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Serial analysis of gene expression of malting barley

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Barley is the world’s fourth most important cereal crop and it’s economic worth to the malting and brewing industries has led to substantial research of the biochemical and physiological processes of barley seed germination. The introduction of large-scale gene expression technologies such as serial analysis of gene expression (SAGE) has enabled the analysis of a large number of expressed transcripts using a quantitative approach. By using a series of enzymatic reactions, cDNA transcripts are processed into short 21bp tags which can be joined by concatenation into longer clones for sequencing (Gowda et al., 2004). Each tag is directly related to a gene transcript enabling quantitative analysis of thousands of expressed genes without any prior knowledge as to their identity. In this study eight LongSAGE libraries were constructed from barley var. Tallon to characterise the transcriptional profile of grain during the malting process (White et al., 2006). One library was constructed from mature un-steeped seed and seven libraries were generated from barley grain at 0, 12, 24, 48, 72, 96 and 120 hours post steeping. From 155,206 LongSAGE tags, 41,909 unique tag sequences were identified (Table 1).

The 100 most abundant tags from each library were analysed to determine the putative function of the most frequent transcripts. Significant functional groups include stress response and cell defence, protein synthesis, cell structure and plant growth, storage proteins and metabolism. Stress response and cell defence transcripts represent 29% of gene expression amongst the 100 most abundant tags (Figure 1). Stress and defence transcripts with high levels of expression include: dehydrin, gamma-thionin, amylase and protease inhibitors, metallothionein, thaumatin-like and glycine-rich proteins.

Transcripts associated with metabolism account for 8% of expression amongst the most frequently encountered tags (Figure 1). Enzymes involved in carbohydrate metabolism account for a large proportion of the metabolic transcripts. This is in accordance with the significant role which they play in the hydrolysis of starch and cell walls, essential in the process of germination. Amongst the most abundant tags we found matches to: alpha-amylase, alpha-glucosidase, (1-3, 1-4)-beta-D-glucanase, beta-1,3-glucanase, beta-1,4-glucanase, beta-glucosidase, a GDSL-motif lipase/hydrolase protein and xyloglucan endotransglycosylase-like protein.

Theses transcripts were substantially up-regulated at 48 h post-steeping and remained appreciably high throughout germination (Figure 2). The increased activity of these enzymes enables the storage reserves of the grain to be released and exploited in the developing seedling. Breakdown of the cell walls by (1-3, 1-4)-beta-D-glucanase allows starch hydrolysing enzymes, such as alpha- and beta-amylase, to enter the cell. By studying the expression level of particular genes, such as those involved in carbohydrate metabolism, we can gain a greater understanding of these systems and their role in barley seed germination and malting.