Identification and characterisation of differentially expressed genes in wheat undergoing gradual water deficit stress.

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Abstract

Gene expression patterns in wheat plants subjected to a gradual water deficit stress initiated from two important developmental stages, terminal spikelet (TS) and boot (BT) are being studied. Two suppressive subtractive hybridisation libraries were prepared between well-watered and water-stressed wheat leaves and used to construct a small microarray containing approximately 300 expressed sequence tags (ESTs). The microarray was screened with RNA isolated from well-watered wheat leaves and wheat leaves from plants subjected to water deficit stress initiated at either TS or BT. Overall, 20% of the genes were significantly up-regulated and 10% were significantly down-regulated under water deficit stress with more changes in gene expression observed in leaves from plants subjected to stress initiated from the BT stage. Clustering analysis revealed five distinct patterns of differential expression, with two clusters showing different patterns of up-regulation, one cluster containing genes significantly down-regulated and two clusters containing genes displaying non-significant differential expression patterns. Northern analysis has confirmed the differential expression of these genes with progressive reduction in leaf relative water content. These genes will be mapped to see if they co-locate with QTLs associated with adaptation to water stress.

Media Summary

Key genes involved in adaptation of wheat to water stress are being studied using an integrated genomics, molecular genetics, physiology and plant breeding approach.

Key Words

Drought, ESTs, micro-arrays, gene expression, Northern

Introduction

Wheat is one of the most important crops worldwide and productivity in Australia and elsewhere is often limited by lack of available water necessary to maximise biomass and complete grain filling (Doyle and Fischer 1979). Abiotic stresses, including drought, induce two broad categories of genes. The first category includes those genes that protect against the stress, including osmoprotectants, turnover proteins, membrane modifiers and detoxification proteins, and the second includes those that regulate signal transduction and gene expression associated with other processes (Shinozaki and Yamaguchi-Shinozaki 2000) including regulatory genes, such as signalling molecules and transcription factors. Genetic control of adaptation to water deficit stress is thus likely to be complex and involve cascades of interactions within and between gene networks.

Microarrays are particularly useful for the study of gene expression changes and can be used to examine where and when specific genes associated with water deficit stress are expressed. Microarrays have been used to study short-term water deficit stress in barley (Ozturk *et al.* 2002), and Arabidopsis (Seki *et al.* 2002, 2003). However, both of these studies focussed on plants left to desiccate on a bench top for anywhere between 2-24 hours; these shock stress conditions are not comparable to a field situation where gradual drought stress affects intact plants in the soil over a period of days and weeks and at different times of the crop growth cycle.

The effect of water stress on wheat yield at different development growth stages has been investigated in the field (Zhang and Oweis 1999), and has shown that wheat yield is particularly susceptible to drought during two important growth periods. The first important growth stage is between terminal spikelet (TS) stage (DC30 in the decimal code of Tottman and Broad, 1987), and boot stage (BT) (DC41-47), and the second is between BT and anthesis (DC65) (Zhang and Oweis 1999).

We have initiated a gene expression analysis project investigating critical physiological stages that influence wheat yield under water-limited conditions. In this paper, we describe differences in gene expression detected in leaves between wheat plants under a 'field-like' slow dry down commencing at the TS (DC30) stage compared to plants under the same water stress beginning at BT (DC45) stage.

Methods

Triticum aestivum cv. Kennedy plants were grown under controlled environment conditions. Water was withheld from the plants from either the terminal spikelet (TS) or boot (BT) developmental stages for a period of 12 days. Flag leaf samples were collected daily from the onset of the stress and sorted according to their relative water content (RWC). RNA was isolated from the four bulked tissue samples (well-watered TS and BT, stressed TS and BT) and used for subtractive library construction, screening microarrays, and Northern blot analysis. Further details on the above methodologies, and on sequencing of ESTs, micro-array construction and analysis, and Northern analysis are available upon request from the corresponding author.

Results

Two subtracted suppressive hybridisation libraries, each containing approximately 2500 clones, were constructed. Sequencing of 500 random clones from the forward library and 100 random clones from the reverse library revealed no overlaps between the two libraries, indicating that the subtraction had been successful. Sequence similarity searches revealed 300 unique ESTs, of which approximately half had been previously described in the literature as drought associated. Of the remaining clones, approximately 25% either could not be assigned a function, or exhibited no similarity to any other sequence in the databases.

Analysis was performed on the microarray using replicate RNA samples isolated from wheat plants subjected to stress initiated at two different growth stages. More genes were significantly up- or down-regulated in leaves of plants where stress was initiated at the BT stage than at the earlier TS stage.

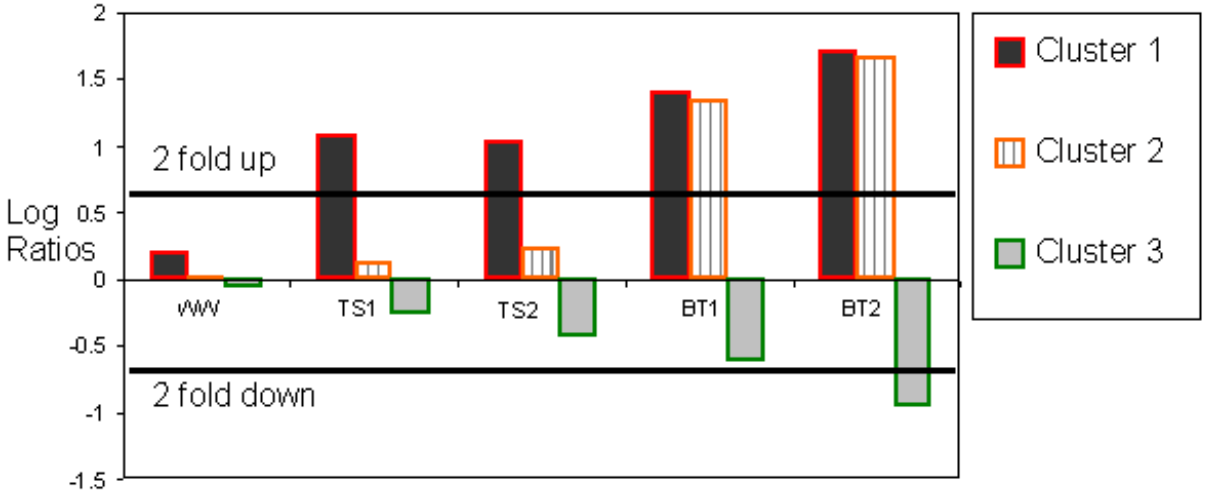

Figure 1. Average expression levels of genes at two different developmental stages for three of the five clusters identified. TS1 and TS2 = Biological replicates of RNA isolated from leaves of

wheat plants stressed from Terminal Spikelet. BT1 and BT2 = Biological replicates of RNA isolated from leaves of wheat plants stressed from Boot. Expression levels above or below the solid lines are >2-fold relative to non-stressed plants.

Cluster analysis was undertaken and identified 5 distinct patterns of gene expression (Figure 1). Cluster 1 contained 17 significantly (greater than 2-fold) up-regulated genes and included genes solely up-regulated under stress, as well as genes up-regulated by both developmental and stress effects. Genes in this cluster included highly expressed genes of unknown function and genes involved in osmoregulation, carbohydrate and protein metabolism. Cluster 2 contained 28 genes that were highly up-regulated under water deficit, especially in tissue from plants stressed from the BT stage. Again, this cluster included genes that were solely stress induced as well as others that were regulated by both developmental and stress effects. Cluster 2 contained 18 known genes which have been previously reported to be up-regulated under drought, including genes involved in amino acid and carbohydrate metabolism and osmoregulation. Cluster 3 contained 31 genes that were predominantly down regulated. This cluster included genes that were solely stress down-regulated, genes that were solely developmental stage regulated and genes that were regulated by both developmental and stress effects. More than half of the genes in this cluster were photosynthesis related; this is consistent with previous reports that have demonstrated that water stress decreases the rate of photosynthesis and starch accumulation in leaves (Seki et al, 2002). Two additional clusters were also identified, containing 96 and 128 genes, respectively (data not shown). Most of the genes in these latter two clusters were not significantly up or down regulated.

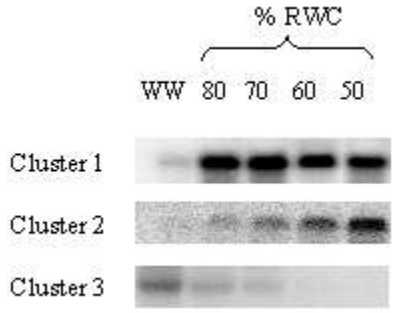

Figure 2. Changes in transcript abundance in wheat leaves during drought stress demonstrated using Northern analysis. One representative from three of the 5 clusters identified by microarray analysis is shown here. RWC = leaf relative water content. WW = well-watered (>95% RWC).

Northern analysis using RNA samples obtained from leaves with decreasing RWC was undertaken to validate the results obtained from the microarray experiment. A representative clone from each of Clusters 1, 2 and 3 was used and all three clones displayed the same trends in expression patterns in the Northern analysis (Figure 2) as they did in the microarray experiments. The clone from cluster 1 appeared to be rapidly induced as up-regulation of its gene expression was observed in the RNA sample isolated from leaves with RWC of 80% when compared to the clone from cluster 2. The clone from cluster 3 hybridised or amplified strongly in the well-watered tissue but was down-regulated with increasing water stress.

A genetic map is currently being constructed in a recombinant inbred line population derived from a cross between Seri and Babax and developed by CIMMYT, Mexico. This population has been selected as its progeny varies in the ability to yield under a range of abiotic stress conditions. The RIL population has been grown at several sites in Australia and Mexico and scored for numerous phenotypic traits, including yield. The above ESTs will be incorporated into the map. QTL analysis will be undertaken to see if any of these ESTs co-locate with QTLs associated with the traits measured in the population.

Conclusions

In this preliminary microarray experiment we have identified numerous genes, including many unknown or novel genes, associated with a slow “field-like” water deficit stress in wheat and have obtained preliminary evidence that water deficit stress has a different effect on gene expression patterns in the two developmental stages studied. This preliminary study provides a basis for future research to advance our understanding of the genetic control of traits that impact on the economic performance of wheat under water-limited conditions.

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