Screening for novel starch traits using NIR spectroscopy

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Introduction

The frequency of type II diabetes, heart disease, obesity and colon and rectal cancers are steadily increasing in Westernised societies and also rising in developing countries. Such diseases are causing major public health problems, resulting in these issues becoming a high priority for governments worldwide. A viable approach for improving public health is to make appropriate changes to food products and give substantiated health benefits while retaining consumer appeal. Numerous studies have indicated that small alterations in grain composition (i.e. starch, beta-glucan and fibre) can deliver significant health benefits, once integrated into food. Consequently, as dietary fibre has been identified as being an important component of the human diet, the potential of barley as a food grain is beginning to be recognised. Barley contains two types of starch, amylose and amylopectin. Three main groups of novel starch barley types have been identified with respect to their amylose content: Low or 'waxy' amylose (0–10%), normal amylose (~25%) and high amylose (>35%). The waxy gene, *wax* (formally called *glx* or *wx*) is located on chromosome 7HS. The waxy phenotype is conferred by various mutations at this locus that either preclude transcription or encode an inactive form of the starch synthase enzyme. The high amylose gene, *amol* is located on chromosome 1H. The genes controlling both these starch mutants are recessive in their genotypic expressions. The selection of these novel starch genotypes in any breeding program is tedious, time consuming, and often involves complicated and destructive laboratory analyses. Furthermore, this conventional selection of either the 'waxy' or high amylose genotype is often delayed until either the intermediate or advanced generation. Near Infrared (NIR) Reflectance Spectroscopy provides the opportunity to screen routinely for these novel starch traits in the early generations of the breeding programme.

Materials and methods

Hulless and covered barley samples were obtained from the University of Adelaide Barley Program, growing seasons 2006 (n = 93) and 2007 (n = 101). Samples were grown at Roseworthy, South Australia (34°31′41″S;138°41′14″E, elevation 97 m). Selections included known waxy, normal and high amylose barley lines. Amylose content ranged from 4.99% to 40.12% and was not correlated to protein, or other carbohydrates tested (beta-glucan and pentosans). Hulless barleys can be susceptible to embryo damage during harvesting. Consequently, gentle harvesting

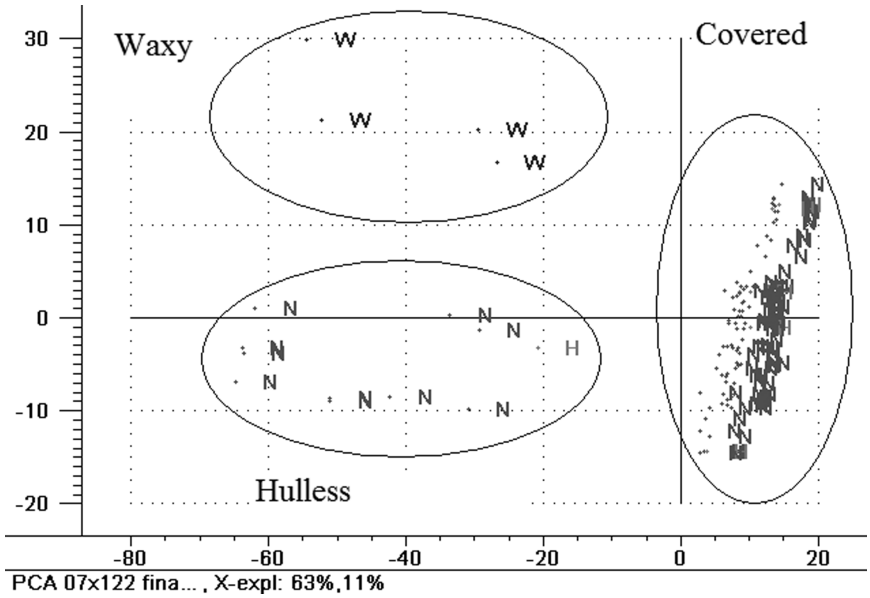

Figure 1. Score plot Factor 1 vs Factor 2 growing season 2006, H = high amylose, W = waxy, N = normal amylose and H = high amylose.

techniques were adopted, resulting in 10–50% of the husk remaining attached to the grains. Barley samples (40 g) were de-hulled using a laboratory scale oat de-huller (except for covered barley). Amylose/amylopectin ratios were measured using the amylose/amylopectin assay procedure (K-AMYL), Megazyme, Ireland. The barley samples were subsequently scanned on a FOSS XDS Near-Infrared Rapid Content Analyser as whole grain in the Natural Products Cup. The reflectance spectra were collected over the range 1100–2500 nm at 2 nm interval, 16 scans/sample. The averaged spectra were analysed using The Unscrambler v9.8 software. The second derivatives of the spectra were obtained by the Savitzky–Golay method, smoothing points 3 and polynomial order 2. Principal component analysis (PCA) was applied to each growing season separately, on centred, weighted spectra (A(Sdev+B)). Full cross validation using the leave one out method was conducted and samples were categorised: Waxy (low amylose) = W, normal amylose = N, high amylose = H.

Results and discussion

The factor plot for growing season 2006 (Figure 1) explains 74% of variation for the first two factors. Three clusters were observed with waxy barley types differentiated from the hulless and covered barley clusters. The growing season 2007 plot (Figure 2) shows the same differentiation of the waxy from non-waxy barley types with 65% of the variation explained. High amylose types could not be spatially separated from normal amylose covered barley. Spatially the factor plots for both seasons would need to include known waxy and hulless barley types to classify the clusters

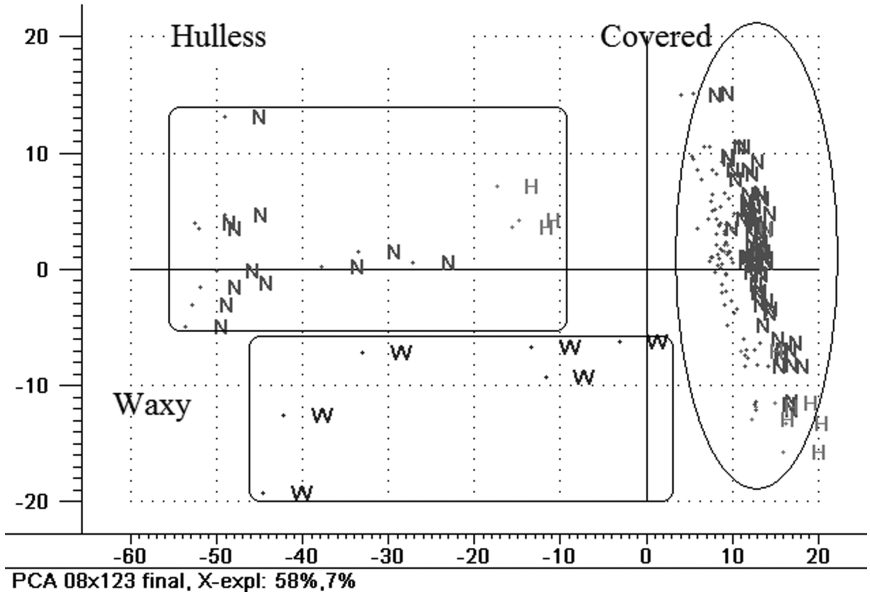

Figure 2. Score plot Factor 1 v Factor 2 growing season 2007, W=waxy, N=normal amylose, H=high amylase.

correctly. Further PCA models for both growing seasons with waxy types removed could not separate the high amylose from normal amylose barley types (plot not shown).

Conclusion

PCA modeling could not identify high from normal amylose covered barley types. Further work to resolve this is planned, and further investigation into spectral differences may narrow the wavelength range and differentiate high from normal amylose content. Waxy barley types can be identified by PCA modeling, with known waxy types identifying the waxy barley type cluster. The clusters for waxy types for both growing seasons were sufficiently separated from the hulless and high/normal groups. Spectra collected from whole grain barley with PCA modeling can be used to screen at early generations for waxy (low amylose) barley lines in a breeding program.