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Improving nitrogen utilisation and amino acid composition in barley used for animal feed

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Short Abstract: Microarray data on the nitrogen utilization in barley have been analysed using clustering of differentially expressed genes. From the annotation of co-regulated genes a map of affected biological processes was drawn and compared with data on Arabidopsis to construct a conjectural overview of the nitrogen utilization in barley.

Long Abstract:

According to the Danish water plan (the Water Plan III) the load of nitrogen fertilizer has to be minimized with 13% before 2015 in Denmark to avoid further environmental nitrogen contamination. To meet the new demands for low input nitrogen fertilization regimes in Danish agriculture the nitrogen utilization efficiency has to be improved.

Therefore, knowledge concerning nutrient uptake, plant component portioning and processes of grain development, especially grain filling are crucial to provide powerful resources for future breeding initiatives to have better seed quality and higher yield. To achieve this objective the elucidation of specific key candidate genes essential for grain filling, regulation of amino acid and prolamin metabolism could be one way to improve breeding of barley with improved nutritional composition and lower environmental impact.
The barley seeds nutritional quality is primarily due to the accumulation of high levels of storage products, mainly starch and protein. Starch and protein account for 50-70% and 10-12% of the grain dry weight, respectively. Developing seeds import both sucrose and amino acids from the phloem. Glutamine and glutamate are the most abundant translocated amino acids, but serine, aspartate and alanine are also found in high amounts in the phloem [Weibull, 1990]. The majority of the translocated amino acids function as building blocks for the synthesis of storage proteins called hordeins. Hordein B and C are the two major groups of storage protein that account for 40-50% of the total seed protein in barley. The B (sulphur-rich) and C (sulphur-poor) hordeins constitute for 95% of the total hordein fractions in relation to the two minor groups termed D and hordeins.

To describe the expression of genes in the developing seeds a set of comprehensive genes has been selected to identify candidate genes using cDNA microarray. Two cDNA libraries from barley containing spikes and seed coat 20 days after pollination (DAP) have been chosen from Clemson University. Approximately 1100 genes have been carefully selected within the carbohydrate and nitrogen metabolism, transport and regulation in the barley grain.

The experimental work was broken into two parts: First a pilot experiment in which the

fabricated cDNA microarray was used for detection of candidate genes in barley seeds grown at three different nitrogen regimes. Second, a larger investigation was planned and carried out building on the observations from the previous experiment. This time a larger number of barley spikes were carefully harvested at different time stages during the grain filling period.

The RNA isolation from the barley seeds from both experiments has undergone initial processing followed by cDNA microarray hybridisations. The resulting hybridisations were scanned and the spots from the image have been analysed. Normalization was performed using the Qspline algorithm which among other things corrects for dye-specific effects [Workman et al. 2002]. Traditional significance testing methods were used to identify a high-confidence set of differentially expressed genes which were extracted for further analysis.

In all cases, co-regulated genes were identified by performing a PAM clustering (Partitioning Around Medoids); a method similar to k-means clustering, but more robust and more reproducible. The functional annotation of genes clustering together was compared against the GeneOntology annotation of all genes and used to identify biological processes whose activity had been affected during the experiment. From this, we constructed a map of what processes were active and under which conditions.

Because the amount of available data on barley usable for cross-referencing and comparison to our data was insufficient, we mapped each of the barley genes from the microarray to the respective equivalent from other organisms (primarily Arabidopsis) using blast. Although this invariably introduces noise and error, it allows us to dramatically increase the amounts of data available to us for comparisons. For example, by examining the response overlap between our data and that observed from publicly available data sets we can classify the responses we observe from our arrays, and subsequently use that knowledge to facilitate the construction of a conjectural overview of the nitrogen utilization in barley.