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STUDIES INTO THE GENETICS OF WHEAT ENDOSPERM RHEOLOGY AND MICROSTRUCTURE

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INTRODUCTION

Despite considerable gains in wheat flour milling yield through conventional breeding strategies and milling technologies, the theoretical maximum yield still has not been attained. Discovery of genes in wheat that control flour yield would provide a means for breeders to develop new wheats that fulfill their potential in relation to this trait. A more targeted approach is to improve the understanding of the role of grain microstructure in determining high flour yield. Previous research has shown that increased flour yield in hard wheat is associated with increased endosperm rheology index, calculated from strength and stiffness as measured by the SKCS (Osborne et al, 2005; Osborne et al, 2007). Edwards et al (2007) demonstrated, through microscopy and particle size analysis of the crushed material from the SKCS 4100 and a first break roll stand, that the SKCS data for unconditioned wheat averaged over 300 grains provided a useful indicator of milling performance of a wheat sample. The *Pina-D1* and *Pinb-D1* alleles, tightly linked to the Ha locus on the short arm of Chromosome 5D, determine the hardness phenotype (Greenwell and Schofield, 1986). However, this does not fully account for the observed genetic variation in hardness, especially within each hardness class, and it is thought that additional modifying genes account for the range of hardness within hard or soft classes (Martin et al, 2001). Others have investigated the relationship between endosperm starch granule size and hardness. Igrejas et al (2002) reported that harder wheat had a higher content of small starch granules but could not find a QTL for starch granule size on the 5D Chromosome; they concluded that "starch size distribution is influenced by genes which have yet to be analysed". The aim of the present study was to test the hypothesis that a genetic association exists in hard wheat between starch granule type and flour yield.

MATERIALS AND METHODS

Samples

Seed samples representing 197 wheat varieties from the Australian Winter Cereals Collection (Tamworth) were used for this study. To obtain sufficient quantities of grain, the seeds were propagated twice. The first propagation took place at Tamworth, NSW in 2004 for an initial study using the SKCS and Environmental Scanning Electron Microscopy (ESEM). The second propagation was carried out at two locations (Biloela, QLD and Narrabri, NSW) in 2005 to produce sufficient quantities of grain for laboratory milling and starch granule size distribution (SGSD). Not all of the varieties grew successfully at all three locations.

Single Kernel Characterization System (SKCS) Analysis

SKCS measurements were carried out on 300 grains from each sample from all three propagations according to AACC Method 55-31. The individual crush response profiles (CRPs) for each sample were first exported from the SKCS 4100 directory in a text file format and then imported into Crush Curve Analysis Software (CCAS - BRI Research, North

Ryde, New South Wales, Australia). CCAS calculated the average CRP for each set of curves then, for each average CRP, calculated the rheological parameters strength (maximum stress, σ) and stiffness (Modulus of Elasticity, calculated as δσ/δε) of the endosperm. The standard SKCS parameters (seed weight, diameter and Hardness Index (HI)), CRPs and the calculated rheological parameters were exported from CCAS in a text file format and further data processing was carried out using Microsoft Excel. The Wheat Rheology Index was calculated as (10 x endosperm stiffness + endosperm strength)/15 (Osborne et al., 2007).

Environmental Scanning Electron Microscopy
Whole wheat grains (three seeds of each selected variety) were manually scored with a scalpel and snapped transversely in two. The fractured grain was mounted on an aluminium stub using double-sided adhesive tape. Images were obtained without further specimen preparation using a Philips XL30 environmental scanning electron microscope (Philips, Denmark) with a gaseous secondary electron (GSE) detector at a pressure of 1 Torr and an accelerating voltage of 15 kV. The working distance was 10 mm. Cell wall and granule diameter measurements were taken using ESEM image scale bars for a general comparison with the laser diffraction method.

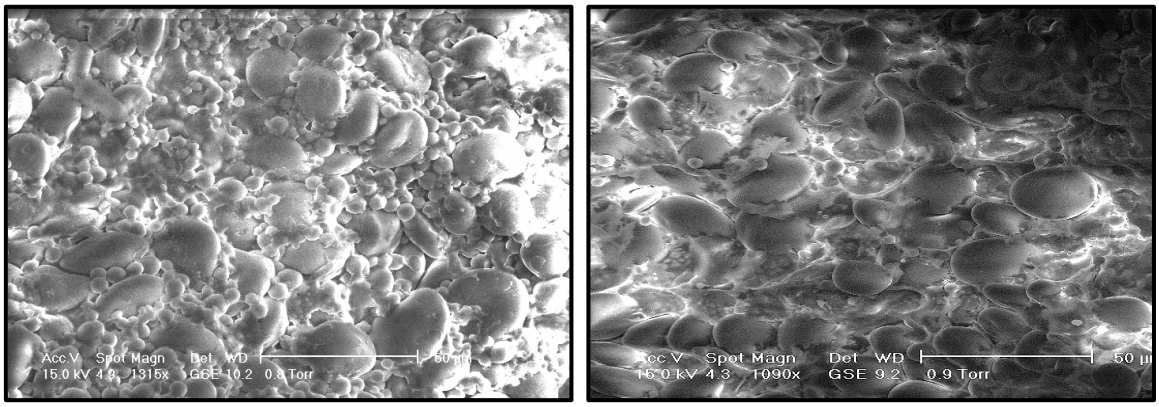
Test milling
Grain samples from the second (Biloela and Narrabri) propagations were available for milling. Cleaned, conditioned wheat was milled using a Buhler MLU 202 Laboratory Mill at a feed rate of 100 $gmin^{-1}$. Bran and pollard fractions were further processed using two passes through a Buhler MLU 203 Laboratory Impact Finisher and the finisher flours were passed through a 150 μm screen before incorporation into the straight-run flour.

Starch extraction
Fifteen wheat varieties grown at Biloela and Narrabri were selected to represent contrasting flour yield. Triplicate subsamples of six grains were taken from each sample. Methods of starch extraction from flour and manually-ground kernels were adapted from Hogg et al (2004), Giroux et al (2003) and Stoddard (1999). The grains were cracked into 2 ml Eppendorf tubes and soaked in 0.7 ml of 0.1M sodium chloride overnight. A plastic Eppendorf pestle attached to an electric drill was used to grind the samples in the soaking medium until the gluten formed a dough ball and the bran was broken into large flakes. The dough ball was pushed to the bottom of the tube and the aqueous starch solution was transferred through a fine sieve (200 μm pore size) to the top of a 2 ml pre-weighed Eppendorf tube containing 1 ml of 80% cesium chloride. The solids were ground again in a further 0.5 ml of 0.1M sodium chloride, the slurry was decanted into the same tube as before, and the solids were ground and slurry decanted for a third time. The starch water suspension and cesium chloride were then centrifuged at 13,000 x g for 3 min. The cesium chloride was decanted and the starch was vortexed with 1 ml water until clumping dispersed (~40 s) and then re-centrifuged. The starch pellet was then washed by 3 min centrifugation at 13,000 G, through a sequence of 2% sodium dodecyl sulfate (SDS) then twice in water.

Laser diffraction analysis
Granule size distribution was quantified in a Malvern Mastersizer 2000 laser-diffraction analyser using the flow-through, 100 ml reservoir. Starch and water (1.7 ml) was vortexed and sonicated for 30 sec at 6W before analysis and added to the reservoir until an obscuration value of between 12–17% was achieved. Settings were optimised for the refractive index of starch in water and an average of three consecutive measurements performed.

RESULTS AND DISCUSSION

A study of the fractured endosperm of hard wheat varieties grouped according to similar rheology index values was performed using ESEM (**Figure 1**). Differing microstructures and fracture patterns were observed between each group. Specifically, the group representing high rheology index had a greater concentration of small starch granules in prismatic cells and fracture along cell walls.



(A) **(B)**

Figure 1. ESEM of fracture surfaces of cracked wheat samples corresponding to (A) high rheology index and (B) low rheology index.

To explore this effect further, samples of diverse wheat germplasm were grown at two sites and subjected to laboratory milling. Starch granule size distribution (SGSD) analysis using a laser diffraction method (LDS) was undertaken on a subset of samples in triplicate representing a range in flour yield.

The results of this study supported an hypothesis for a significant influence of SGSD on flour yield of hard wheat varieties. In addition, a significant part ($R^2 > 0.40$ ($p < 0.05$) at two sites) of the association appeared to be under genetic control despite known environmental effects on small granule number. Results indicate a more even gradation of granule sizes involving an increase in the sample volume % of small granule (types B and C) and decrease in type A granules. This was associated with increased rheology index values and higher flour yield. In composite materials such a particle distribution produces a denser and stronger structure. In wheat endosperm with strongly bonded granules or associated low levels of puroindoline, fracture along cell walls and through starch granules may be enhanced. The ratio of type A:C starch granule accounted for up to 56% ($p < 0.05$) of the variation in flour yield in the samples studied.

Thus, rheological parameters measured using a rapid SKCS screening method can now be linked to the genetic regulation of SGSD with implications for the improvement of milling performance of hard wheat. Further confirmation is required on a wider sample set also accounting for varietal differences in granule bond strength. Studies into the regulation of starch granule classes and their influences on the processing qualities of other cereals may be challenging.

CONCLUSIONS

ESEM observations on samples of hard wheat varieties confirmed the degrees of variation in endosperm structure ranked by SKCS crush response profile characteristics. The endosperm rheology associated with high flour yield was characterised by fracture along cell walls and the presence of a higher proportion of small spherical bodies, possibly type C starch granules in prismatic cells. These observations have been confirmed by quantitative starch granule size distribution analyses which indicate that a higher milling quality results from a more evenly graded granule size distribution. This may provide a way of effecting genetic improvement in wheat flour yield through manipulating the genes regulating starch granule size distribution. Further confirmation is required on a wider sample set also accounting for varietal differences in granule bond strength.

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REFERENCES

Edwards, M.A., Osborne, B.G. and Henry, R.J., (2007). J. Cereal Sci. 46: 64-74.
Giroux, M.J., Sripo, T., Gerhardt, S. and Sherwood, J. (2003). Biotechnology & Genetic Engineering Reviews 20: 277-290
Greenwell, P. and Schofield, J.D. (1986). Cereal Chem. 63: 379-380.
Hogg, A.C., Sripo, T., Beecher, B., Martin, J.M. and Giroux, M.J., (2004). Theor. Appl. Genet. 108: 1089-1097.
Igrejas, G., Leroy, P., Charmet, G., Gaborit, T., Marion, D. and Branlard, G. (2002). Theor. Appl. Genet. 106: 19-27.
Martin, J.M., Frohberg, R.C., Morris, C.F., Talbert, L.E. and Giroux, M.J. (2001). Crop Sci. 41: 228-234.
Osborne, B.G., Anderssen, R.S. and Huynh, H.N. (2005). In: Cauvain, S.P., Salmon, S.S. and Young, L.S., Using Cereal Science and Technology for the Benefit of Consumers. Proc.12th International ICC Cereal and Bread Congress, Harrogate, UK, 23-26th May 2004, pp. 207-211.
Osborne, B.G., Henry, R. and Southan, M.D. (2007). J. Cereal Sci. 45: 122-127.
Stoddard, F.L. (1999). Cereal Chem. 76: 145-149.

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