The Dof transcription factor family in wheat

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ABSTRACT

Plant specific Dof (DNA binding with One Finger) proteins play a diverse range of roles in the areas of plant growth and development, from regulation of the C_4PEPC gene promoter to the regulation of seed storage proteins. However, at present very little is known about the Dof family in wheat with only two proteins having been identified to date. This study has identified the Dof family members in wheat from available sequence data. This family was then analysed across publicly available datasets from Affymetrix wheat genome arrays. Organ specificity, grain and anther development and responses to stresses such as salt on Dof gene expression were analysed. Two genes were identified displaying high expression in stems and leaf sheaths and with high correlation to sucrose metabolic enzymes.

INTRODUCTION

Dof proteins are zinc finger transcription factors that are exclusively found in the plant kingdom, with the occurrence of dof-encoding sequences ranging from unicellular algae Chlamydomonas reinhardtii to angiosperms such as wheat. This indicates an ancient origin and the possibility of diversification throughout plant evolution[1]. Members of the Dof transcription factor family play important roles in plant growth and development with all biological processes speculated to be mediated by Dof proteins being plant specific. Roles range from key regulators in the light response of the C_4PEPC gene promoter [2,3] to regulation of genes that encode seed storage proteins and germination [4-6].

Despite the important roles of Dof proteins in plants and the importance of wheat as a food crop, this family has been studied to a very limited extent in wheat. To date, only two Dof proteins from wheat have been investigated, WPBF[5], an activator of prolamin gene expression and TaDof1[7] whose function is unknown. This project aims to identify all Dof family members from available sequence data and to investigate their role in plant growth and development in wheat.

Most known transcription factors can be grouped into families according to their DNA binding domain[8]. Members of the Dof family all contain the highly conserved 52 amino acid Dof domain, including a single zinc finger, in the N-terminal region. Outside of the Dof domain the proteins are highly divergent. With the completion of the Arabidopsis and rice genome, bioinformatic analysis has been used to identify the dof families in these two model species[1,8]. While the genome has not been fully sequenced for wheat a large amount of EST sequence information is available and can be used to identify potential Dof proteins.

METHODS

Nucleotide and deduced amino acid sequences of the Dof domains and full length proteins were obtained from the Database of Arabidopsis Transcription factors (http://datf.cbi.pku.edu.cn/) and the Rice Transcription Factor Database (http://ricetfdb.bio.uni-potsdam.de/v2.1/) and used to perform independent BLAST searches through several wheat databases including NCBI (http://www.ncbi.nlm.nih.gov/), the Plant Genome Database (http://www.plantgdb.org/) and Plant Gene Indices (http://compbio.dfci.harvard.edu/tgi/plant.html). With the aid of NCBI's UniGene contigs were assembled with sequences considered to be the same gene. Assembled contigs and identified singletons were aligned and sequences more that 98% identical were considered the same and the best representative contig selected.

The finalised sequences were used to BLAST the wheat Affymetrix database (http://www.affymetrix.com/index.affx) and representative probes selected. Expression patterns of these genes were then analysed across 6 publicly available datasets of Affymetrix wheat genome arrays. Data was normalised against 5 control genes (accession numbers BG906441, CJ649721, CJ555902, BJ250565 and BE404167).

RESULTS

Using the presence of a Dof domain as the defining feature of a Dof protein, currently available sequences from wheat were screened and 35 putative wheat dof proteins identified (data not shown). A large percentage of ESTs do not have a full length representative. Therefore, it is likely that some Dof proteins have not been included in this analysis due to the absence of the Dof domain in the database sequence.

As a result of the high level of conservation in the Dof domain most Dof proteins share similar target sequences [9,10]. Therefore, their tissue and cell specific localisation

is very important in determining their specificity of action. Fourteen Affymetrix probes each representing at least one Dof gene were identified to be present on the Affymetrix chip. Publicly available Affymetrix data were used to identify which organs the selected Dof proteins were expressed in.

Two probes were assigned to two selected Dof genes, accession numbers AY955493 (TaDof1)[7] and TC266812, and the transcript expression profiles of these two genes are investigated further in this communication. Figure 1 illustrates the expression profile for these two genes in different organs. Both were found to be expressed in the roots, shoots, anthers, leaves and predominately in the stem/leaf sheaths. They are however, not expressed in grain at 14 to 17 days post anthesis. These two proteins share a very high level of homology at 95.4 percent identity at the nucleotide level and possibly have a similar function. AY955493 has been further characterised here to elucidate a possible biological function by analysing levels of expression in response to stress and correlation of expression levels with other genes. Expression of AY955493 was found to be upregulated in response to salt stress in the shoots (Figure 2). In addition expression of AY955493 was found to be significantly correlated with expression levels of a number of sucrose metabolic enzymes including an apoplastic invertase 1 homologue and sucrose phosphate synthase (Figure 3).

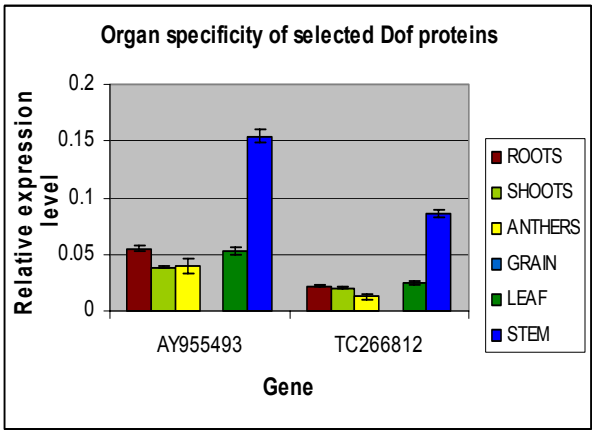


Figure 1: Expression analysis of AY955493 and TC266812 in wheat organs. Both AY955493 and TC266812 mRNA are expressed in all organs except grain. Highest expression is in the stem and leaf sheaths.

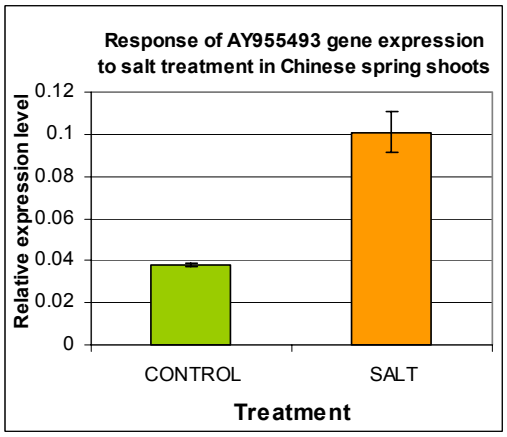
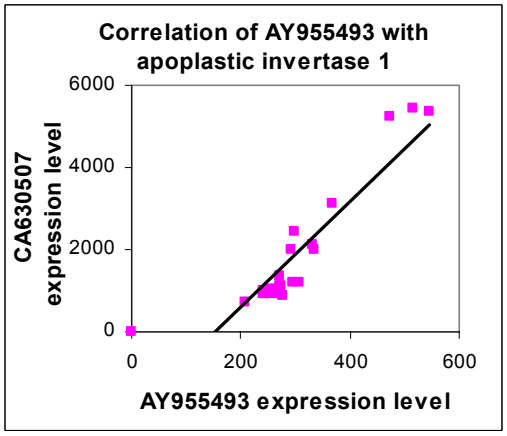


Figure 2: Expression analysis of AY955493 with salt treatment in Chinese Spring shoots. Expression of AY955493 mRNA dramatically increased with salt treatment.



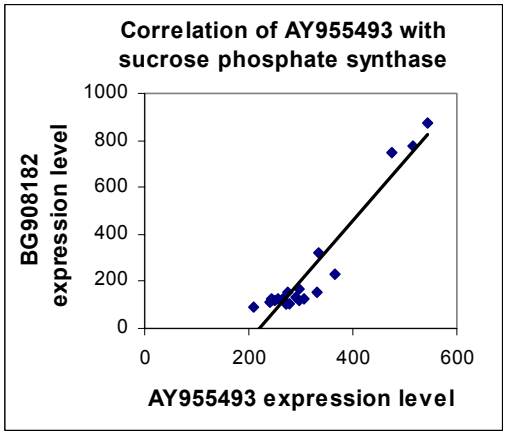


Figure 3: Correlation anlaysis of AY955493 expression patterns in the anthers. Expression of AY955493 is highly correlated with an apoplastic invertase 1 homologue (accession number CA630507) and sucrose phosphate synthase (accession number BG908182).

DISCUSSION

Previous bioinformatic analysis has identified 30 Dof proteins in rice[1] and 37 in Arabidopsis[8]. This analysis identified 35 putative Dof proteins in wheat. Using Affymetrix data, members of the Dof family were analysed and the expression patterns of two, AY955493 (TaDof1) and TC266812 are described here. Both were found to be expressed in all organs except grain with the highest levels in stems/leaf sheaths. This data is in agreement with previous organ expression profiling of these genes using quantitative RT-PCR (data not shown). AY955493 was also upregulated with salt treatment and highly correlated with a number of sucrose metabolic enzymes. These results await further investigation but indicate a possible involvement of these genes in stem development or in regulating carbon metabolism in the stem.

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