Water soluble carbohydrate accumulation in *Triticum aestivum*

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ABSTRACT

Water soluble carbohydrates (WSC) accumulated in wheat stems are an important carbon source for grain filling. Variation in stem WSC concentration among wheat genotypes is one of the genetic factors thought to influence grain weight and yield under water-limited environments. Here, we describe the molecular dissection of carbohydrate metabolism in the stems of *Triticum aestivum* at the WSC accumulation phase. Gene expression profiling analysis of carbohydrate metabolic enzymes revealed that the mRNA levels of two fructan synthetic enzyme families (1-SST and 6-SFT) in the stem were positively correlated with stem WSC and fructan concentrations, while the mRNA levels of enzyme families involved in sucrose hydrolysis were inversely correlated with WSC concentration. The transcript levels of two SNF1-related protein kinases were also positively correlated with stem WSC concentration and appeared to be co-regulated with the expression levels of 6-SFT and 1-SST in wheat stems. The data suggest that differential carbon partitioning in the wheat stem is one mechanism that contributes to variation in WSC accumulation.

INTRODUCTION

Water soluble carbohydrates (WSC) accumulate in the stem and leaf sheath of wheat during the early reproductive phase of wheat and other cool-season cereals and serve as temporary carbohydrate reserves. WSC in wheat stems is mainly composed of fructans, sucrose, glucose and fructose, with fructans being the major component at the late stage of the WSC accumulation phase (Ruuska et al., 2006). Fructans are soluble linear or branched β-2,1- or β-2,6-linked fructosyl-oligosaccharides, that are derived from sucrose and synthesized in the vacuole (Van Laere and Van den Ende, 2002; Chalmers et al., 2005).

WSC can accumulate in wheat stems to more than 40% of total stem dry weight (Housley, 2000). WSC mobilises from the stem during the later phase of grain filling and thus can become an important source of assimilate for grain yield in wheat under terminal drought stress conditions (Blum, 1998). Stem WSC accumulation is influenced by environmental factors (Blum, 1998; Ruuska et al., 2006, 2007). However, considerable genotypic variation in stem WSC concentration has been observed in wheat (Ruuska et al., 2006; Xue et al., 2008). Genotypic ranking among wheat genotypes in stem WSC concentration is generally consistent across environments, with large broad-sense heritability (H = 0.9) (Ruuska et al., 2006). Genotypic differences in stem WSC concentration at anthesis are attributed mainly to the fructan component (Ruuska et al., 2006; Xue et al., 2008). Positive relationships between stem WSC concentration at anthesis and wheat grain weight, or yield under certain water-limited environmental conditions, have been observed in several studies (Asseng and van Herwaarden, 2003; Ruuska et al., 2006, Xue et al., 2008). Therefore, high WSC concentration is considered to be a potentially useful trait for improving grain weight and yield in water-limited wheat production environments (Blum, 1998; Asseng and van Herwaarden, 2003; Ruuska et al., 2006; Foulkes et al., 2007). To understand the molecular mechanisms underlying the WSC trait, detailed molecular studies have recently been performed in wheat and these studies have shed some light on genotypic differences in stem WSC accumulation (Xue et al., 2008; and Ruuska et al., 2008).

GENOME-WIDE EXPRESSION ANALYSIS FOR DISSECTING GENOTYPIC VARIATION IN EXPRESSION LEVELS OF CARBOHYDRATE METABOLIC GENES RELATED TO WSC ACCUMULATION

To dissect the molecular basis of genotypic variation in stem WSC concentration, Affymetrix GeneChip expression analysis was performed using the stems of recombinant inbred SB (Seri/Babax) lines of bread wheat, varying in stem WSC concentration (Xue et al., 2008). This study focused on genes involved in metabolic pathways of glycolysis, gluconeogenesis, and sucrose and fructan synthesis and hydrolysis. This expression analysis revealed that the mRNA levels of 11 enzyme families showed significant correlations with stem WSC concentrations in 8 SB lines (Table 1). Among these, 4 enzyme families (fructose bisphophatase, sucrose phosphate synthase, Ta1-SST and Ta6-SFT) that have a role in shifting the metabolic flow of carbon towards sucrose and fructan synthesis appear to be positively correlated with the stem WSC concentrations. In contrast, the transcript levels of the enzyme families that are involved in hydrolysis of sucrose (acid invertase and sucrose synthase) or diverting carbon away from the sucrose synthetic pathway (e.g., fructokinase, pyruvate dehydrogenase and UDP-glucose 6-dehydrogenase) are inversely correlated with the stem WSC concentrations in the SB lines. The WSC-correlated expression patterns of these enzymes were also observed in an extended group of SB lines (16 lines) using quantitative RT-PCR (Xue et al., 2008). These data provide evidence on the mRNA regulation of

carbohydrate metabolic enzymes as a means for modulating WSC accumulation.

Table 1. Correlation analysis between the transcript levels of enzyme families and WSC concentrations in the stems of 8 SB lines at anthesis. The total mRNA level of an enzyme family in each line is the sum of the hybridisation signals of all isoenzymes from each family, derived from Affymetrix GeneChip analysis. The mean value of hybridisation signals from 2 field replicates of each line was used for correlation analysis between the total mRNA levels of each enzyme family and WSC concentrations at the genotypic level (n = 8). Correlations between in the mRNA levels of these families and WSC concentrations were also analysed at the genotype/replicate level (n =16, i.e., 8 genotypes × 2 field replicates), which provide further support for the significant corrections at the genotypic level. * ($p < 0.05$); ** ($p < 0.01$).

Enzyme name	Correlation coefficient between total mRNA level and WSC	
	Genotype (n = 8)	Genotype & replicate (n = 16)
Mitochondrial pyruvate dehydrogenase complex - E1β	-0.79*	-0.64**
Mitochondrial pyruvate dehydrogenase complex - E3	-0.80*	-0.61*
Fructose bisphophatase	0.72*	0.66**
UDP-glucose 6-dehydrogenase	-0.88**	-0.68**
UDP-glucuronate decarboxylase	-0.86**	-0.67**
Cellulose synthase – CesA1	-0.84**	-0.57*
Cellulose synthase – CesA4-like	-0.82**	-0.62*
Cellulose synthase – CesA10	-0.86**	-0.68**
Sucrose phosphate synthase	0.82**	0.69**
Sucrose synthase	-0.77*	-0.70**
Fructokinase	-0.75*	-0.56*
Soluble acid invertase	-0.87**	-0.74**
Sucrose:sucrose 1-fructosyltransferase (Ta1-SST)	0.86**	0.85**
Sucrose:fructan 6- fructosyltransferase (Ta6-SFT)	0.92**	0.86**

GENOTYPIC VARIATION IN THE ENZYME ACTIVITIES OF SUCROSE SYNTHASE, SOLUBLE ACID INVERTASE AND SUCROSE:SUCROSE 1-FRUCTOSYLTRANSFERASE IN WHEAT STEMS

Sucrose synthase, soluble acid invertase and Ta1-SST are three important differentially expressed enzymes with relatively high genotypic differences in expression level between high and low WSC SB lines (Xue et al., 2008). To ascertain whether genotypic differences in the transcript levels of these enzyme families are associated with these enzyme levels, the activities of the three enzymes in the stem of the 8 SB lines that were used in Affymetrix GeneChip analysis were examined. The enzyme activities of these three enzymes in the 8 SB lines were significantly correlated with the total mRNA levels of their respective enzyme families derived from the Affymetrix GeneChip data (Table 2). The activities of sucrose synthase and soluble acid invertase were inversely correlated with the stem WSC concentrations in these SB lines (Table 2). The relative enzyme activity of Ta1-SST in the stem of 8 SB lines was also significantly correlated with the stem WSC and fructan concentrations (Table 2). These biochemical data further support that the role of these enzymes in controlling the levels of stem WSC accumulation.

Table 2. Genotypic variation in the activities of sucrose-utilising enzymes in the stems of SB lines at anthesis

Enzyme	Correlation coefficient		
	Enzyme activity and mRNA level	Enzyme activity and WSC level	Enzyme activity and fructan level
Sucrose synthase	0.76*	-0.74*	
Soluble acid invertase	0.80*	-0.71*	
Ta1-SST	0.74*	0.84**	0.82*

POTENTIAL INVOLVEMENT OF SNF1-RELATED PROTEIN KINASE SUBFAMILY 3 (SnRK3) GENES IN CONTROLLING GENOTYPIC DIFFERENCES IN FRUCTAN ACCUMULATION

The SnRK family are known to be involved in regulating carbon metabolism in plants (Halford et al., 2003). Comparative analysis of global expression profiles of the stem samples from SB lines varying in WSC concentration revealed that the mRNA levels of two members (WPK4 and TaPK7) of the SnRK3 subfamily were highly correlated with the stem WSC levels, with correlation coefficients of 0.88 for TaPK7 and 0.81 for WPK4. This positive relationship between the TaPK7 and WPK4 expression and WSC level appears to be associated with strong correlations in expression levels between these kinases and fructan synthetic enzymes (Ta6-SFT1 and Ta6-SFT2 and TaSST2 as shown in Figure 1). The positive association between the levels of these two kinase transcripts and stem fructan or fructosyltransferase transcripts was also observed in wheat plants grown with the different levels of nitrogen (Ruuska et al., 2008). Plants grown under a low nitrogen level accumulate a much higher level of stem fructan than those with a high nitrogen level. The enhanced fructan accumulation in the low-nitrogen-treated plants is accompanied with the high transcript levels of WPK4, TaPK7 and fructosyltransferase genes. In addition, WPK4 and fructosyltransferase genes are all up-regulated by sucrose (Ruuska et al., 2008). These expression data indicate that WPK4 and TaPK7 are potentially involved in controlling fructan synthesis in the stem. However, whether this control is executed at

the fructosyltransferase transcriptional or enzyme level is currently unknown.

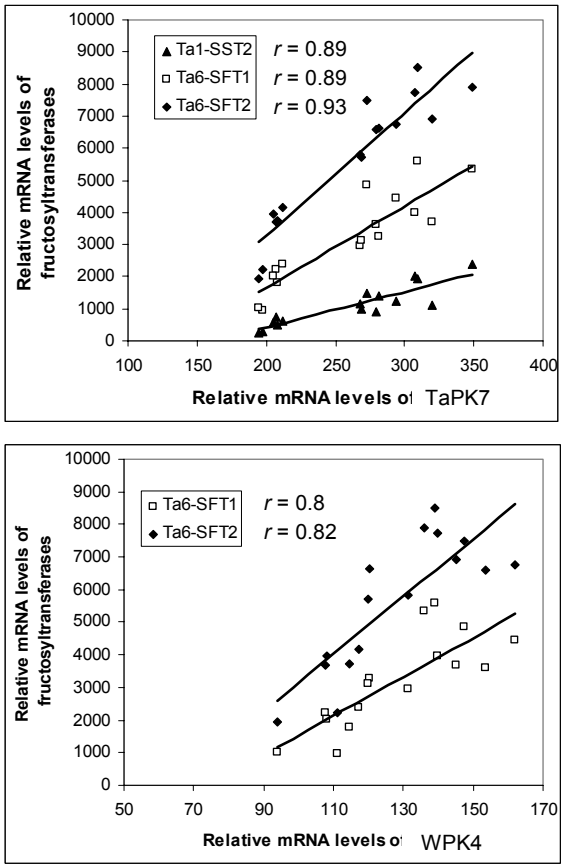

Figure 1. Co-regulation of SnRK3 and fructosyltransferase genes in the stems of SB lines at anthesis. Data are derived from Affymetrix GeneChip analysis (Xue et al., 2008). WPK4 (AB011670); TaPK7 (TC253507).

Overall, the data derived from a combined analysis of global transcript profiling and key enzyme activities or end product contents in stems suggest that the high stem WSC trait in wheat is associated with enhanced fructan deposition and reduced rates of sucrose hydrolysis and carbon partitioning into other cellular processes. These molecular studies also pinpoint some potential targets for metabolic engineering to improve stem WSC concentration in wheat. However, a complete picture of the molecular basis of the WSC trait and its contribution to grain weight and yield awaits further molecular analyses of genotypic differences in WSC metabolism in the source leaf organ and its mobilization from stem to grain during the grain filling period.

ACKNOWLEDGEMENT

This study was supported by the Australian Grains Research & Development Corporation.

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