



Functional genomics in the growth and end-use quality of cereals.

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Abstract

The international research trend towards functional genomics of cereals has been demonstrated by the huge commercial and public investment in this technology around the world. Access to and control of the technology, together with the discovery of gene systems that will flow from cereal genomics work, is seen as a key to the future of cereal production. While cereal research in Australia has an excellent international reputation, this is threatened by the sheer scale of investment in functional plant genomics in Europe and North America. The development of an integrated research effort in cereal genomics offers us a means for creating a national research focus that will enhance Australia's capacity not only to be part of the current revolution in agricultural biotechnology, but also to remain highly competitive in an existing area of research strength. In 2000, the Grains Research and Development Corporation commenced funding a research centre for Functional Genomics in the Growth and End-use Quality of Cereals at the Universities of Adelaide and Melbourne, with collaborative links into international functional genomics programs in both the private and public sectors.

Introduction

The overall aim of the Centre for Functional Genomics in the Growth and End-use Quality of Cereals is to apply emerging tools, technologies and expertise in cereal genomics to specific targets of relevance to Australian cereal industries, namely the definition of factors that control early seedling growth and vigour, and grain quality. There are two major components of the Program; the use of genomics to analyse the genes and enzymes controlling cell wall development in cereals and the identification of genes controlling the early stages of embryo and endosperm development. An outline of the objectives and progress in work on the embryo and endosperm development projects is covered in a separate paper. This article will focus on the central role of cell walls, which are primary determinants of seedling growth and

development. Walls are also key components in the resistance of plants to pathogens, in human nutrition, and in determining many key quality characteristics of the mature grain.

Subtle variations in the fine structures of major wall polysaccharides can have a dramatic effect on the functional/rheological properties of cereal-based products. The major wall components in young vegetative tissues and in grain are arabinoxylans and (1,3;1,4)- β -glucans; other wall components include cellulose, some protein and other minor polysaccharides. Although their distribution in higher plants is restricted to cell walls of grasses, the (1,3;1,4)- β -glucans are constituents of most human diets and many animal feed formulations, because the grasses includes cereals such as wheat, rice, maize, barley, rye, sorghum and millet. As cell wall components, the (1,3;1,4)- β -glucans usually make a relatively minor contribution to the total weight of cereal grains, but they can have a disproportionately large impact on grain technology, utilisation and nutrition. This impact is largely attributable to the propensity of these polysaccharides to be extracted from walls with aqueous solvents and thereafter to form solutions of high viscosity. Feruloylated arabinoxylans exhibit similar physicochemical properties. Thus, in baking with cereal flours, the (1,3;1,4)- β -glucans and arabinoxylans will influence dough rheology, and in malting and brewing they can adversely affect the efficiency of malt extraction, filtration processes and the quality of the final beer. Similarly, (1,3;1,4)- β -glucans can have undesirable effects on the digestibility of cereal-based stockfeeds by monogastric animals such as pigs and poultry. In contrast, they are important constituents of the 'dietary fibre' component of human foods, which is considered to be of salutary importance in several areas of human digestion and health (Bhatty 1993).

Despite the importance and contributions of different wall components to agro-industrial processes such as paper and pulping, food quality and texture, dietary fibre and ruminant digestibility, genetic manipulation of the major wall components, the polysaccharides, has been hampered by the paucity of our knowledge of the mechanism(s) and control of the biosynthetic steps and, until recently, the lack of any clone for a plant polysaccharide synthase. The relatively recent cloning of a plant cellulose synthase gene (Pear *et al.*, 1996; Arioli *et al.*, 1998) and the identification of mutants in cell wall components (Reiter, 1998) should pave the way for genetic tailoring of wall phenotypes for specific cereal-based processes.

As plants grow, cells in the young vegetative tissues elongate in the direction of the growth. For this to occur, the cell walls which surround the elongating cells are "loosened", in part *via* partial hydrolysis of polysaccharides that non-covalently cross-link cellulosic microfibrils in the wall. Turgor pressure then forces the cellulose microfibrils to slip past each other as the strength of the cross-linking or matrix phase between the microfibrils is weakened (Cosgrove, 1999). Thus, these changes in cell wall structure and the fine structure of constituent polysaccharides are key determinants of seedling growth and development. The genes and enzymes that control growth are largely unknown. Identification of these genes will enable us to define factors that influence early growth of the coleoptile, coleorhiza and young seedling generally. A detailed understanding of genes required for rapid seedling establishment, together with those that cause growth to cease in the face of abiotic stresses, would provide opportunities for the enhancement of early vigour in young cereal seedlings.

Methods

The Centre's research program is divided into six projects:

- Project 1. Genes and enzymes responsible for cell wall synthesis in cereals
- Project 2. Coordination of gene expression and enzyme activity during growth of young seedlings
- Project 3. Coordination of gene expression and enzyme activity during differentiation in developing cereal grains
- Project 4. Construction of cell and tissue specific libraries from wheat meiocytes and components of the developing wheat grain
- Project 5. Transcript analysis from specific cells and tissues
- Project 6. Identification and analysis of genes controlling early embryo and endosperm development

Research in the Centre is integrative and multidisciplinary. Seedling vigour, growth and grain quality are examined, not only with a view to the discovery of genes and the mechanisms of their regulation, but also to define the key enzymes and proteins that ultimately control seedling growth and grain development, and to determine the 3-D structure of these enzymes and proteins. In this way we can go beyond gene sequence and define the entire process of wall assembly and modification. Thus, the research will define seedling growth and grain development all the way from the gene level (genomics), through to the enzymes involved in wall synthesis in the cell (proteomics), and finally to the chemical structures of cell wall components themselves. Although the current focus is on the role of cell walls during seedling growth and endosperm development, flexibility will be maintained to ensure that the integrated experimental approach developed in the program can be readily transferred and applied to new targets identified by the cereals industries.

Results and Discussion

The research activities of the Program began in January 2000, and significant progress has been made in the generation of EST libraries, the development of new methods, including 2D protein separations and transcript imaging, analysis of cell walls and the identification of candidate genes for further analysis. Highlights of recent research include:

- barley suspension cultures have been established and protoplast production has begun
- wall development in elongating coleoptiles has been monitored, polysaccharide analyses performed, the first EST sequences have been generated, and cDNA libraries have been sent for sequencing
- initial 2-D gel electrophoresis experiments have been performed for the proteomics components of the Program. The first amino acid sequences of proteins eluted from the gels have been generated.
- all the polysaccharide analytical techniques are established and exchange of personnel and samples is working effectively

- new techniques in transcript analysis have been developed and are again producing encouraging results
- database mining has been used to find genes involved in cell wall synthesis, and in early endosperm and embryo development. The need to obtain the maximum benefit from existing databases, available in both the public and private sectors, has been emphasised
- virus-induced gene silencing systems have been established for the functional analysis of candidate genes
- other model plant systems have been used as experimental tools to the advantage of the Program. In particular, closer links with rice and *Arabidopsis* functional genomics programs and the use of these plants in transformation experiments has significantly advanced progress towards our research objectives
- significant progress has been made in the microdissection of endosperm and embryo tissues during the very early stages of grain development. cDNA libraries have been generated for EST sequencing.

Our bioinformatics system is currently under development, but further work and investment in this area will be necessary to efficiently store, process and analyse the large volumes of data that are being generated. A key early requirement in the establishment of a bioinformatics capability is an efficient "laboratory information management system" (LIMS). The LIMS is central not only to the management of vast amounts of electronic information generated during EST sequencing, database mining, transcript analysis, proteomics and gene discovery activities in general, but is also crucial for the effective management of financial/purchasing activities and communication between the research groups.

To enhance and facilitate communication within the Program, a web page has been established by Dr. Ute Baumann and will be developed further in the immediate future. The web page has two components. The first has provides general information about the FG Program. The page is generally accessible at <http://planta.waite.adelaide.edu.au/genomics/>. This component is seen as a vehicle to publicize the Program through the scientific and agricultural biotechnology community, as well as for attracting students and postdoctoral fellows. The other component will be password-protected, for research staff only. Experimental results and other research information will be accessible to all staff at both institutions through this page.

Conclusions

At this stage many candidate genes encoding enzymes involved in cell wall biosynthesis in barley have been identified and isolated. These include members of the putative cellulose synthase gene family (*CesA*), cellulose synthase-like genes (*Csl*) and genes encoding polysaccharide hydrolases that might also participate in wall synthesis and re-modeling. The identification has relied heavily on the establishment of new methods for transcript analysis and for the generation of cDNA libraries from very small samples of tissues in young embryos and early endosperm of the grain. As a result of the rapid identification of candidate genes, the emphasis of the program

will gradually shift to the development of rapid functional analysis systems for defining the exact role of individual candidate genes in wall development.

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