

Genetical Genomic Approaches to Candidate Gene Marker Identification for a Drought-Adaptation Trait in Wheat

C Lynne McIntyre¹, Gang-Ping Xue¹, Anthony van Herwaarden¹ and Ray Shorter¹

¹CSIRO Plant Industry, 306 Carmody Rd., St Lucia, Qld. 4067, Australia Email lynne.mcintyre@csiro.au

Abstract

Mid-season and terminal drought is commonly experienced by wheat grown in the northern Australian cropping region. Traits such as water soluble carbohydrates (WSC) in wheat stems, which enable plants to remobilise carbohydrates stored around anthesis to developing grain, are advantageous. We are using a genetical genomic approach that aims to elucidate the genetic and genic basis of this trait with a view to improving selection efficiency for this trait. This research is part of a broader CSIRO Plant Industry wheat yield under drought initiative. Using a recombinant inbred line (RIL) population evaluated over several sites and years, we have confirmed that WSC is a polygenic trait, with many QTL of small effect. High yielding progeny selected from the population were found to be enriched for QTL for higher WSC. RNA from the high and low WSC tails of this RIL population, sampled at anthesis from a rainfed field trial, was screened over wheat Affymetrix GeneChips to identify differentially expressed genes. Many of these genes map to regions to which QTL for WSC have been located. The association between expression of these and other carbohydrate metabolism genes and WSC has been further confirmed using qRT-PCR. Our current research is focussing on confirming the value of QTL for WSC and on elucidating the role of these differentially expressed genes in the WSC trait.

Media summary

Genes and QTLs for water soluble carbohydrates in wheat have been identified that should assist in developing improved cultivars for dry environments.

Key words

Wheat, drought, grain weight, grain yield, WSC, QTL, genomics

Introduction

Rainfall variability is the dominant factor causing yearly and regional variability in Australian wheat yields, with drought frequently causing lower yields (Potgieter *et al.* 2005). In the north-east wheat zone (latitude 23^o – 30^o S) some degree of post-anthesis moisture stress occurs in 50-70% of seasons. Under these moisture limited conditions, post-anthesis assimilation can be restricted as a result of photosynthesis inhibition and reduced leaf area which in turn limits supply of carbohydrates to the developing grain. This reduced supply of carbon can restrict grain development and lead to smaller and shrivelled grain and lower yields. Water-soluble carbohydrates (WSC) accumulated pre-anthesis and stored in the stem mainly as fructan with some sucrose and hexose provide an alternative source of assimilates to current photosynthesis for grain filling.

Genetical genomics (Jansen and Nap 2001) is an approach that harnesses the discipline of genetics with the raw power of genomics. It utilises genetic populations such as recombinant inbred lines (RILs), double haploid lines (DH), and near-isogenic lines, or segregating pedigrees such as those used in animal and human studies, as the source of material for genomic studies to identify the genetic basis of gene mRNA expression differences. Such genetic variation can potentially be exploited in plant improvement programs. Furthermore, it enables both functional and regulatory genes to be identified as associated with a specific trait or a specific chromosomal region, providing valuable information on gene regulatory

networks and pathways and the interrelation of gene products. There are few genetical genomics papers in wheat; for example (Guillaumie *et al.* 2004) on seed storage protein fractions and two from our laboratory: (Xue *et al.* 2006) on transpiration efficiency and (Xue *et al.* 2008) on WSC. In this paper, we describe a genetical genomics approach using tails of a segregating population to identify candidate genes for stem carbohydrates in wheat and their co-location with QTLs.

Materials and methods

From an evaluation of a Seri-Babax population of 194 recombinant inbred lines (SB RILs) (Olivares-Villegas *et al.* 2007) in north-eastern environments of Australia in 2002 and 2003, eight high (223 mg g⁻¹) and eight low (142 mg g⁻¹) RILs for WSC concentration were selected. These 16 RILs were evaluated under rainfed conditions in 2005 in a two replicate field trial at the CSIRO Cooper Laboratory at Gatton (latitude 27° 34' S, longitude 152° 17' E). The trial was sown 12 July and suffered pre- and post-anthesis moisture stress, although a rainfall event just prior to anthesis meant that plant samples taken at anthesis were not stressed. Grain yield (g m⁻²) and individual grain weight (mg) was recorded for each plot. The upper two (peduncle and penultimate) internodes, with leaf sheath attached, from 7-8 fully developed stems were randomly sampled at anthesis from each plot and immediately dropped into liquid nitrogen. These samples were used for RNA isolation, WSC, enzyme and cell wall analyses.

A subset of the 16 SB RILs (four high and four low) covering the range of WSC levels was selected for Affymetrix GeneChip analysis, and differentially expressed (DE) genes identified of which a high proportion encoded enzymes involved in carbohydrate metabolism (Xue *et al.* 2008). Quantitative RT-PCR, using gene-specific primers designed for the differentially expressed carbohydrate metabolic genes, was then used to validate the association of levels of gene expression and stem WSC concentration in the wider set of 16 SB RILs. The enzyme activities of three important differentially expressed enzyme families (sucrose synthase, soluble acid invertase and sucrose:sucrose 1-fructosyltransferase) were measured in the eight lines used in the Affymetrix GeneChip experiments. Hemicellulose and cellulose contents were measured in the three highest WSC and three lowest WSC SB lines (Xue *et al.* 2008).

The 194 RILs in the SB population were used to score 587 SSR, AFLP and DArT markers (McIntyre *et al.* 2006) of which 425 were used to construct a map (data not shown). QTL analyses of WSC concentration, measured in the SB population in trials over 2002- 2006, were undertaken using QTL Cartographer v2.0 (Bioinformatics Research Centre, North Carolina State University, USA). Putative QTL were identified at LOD>3.0 as either multi-site (two or more trials) or single site QTL; suggestive QTL 2.0<LOD<3.0) were also noted.

Results and discussion

The high WSC progeny group had significantly higher WSC concentrations in the upper two internodes (195 mg g⁻¹ dry wt) than the low WSC progeny group (164 mg g⁻¹ dry wt). Over the 16 SB RILs, WSC concentration was positively correlated with individual grain weight ($r = 0.72$, $p < 0.01$) and grain yield ($r = 0.65$, $p < 0.01$).

Of the 325 genes identified through Affymetrix GeneChip analysis as differentially expressed between four high and four low WSC SB progeny lines, 127 were up-regulated in high vs. low WSC lines and 198 were down-regulated; approximately 25% of these were genes encoding enzymes involved in carbohydrate metabolism. Quantitative RT-PCR analysis across the 16 SB RILs revealed that mRNA expression levels of genes encoding enzymes involved in fructan synthesis, such as sucrose:sucrose 1-fructosyltransferase (1-SST) and sucrose:fructan 6- fructosyltransferase were positively correlated with WSC concentration. In contrast mRNA levels of genes encoding enzymes involved in sucrose hydrolysis (sucrose synthase, SS and soluble acid invertase, SAI), sucrose catabolism (fructokinase and pyruvate dehydrogenase) or capable of diverting carbon to cell wall synthesis pathways (UDP-glucose 6-dehydrogenase, UDP-glucuronate decarboxylase and cellulose synthase) were negatively

correlated with WSC levels (Figure 1). Enzyme activities of 1-SST, SS and SAI were found to be positively correlated with their mRNA levels (Figure 2). This enzyme activity analysis thus supports the observed genotypic variation in mRNA levels of genes encoding these enzymes. Cellulose and hemicellulose content of the stem of three high WSC lines was found to be lower than that in low WSC lines. This is consistent with the negative correlation observed between mRNA levels of genes encoding enzymes involved in stem wall polysaccharide synthesis and WSC levels (Figure 1). Together these data suggest potentially differential partitioning of carbon to cell wall components between high and low WSC lines.

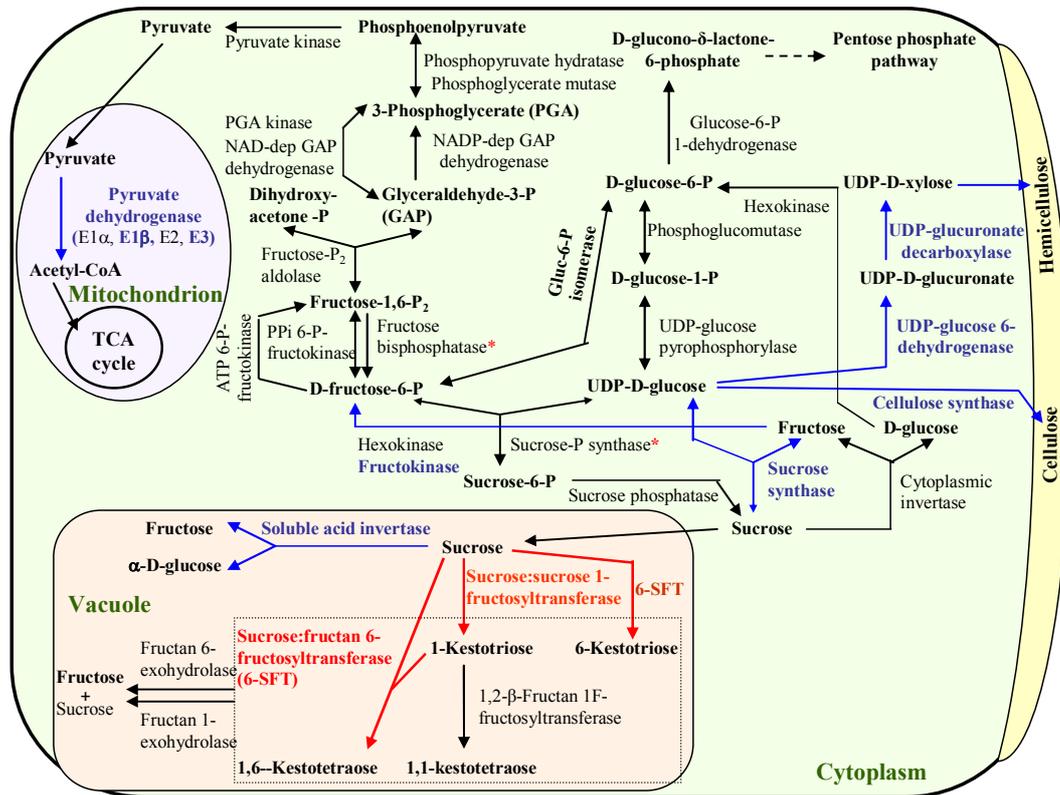


Figure 1. Illustration of major WSC metabolic pathways and WSC-correlated enzyme families. The total mRNA levels of individual enzyme families were determined by Affymetrix GeneChip analysis. **Red** colour indicates enzyme families with the total mRNA levels positively correlated with stem WSC concentrations; **blue** indicates enzyme families with the total mRNA levels inversely correlated with WSC; **black** indicates enzyme families that showed no significant correlations. After Xue *et al.* (2008).

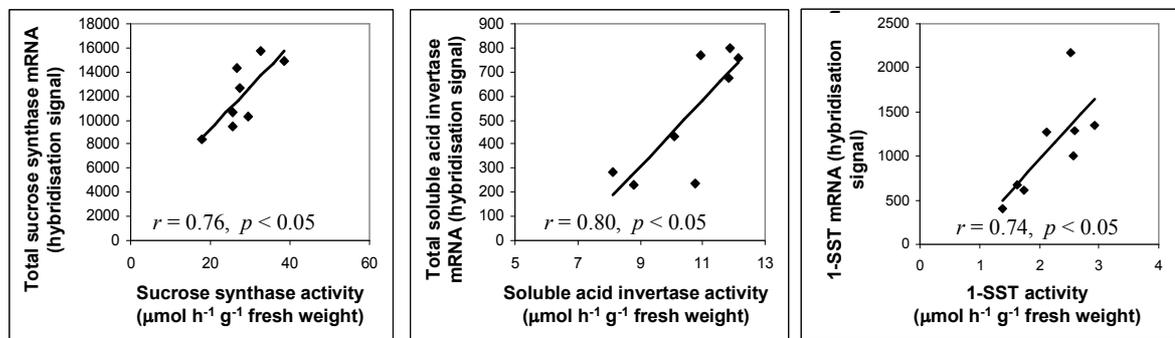


Figure 2. Total mRNA levels of sucrose synthase, soluble acid invertase and sucrose:sucrose 1-fructosyltransferase are correlated with their enzyme activities in 8 SB lines. After Xue *et al.* (2008).

QTL analysis of WSC in the SB population identified five multi-site putative QTL on chromosomes 1D, 4A, 6B, 7A and 7D, each explaining between 4-13% of the phenotypic variation. For all five QTL, the Seri alleles favoured increased levels of WSC. Approximately one-third of the genes encoding enzymes involved in carbohydrate metabolism (identified in the genomics studies above) have been deletion-bin mapped. Of these, approximately one-quarter map to one of the five chromosome arms in which a QTL for WSC was identified in the SB population (data not shown). In addition, approximately one-third of the non-carbohydrate metabolism genes that were differentially expressed mapped to these 5 chromosome arms (data not shown), suggesting that the genetical genomics approach has identified an enriched set of genes that potentially underpin genetic variation in the WSC trait.

Conclusions

The research presented in this paper is part of a larger field-to-laboratory study that aims to identify the physiological, genetic and genic bases of high yield and larger grain size under water-limited conditions. Genetical genomics integrates genetic (segregation in populations) and genomic (large scale gene expression) studies to assist in our understanding of the functional and regulatory networks that underpin key traits that are relevant to adaptation in water-limited environments. The approach is being used for other traits, including transpiration efficiency, in our laboratory. Future laboratory work on WSC will focus on regulation of genes involved in the WSC trait, including the mapping of expression QTL.

References

- Guillaumie S, Charmet G, Linossier L, Torney V, Robert N, Ravel C (2004) Colocation between a gene encoding the bZip factor SPA and an eQTL for a high-molecular-weight glutenin subunit in wheat (*Triticum aestivum*). *Genome* **47**, 705-713.
- Jansen RC, Nap JP (2001) Genetical genomics: the added value from segregation. *Trends in Genetics* **17**, 388-391.
- McIntyre CL, Chapman SC, Mathews KL, van Herwaarden AF, Reynolds MP, Shorter R (2006) Identification of germplasm and genomic regions for traits relevant to wheat production in drought environments. In 'Proceedings 13th Australasian Plant Breeding Conference'. Christchurch New Zealand.
- Olivares-Villegas JJ, Reynolds MP, McDonald GK (2007) Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Functional Plant Biology* **34**, 189-203.
- Potgieter AB, Hammer GL, Meinke H, Stone RC, Goddard L (2005) Three putative types of El Nino revealed by spatial variability in impact on Australian wheat yield. *Journal of Climate* **18**, 1566-1574.
- Xue GP, McIntyre CL, Chapman S, Bower NI, Way H, Reverter A, Clarke B, Shorter R (2006) Differential gene expression of wheat progeny with contrasting levels of transpiration efficiency. *Plant Molecular Biology* **61**, 863-881.
- Xue GP, McIntyre CL, Jenkins CLD, Glassop D, van Herwaarden AF, Shorter R (2008) Molecular dissection of variation in carbohydrate metabolism related to water-soluble carbohydrate accumulation in stems of wheat. *Plant Physiology* **146**, 441-454.