et al 1986) and this appeared to have a direct, deleterious effect on the rate of modification during malting (Swanston 1995). High levels of β-glucan were also problematic when barley was used as a feed for nonruminants, especially poultry (Gohl et al 1978).

The precise nature of the association between the waxy starch character and high levels of β-glucan has not yet been demonstrated, but Stahl et al (1996) considered progeny from five backcrosses and concluded that the association could not be broken. This was supported by data from inbred lines produced by single-seed descent (Swanston 1996), which showed waxy lines to invariably demonstrate slower cell wall modification.

The genotype Chalky Glenn is a mutant with slightly thinner cell walls and a lower β-glucan content than its parent cultivar Glenn (Aastrup et al 1985), which derived from a mutation breeding program (Eslick 1981). Although there is some variation in the expression of thin cell walls across the endosperm (Miller and Fulcher 1994), Aastrup et al (1985) suggested that Chalky Glenn showed slightly faster cell wall modification than its parent. Such differences were not confirmed under Scottish growing conditions (Swanston et al 1995a). It was, however, decided to produce inbred lines from a cross between Chalky Glenn and a waxy cultivar to investigate whether this offered a potential way to reduce β-glucan content in waxy genotypes.

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MATERIALS AND METHODS

A cross was made between the genotypes Chalky Glenn (six-row) and Waxy Hector (two-row) and inbred lines were produced by single-seed descent (Goulden 1939). This approach permits several generations of selfing, during which loose genetic linkages will be broken. Lines from the F7 generation were sown as 2-m rows at the Scottish Crop Research Institute (SCRI), Dundee, in 1993, and protected from foliar pathogens by appropriate fungicide treatment. Following harvest, grain was milled to pass through a 0.5-mm screen, and the flour was tested by the iodine-staining technique of Hovenkamp-Hermelink et al (1988) with modifications suggested by Swanston et al (1995b). Of 68 lines tested, 41 were waxy, of which 21 were of the two-row type. Of the 27 nonwaxy lines, 10 were of the two-row type. As grain quality comparisons would be subject to effects on nitrogen content associated with six-row versus two-row crosses (Day and Dickson 1957, Swanston 1983), initial studies were based only on the two-row types, which are more representative of the genotypes usually grown in Scotland.

Grain samples were used to determine 1,000 kernel weight, grain nitrogen by the Dumas combustion method (Buckee 1994), and milling energy (Allison et al 1979), which determines the mechanical energy expended in milling a given quantity of grain. β-Glucan contents were determined by an enzymatic method (McCleary and Glennie-Holmes 1985). A number of selected samples were malted by the method of Swanston and Taylor (1990) using two air rests. The extent of endosperm cell wall modification was determined using the Carlsberg malt modification analyzer (Aastrup et al 1981).

In 1994, the 31 two-row genotypes were included in a trial of two replicates grown at SCRI, with an appropriate fungicide regime again employed. Plots were 2 m long and 1.5 m wide. Following harvest, grain samples were cleaned and assessed for milling energy and β-glucan contents as described above.

RESULTS

1993 Grain Quality Data

The waxy types had higher mean β-glucan content and higher mean grain nitrogen than nonwaxy types (Table I). Previous experiments (Swanston et al 1995b, Swanston 1996) did not find significant differences in grain nitrogen between waxy and nonwaxy types, but those results were derived from different sets of germplasm. Here, grain nitrogen contents were raised as the population comprised two-row progeny from a six-row versus two-row cross, so differences between the two populations may be amplified. Waxy lines had slightly, but significantly lower 1,000 kernel weights than nonwaxy lines, so differences in grain nitrogen content may also reflect, at least in part, differences in grain filling. Differences in 1,000 kernel weights were of a magnitude similar to those shown by Swanston (1996), although that study also showed a seasonal effect on variations in grain size.

A major difference between the results here and those found by Swanston (1996) was that mean milling energies were not significantly different between the two groups. Swanston et al (1995b) also suggested that the waxy gene was associated with an increase in milling energy. Here, however, there was considerable overlap between the milling energy values of the waxy and nonwaxy populations. There was also overlap with regard to the β-glucan content, indicating that it might be possible to observe waxy types with β-glucan contents similar to those of nonwaxy types.

Correlations between β-glucan content and other quality parameters (Table II) were nonsignificant, with the exception of that between β-glucan and milling energy in the waxy types. This was in agreement with earlier data (Swanston 1996) suggesting a stronger association between milling energy and cell wall modification in waxy types as compared to nonwaxy types. The association between milling energy, β-glucan content, and cell wall modification was further investigated by malting the eight waxy lines with highest milling energy and the eight with the lowest milling energy.

When cell wall modification in the malts was plotted against milling energy (Fig. 1), it was clear that there were two distinct groups. Differences between the groups in milling energy were reflected in the relative ease of cell wall modification although, within the two groups, differences in cell wall modification did not explain variation in milling energy. In the low milling energy group, there was a similar relationship between cell wall modification and β-glucan content (Fig 1). The high milling energy group was again distinct, but showed a much wider range in β-glucan content. While ease of cell wall modification was associated with low levels of β-glucan, poor cell wall modification may not be caused only by high β-glucan content. It is likely that molecular structure and association with other cell wall components are also of considerable importance.

1994 Trial Data

Swanston (1996) showed that the grain quality of waxy compared to nonwaxy populations was subject to seasonal effects. In addition, Ellis et al (1989) suggested that correlations between seasons, for quality parameters, could be quite variable. For those reasons, it was necessary to extend observations on milling energy and β-glucan content for a further year. Trial results (Table III) showed good agreement between replicates and significant differences between genotypes. Mean values were, therefore, plotted against data from the previous season for waxy and nonwaxy lines.

For milling energy (Fig. 2), both waxy and nonwaxy types showed some variation between seasons in the degree of scatter, but correlations were highly significant for both populations. In the waxy types, there were two distinct clusters of high and low milling energy, respectively, with four samples falling between the two main groups. The data for β-glucan (Fig. 3) showed greater scatter, especially for the nonwaxy population where there also appeared to be two distinct clusters. Variation among waxy lines appeared to be more continuous.

The waxy lines were divided into two groups; the low milling energy lines, as shown in Fig. 2, were analyzed separately from the others. In addition to lower milling energy, they had significantly lower levels of β-glucan when compared to the other waxy lines (Table IV) and were comparable to the nonwaxy types. The populations described here were too small for firm conclusions to be drawn about the mode of inheritance, but it was clear that waxy lines with low levels of β-glucan were identified and shown to perform consistently between seasons.

TABLE I
Means and Standard Deviations (SD) for Quality Characters Measured on Waxy and Nonwaxy Populations from the Cross of Chalky Glenn (CG) and Waxy Hector(WH) Grown in 1993

	Waxy		Nonwaxy		Parents	
	Mean	SD	Mean	SD	CG	WH
Milling energy (J)	739.8	80.00	704.3	69.09	621.9	800.7
β-Glucan (%)	4.64	0.777	3.89***[a]	0.341	3.56	4.68
Grain nitrogen (%)	2.28	0.299	1.87***	0.189	1.95	1.74
1,000 kernel wt (g)	49.97	4.950	53.15*	6.116	46.48	50.20

[a] *** = Significant at 0.1% level, * = significant at 5% level.

TABLE II
Correlation Coefficients Between β-Glucan Content and Other Quality Characteristics Measured on Waxy and Nonwaxy Populations from the Cross of Chalky Glenn and Waxy Hector Grown in 1993[a]

	Waxy	Nonwaxy
Milling energy	0.883***[b]	0.495
Grain nitrogen content	–0.258	–0.020
1,000 kernel weight	0.378	0.527

[a] Waxy (n = 21), nonwaxy (n = 10).
[b] *** = Significant at the 0.1% level.

DISCUSSION

Recent interest in waxy barley has centered on its potential for human nutrition due to the hypocholesterolemic effect associated with the high levels of β-glucan, a component of soluble fiber (Newman et al 1989). However, as the qualities that enhance food use specifically exclude genotypes from the malting and brewing industries, such barleys are likely to find only small niche markets. For waxy barley to be widely grown, it would have to meet

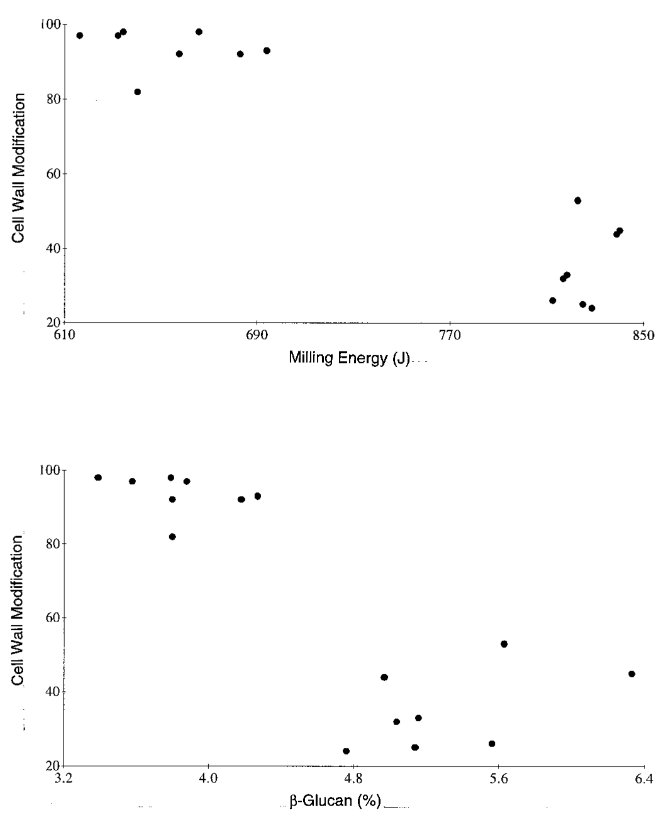

Fig. 1. Cell wall modification (%) plotted against milling energy (top) and β-glucan content (bottom) for 16 waxy lines from the cross Chalky Glenn and Waxy Hector grown in 1993. Lines were selected as the eight highest and eight lowest in the milling energy test.

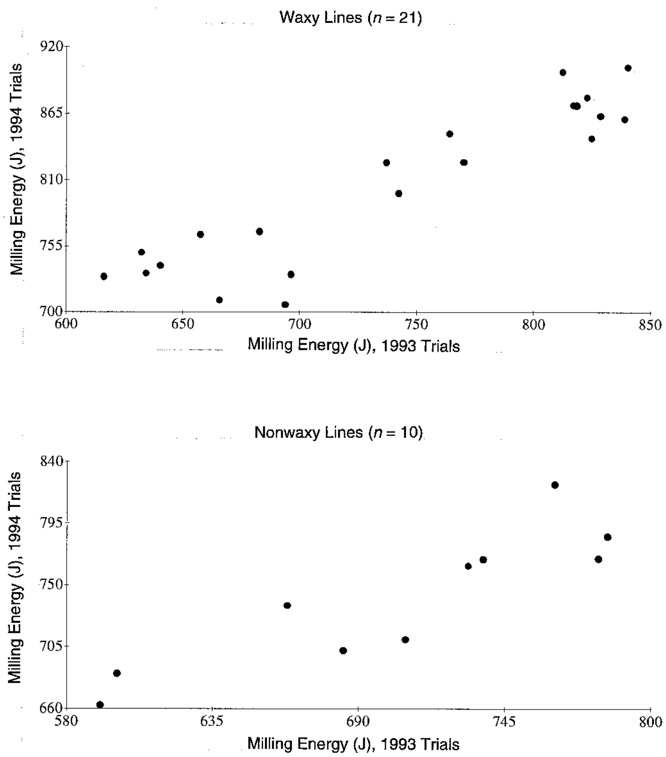

Fig. 2. Mean milling energies from 1994 trial data plotted against 1993 results for waxy (top) and nonwaxy (bottom) lines from the cross between Chalky Glenn and Waxy Hector. Correlation coefficients (r) are: $r = 0.922$, $P < 0.001$ for waxy, and $r = 0.835$, $0.001 < P < 0.01$ for nonwaxy.

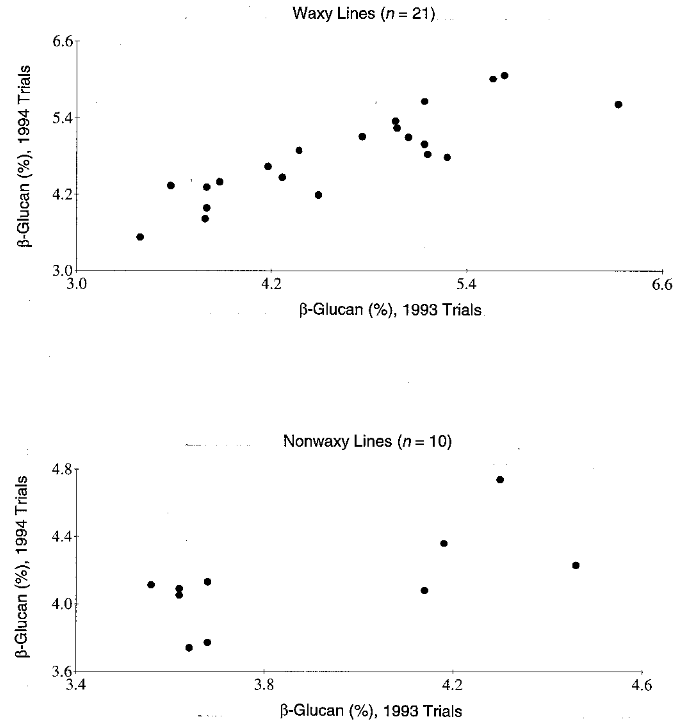

Fig. 3. Mean β-glucan contents from 1994 trial data plotted against 1993 results for waxy (top) and nonwaxy (bottom) lines from the cross between Chalky Glenn and Waxy Hector. Correlation coefficients (r) are: $r = 0.868$, $P < 0.001$ for waxy, and $r = 0.673$, $0.05 < P < 0.01$ for nonwaxy.

TABLE III
Analysis of Variance for β-Glucan Contents and Milling Energy of Inbred Lines Grown in 1994 Trial

		Mean Squares	
	df[a]	β-Glucan	Milling Energy
Between replicates	1	0.094	165.3
Between genotypes	30	0.916***[b]	8,656.8***
Residual	30	0.028	487.3
Total	61		

[a] Degrees of freedom.
[b] *** = Significant at the 0.1% level.

TABLE IV
Milling Energy and β-Glucan Content, Means and Standard Deviations (SD) of Three Groups of Inbred Lines Grown in 1994 Trial

	Milling Energy		β-Glucan	
	Mean	SD	Mean	SD
Waxy (low milling energy)	736.6	21.26	4.19	0.350
Waxy (medium to high milling energy)	857.5***[a]	31.04	5.30***	0.442
Nonwaxy	749.2	44.43	4.13	0.302

[a] *** = Significantly different from the nonwaxy group in the same column at the 0.1% level. Differences between low milling energy waxy and nonwaxy groups were not significant.

major, existing, as well as novel, market requirements. Waxy starch hydrates more readily (Eslick 1981) and gives more viscous gels (Vasanthan and Bhatty 1996) than normal starch, so it is more suited as a thickener in both food and nonfood applications. It is also particularly suited to the production of high maltose syrups (Goering and Eslick 1989) and, hence, has potential for fuel alcohol production.

Swanston (1996) suggested that waxy types with acceptable levels of hot water extract could be produced, although they were likely to experience problems with wort viscosity. The reduction in grain size associated with waxy types is less than that observed with certain dwarfing genes that have been incorporated into successful cultivars (Swanston et al 1995b) and waxy types with acceptable grain size have been produced (Stahl et al 1996). The parents used in this experiment would not be expected to produce progeny of good malting quality and, in addition, the high levels of grain nitrogen observed in 1993 would be problematic. However, lines have been developed in which a genetic factor responsible for reduced β-glucan content is expressed in a waxy background. It may be possible to produce waxy barleys that not only serve a number of niche markets where high fiber content is not required, but that could also be suitable for malting and brewing.

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