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Manipulation of the cell wall composition of wheat endosperm cell walls to improve nutritional properties

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Introduction

The cell walls of cereal endosperms are major components of the dietary fibre consumed by humans. In wheat they account for 2-3% of white flour and comprise principally arabinoxylan (AX) (65-70%) with ~20% of (1→3)(1→4) β-glucan and smaller amounts of glucomannan and cellulose (Shewry and Morell, 2001). Arabinoxylans consist of chains of xylose units with substitutions of arabinose molecules and ferulic acid cross-links. They include water-soluble and insoluble polymers which have high viscosity and affect processing and nutrition. In rice and barley, families of cellulose synthase (CesA) genes (Burton *et al.*, 2004) encode proteins which associate to form large complexes (rosette structures) which are responsible for the formation of microfibrils. The enzyme system involved in the biosynthesis of the (1→3, 1→4)-β-D-glucan homopolymer, with its unique organization of the two linkage types in a chain, involves at least one cellulose synthase-like enzyme, encoded by a CslF gene, one of two members of the Csl group found in monocotyledons that encode GT2 glycosyl transferases (Burton *et al.*, 2006). CslA family members from rice encode (1→4)-β-D-mannan synthases (Leipmann *et al.*, 2005).

We aim to manipulate the fibre composition of wheat flour to improve its contribution to human health, by identifying and manipulating the expression of genes encoding enzymes involved in arabinoxylan and β-D-glucan biosynthesis and determining the impact of these modifications on the composition and properties of the cell wall. Although relatively few plant cell wall biosynthetic genes of known function have been identified, it is possible to identify candidates for other steps by sequence similarity. In particular, the complete *Arabidopsis thaliana* genome is useful when mining for these. However, *Arabidopsis* is genetically very distant from wheat and we are therefore also exploiting the genome sequence of rice which is more closely related.

Results and discussion

A number of related genes that encode true cellulose synthases (CESA) and related proteins (cellulose synthase-like, CSL) have been identified in *Arabidopsis*, the latter being presumed to include xylan synthase genes as well as β-D-glucan synthase genes. We therefore used a new bioinformatics resource (WhETS) (Mitchell, 2006) to identify corresponding genes in the genome of rice and ESTs in databases of wheat sequences (Fig. 1).

ESTs (expressed sequence tags) represent mRNA sequences and their relative abundances in libraries represent transcript abundances in the tissues from which the libraries were made. Counts of ESTs corresponding to CSL genes in EST libraries made from developing wheat grains showed that sequences related to CSLF and CSLD were particularly abundant and this abundance was confirmed by expression profiling using cDNA arrays. In order of expression, three of the most abundant ESTs were chosen as candidate genes for further analysis.

Matching sets of wheat ESTs were assembled for each candidate gene and alignments of the resulting contigs and singlets to the rice template were displayed (Fig. 2). Oligonucleotide primers were then designed to amplify the corresponding sequences using the Polymerase Chain Reaction (PCR) with cDNA prepared from developing grain of wheat cv. 'Cadenza' as a template.

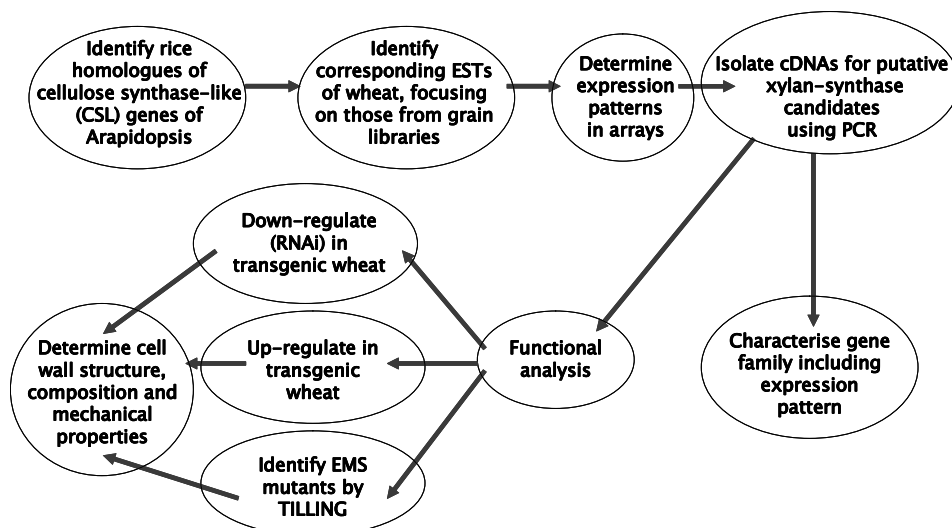


Fig. 1. A molecular approach to identifying and characterising xylan and glucan synthesis.

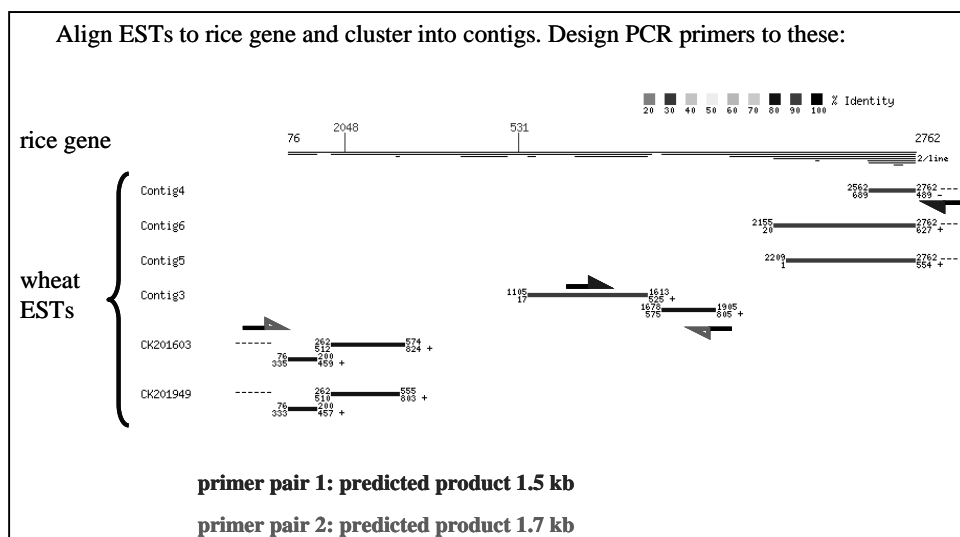


Fig. 2. Alignment of wheat ESTs to candidate rice gene. (Arrows showing two pairs of oligonucleotide primers).

The cDNA products were isolated and cloned into vectors. Clones from each product were sequenced (DNA Sequencing Facility Oxford) (Fig. 3).

Shorter cDNA sequences are required to design RNAi constructs for gene knockout. The same method was therefore used to design one set of primer pairs for each candidate gene based on the conserved regions of the sequences.

The RNAi vectors are being used to transform bread wheat cv. 'Cadenza' using the *Agrobacterium*-based system established at Rothamsted (Jones *et al.*, 2005) using the starchy endosperm-specific promoter of the HMW subunit 1Dx5 gene to drive expression (Lamacchia *et al.*, 2001). The transformed plants will then be analysed to determine the effects of the manipulations on the composition and mechanical properties of the cell walls.

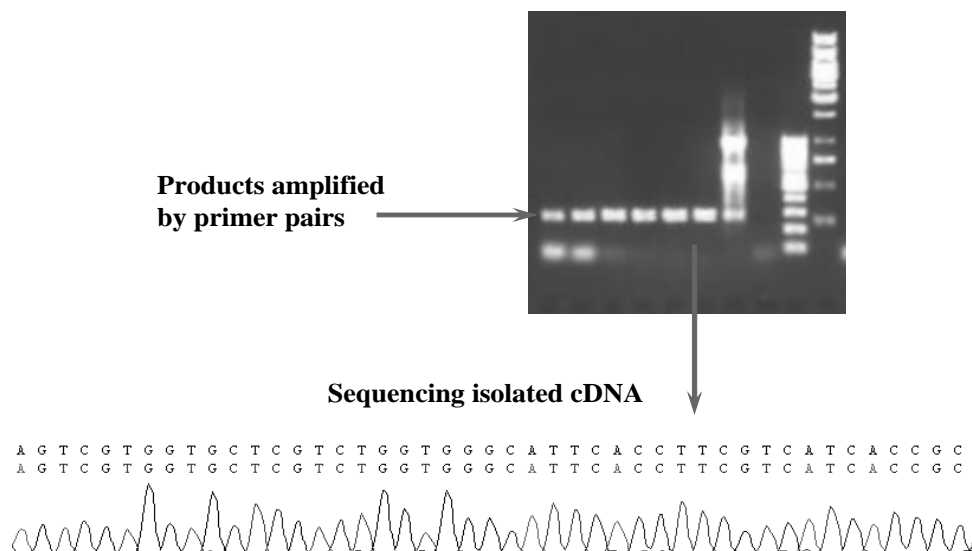


Fig. 3. PCR amplified cDNA products on agarose gel and part of the sequence of the same product.

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