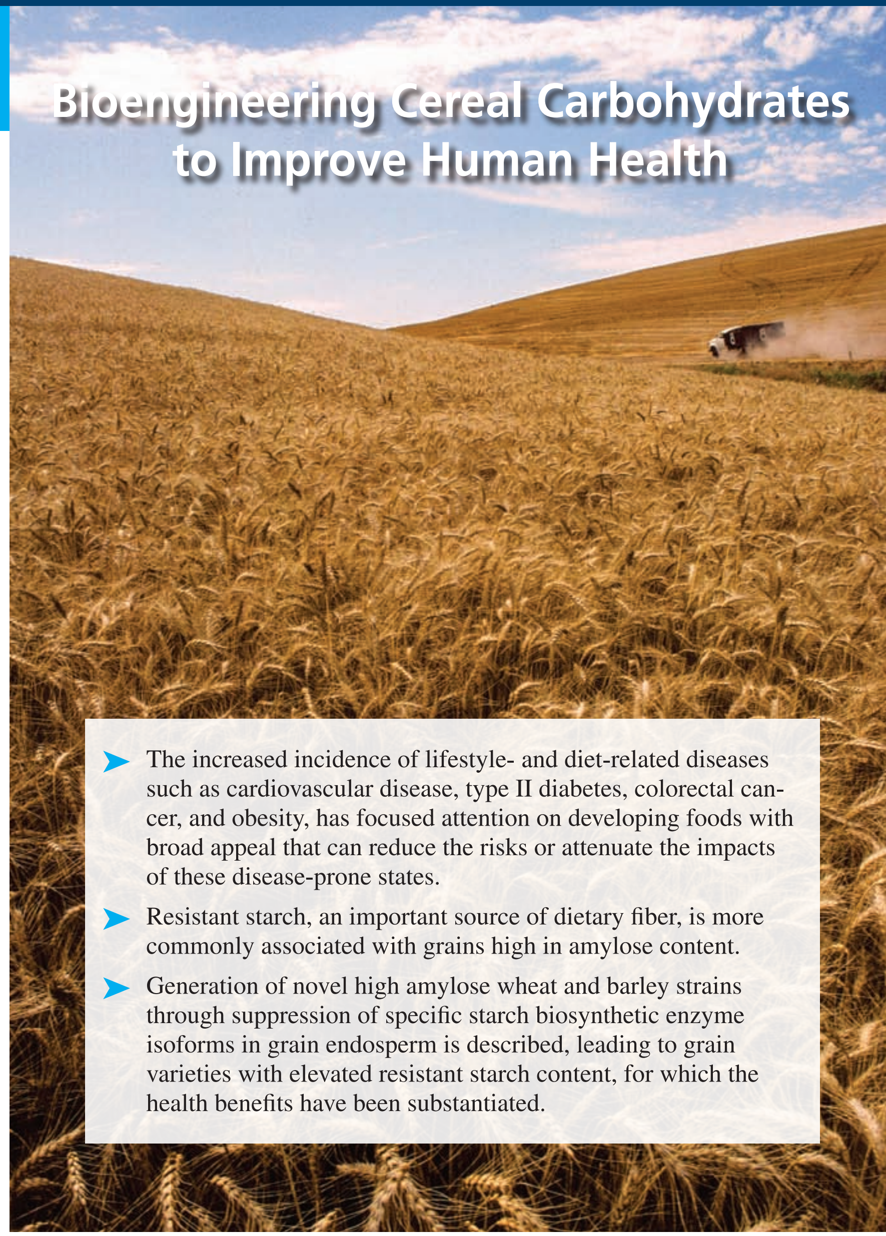
Bioengineering Cereal Carbohydrates to Improve Human Health

A. Regina[1,2]
A. R. Bird[1,3]
Z. Li[1,2]
S. Rahman[1,2]
G. Mann[1,2]
E. Chanliaud[4]
P. Berbezy[4]
D. Topping[1,5]
M. K. Morell[1]



Courtesy of the Agricultural Research Service.

- The increased incidence of lifestyle- and diet-related diseases such as cardiovascular disease, type II diabetes, colorectal cancer, and obesity, has focused attention on developing foods with broad appeal that can reduce the risks or attenuate the impacts of these disease-prone states.
- Resistant starch, an important source of dietary fiber, is more commonly associated with grains high in amylose content.
- Generation of novel high amylose wheat and barley strains through suppression of specific starch biosynthetic enzyme isoforms in grain endosperm is described, leading to grain varieties with elevated resistant starch content, for which the health benefits have been substantiated.

Diet- and lifestyle-related diseases such as cardiovascular disease, certain cancers (especially of the large bowel), inflammatory bowel diseases (IBD), and diabetes are established major public health problems in industrialized countries and are also emerging in developing ones with greater affluence through industrialization and wealth (11,14). The ageing of populations and the rising prevalence of obesity are compounding the impact of these disease states. Tackling these socio-economic issues has become a priority for governments worldwide, with primary prevention a frontline strategy of public health campaigns.

It is well established that diet and lifestyle play a major role in the development of these illnesses and dietary modification is an established means of risk reduction (1). However, individual behavior change seems unlikely to translate to a meaningful improvement in public health. This conclusion is supported by the global rise in obesity, despite the concerns of national and international health authorities. One viable strategy for improving public health is appropriate modification of the food supply to give products that deliver substantiated health benefits while retaining consumer appeal. Cereals are prime targets in this regard. As dietary staples, relatively small improvements in grain composition (especially in starch and fiber) have the potential to translate into significant health gains at the population level when they are incorporated into food.

Whole grains are acknowledged as important contributors to dietary improvement, but generally they are less attractive to consumers compared to more refined or processed starchy foods. This is despite the negative comments that the latter have attracted, with some health authorities even advocating their restricted consumption. These foods include bread, pasta, breakfast cereals, and other items that are widely consumed. One of the key criticisms of these products is that their starch component is digested rapidly and almost completely in the small intestine.

The rate at which glucose is digested in the upper gut and absorbed into the blood circulation is thought to be critical to health. The rise in blood glucose provokes increased pancreatic insulin secretion in rough proportion to the quantity absorbed. This has obvious linkages to diabetes—a condition characterized by impaired insulin secretion and/or action. Sustained hyperglycemia results in potentially dele-

[1] Commonwealth Scientific and Industrial Research Organization, Food Futures National Research Flagship, P.O. Box 93, North Ryde 1670, NSW, Australia.
[2] CSIRO, Plant Industry, G.P.O Box 1600, Canberra ACT 2601, Australia.
[3] CSIRO, Human Nutrition, P.O. Box 10041, Adelaide BC 5000, SA, Australia.
[4] ULICE, ZAC les Portes de RIOM, 63204 RIOM cedex, France.
[5] CSIRO Preventative Health National Research Flagship, CSIRO Human Nutrition, P.O. Box 10041, Adelaide BC 5000, SA, Australia.

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terious cellular changes leading to long-term impairment in health status and increased risk of diseases, especially those of the circulatory system. Starches are major sources of dietary glucose, and as such, they are significant determinants of postprandial fluctuations in blood glucose. Food choices that favor more slowly digestible starches clearly have a benefit for the promotion of long-term health and minimizing the risk of type II diabetes and cardiovascular disease (27).

Whole grain foods elicit relatively modest postprandial glycemic responses, yet the starches they contain are, potentially at least, readily digestible. The reason for this apparent anomaly is that essentially indigestible botanical structures (i.e., plant cell walls) in intact grains limit or prevent access of α-amylases from reaching their substrate. Milling removes these barriers, as evidenced by the fact that the glycemic index (GI; a common comparative metric of the glycemic response to food carbohydrates) of white and whole meal wheat bread are similarly high.

Soluble nonstarch polysaccharides (NSP; the major component of dietary fiber) in common cereal grains, such as oats and barley, increase the viscosity of the luminal contents of the gut and thereby impede starch hydrolysis as well as slow diffusion of glucose to the intestinal wall. Starchy foods containing mixed link β-glucans, such as oats and barley, are usually low GI (23) and long-term human intervention studies have shown that blood glucose and insulin responses are reduced in normoglycemic individuals when foods and diets containing either these particular whole grains (9) or β-glucan isolates were consumed (3). Wheat also contains soluble NSP, the arabinoxylans, and although these polysaccharides are capable of forming viscous solutions, their levels in contemporary cultivars seem to be too low to evoke a clinically meaningful response (12).

Aside from the importance of slowing the rate of starch digestion and absorption for consumer health, shifting the site of starch digestion from the small intestine to the large bowel provides additional health benefits (4). It is known now that not all starch eaten by humans is digested to completion, but a variable fraction known as resistant starch (RS) enters the large bowel (2). In the latter intestinal compartment it is fermented by the resident microflora, primarily to short chain fatty acids (SCFA). There is mounting evidence that RS may have a wide range of health benefits with potential roles in promoting bowel health and possibly protecting against serious diseases such as colorectal cancer and IBD.

One of the changing paradigms in human carbohydrate digestion and metabolism is the emergence of large bowel bacterial fermentation as an important contributor to normal visceral function. While NSP contribute to large bowel function largely (but not exclusively) through physical bulking, RS acts substantially through its fermentation products, i.e., SCFA (24). These acids are absorbed and provide the large bowel and liver with energy. However, of the three major acids which are formed (acetate, propionate, and butyrate), butyrate has attracted the most attention. Experimental studies have shown that it has multiple actions that suggest it is pivotal in maintaining a normal population of colonocytes. These are the cells that line the large bowel and their health is central to lowering the risk of cancer and IBD. RS fermentation seems to favor butyrate production relative to that of NSP, suggesting that it is more important in the promotion of large bowel health. Evidence from early survey studies indicated that populations at low risk of noninfectious disease (such as native Africans in south east Africa) consumed unrefined cereal foods and so ate more fiber than Europeans living in the same region who ate refined foods. However, more recent data show that these natives did not have high fiber intakes, but that their foods were enriched in RS.

The proportion of ingested starch that actually escapes digestion in the small intestine is dictated by a multitude of factors. Extrinsic influences include methods used to prepare, process, cook, and store foods. Structural characteristics of the starch itself (notably amylose content) as well as interactions with NSP and other components of the food matrix are also important, and it is on this basis that four categories of RS have been identified: 1) physically inaccessible starch (RS 1), 2) starch granules that resist digestion (RS 2), retrograded starch (RS 3), and chemically modified starch (RS 4). A fifth category, termed V-form RS, has recently been proposed (7). RS encompasses a wide range of products; most starchy foods contain a mixture of different types of RS, and there is limited preliminary evidence that the physiological properties vary depending on RS type (4).

These considerations suggest that in addition to the traditional cereal quality attributes, such as protein and grain hardness, starch and NSP content and properties should be given more attention as important cereal-breeding targets. Objectives should not just be about raising the content of these and other protective constituents, but should also address enhancing their physiological functionality to ultimately produce food products capable of providing better health for consumers. This approach also affords the opportunity to expand and diversify the range of cereal-based food products available to consumers, thereby making it easier for them to make healthier food choices.

Understanding Starch Synthesis in Cereals

Extensive efforts during the past several decades on understanding cereal starch biosynthesis have presented a clearer picture of the enzymes that have a direct role in the synthesis of these complex molecules. Synthesis of starch in plants takes place within organelles called plastids, which can be either photosynthetic (e.g., leaf chloroplast) or nonphotosynthetic (e.g., endosperm amyloplast). Starch synthesized within the chloroplast during the day, called transitory starch, is broken down at night to maintain the supply of sugars for survival and growth in the dark, whereas starch synthesized within the amyloplast in the seed endosperm (reserve starch) is for long-term storage and will be used for energy supply during seed germination. A schematic representation of the key steps in the cereal grain starch biosynthetic pathway is depicted in Figure 1. Synthesis of starch requires four biochemical steps; substrate activation, chain elongation, chain branching, and chain debranching (16).

The substrate activation step is carried out by the enzyme ADP glucose pyrophosphorylase (AGPase), which catalyzes the synthesis of ADP glucose from glucose-1 phosphate and ATP. As the first committed step in the pathway, the activity of AGPase is under complex control in order to determine the flux into starch. Modifications of AGPase, such as

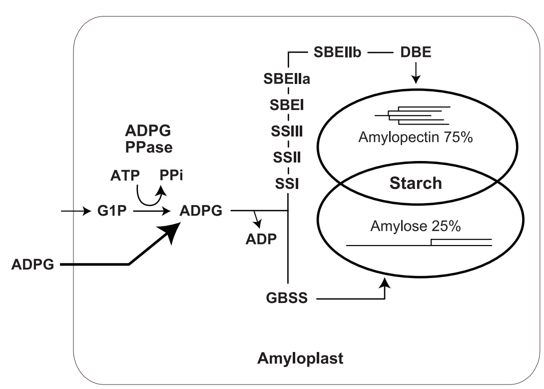

Fig. 1. A schematic representation of starch biosynthetic pathway in cereal.

increasing the enzyme activity or reducing the sensitivity of the enzyme to inhibitors, increase the amount of starch produced in maize endosperm (8,10) and in wheat (22). The next stage of starch biosynthesis, chain elongation, is carried out by starch synthases (SSs), which transfer the glucose residue of ADP glucose to the nonreducing end of a preexisting α-1,4 glucan chain. There are two broad classes of SSs in plants, starch synthases essential for amylose synthesis, the granule-bound starch synthases (GBSS), and starch synthases responsible for amylopectin synthesis. Four classes of enzymes responsible for amylopectin synthesis are present in the endosperm, SSI, SSII, SSIII, and SSIV. The chain-branching step is catalyzed by starch branching enzymes (SBEs) that cleave linear regions of α-1,4 glucan, and form a new α-1,6 branch point through transfer of the cleaved chain to a second chain. In dicots, there are two classes of this activity, while in monocots, three classes are found (SBEI, SBEIIa, and SBEIIb). The final required activity, chain debranching, remains somewhat of an enigma. While the genetic evidence for the requirement for debranching activity in starch synthesis is very clear, the mechanism through which debranching enzymes participate in starch synthesis remains to be fully defined (16).

Developing Novel High Amylose Cereals

One of the important routes to modifying the RS content of processed foods is to raise the level of amylose in the parent grain. The relationship between RS content and amylose content has been proposed to arise because of the inhibitory effects of amylose on the swelling of the starch granule in cooking, and because it retrogrades rapidly. Three mechanisms have been demonstrated to generate high amylose phenotypes in cereals.

1) A reduction in starch branching leads to conversion of amylopectin molecules into amylose and lower molecular weight, lower branch frequency amylopectin-derived molecules with amylose-like functionality.

2) A reduction in starch synthase activity results in increases in the amylose to amylopectin ratio through a reduction of amylopectin synthesis.

3) Increases in amylose content by up-regulating GBSS activity have been reported, although increases in amylose contents via this mechanism have not been demonstrated to reach the high levels achieved using the other mechanisms. Studies from a range of plant species have indicated that the broad biosynthetic roles of the key enzymes are conserved; absence of any one or a combination of these enzymes leads to variation in starch structure and properties that are essentially consistent between species. However, we have found two important examples in cereals where there are very critical differences between cereals in the degree to which individual genes control the structure of starch, and the exploitation of these differences has led to the creation of modified starches with important potential in the food industry and in human nutrition.

High Amylose Wheat

As noted above, three isoforms of SBE, SBEI, SBE IIa, and SBE IIb are reported in cereals. Lack of expression of different isoforms of SBE influenced the starch phenotype differently in different species. Mutations in SBE I in both wheat and maize did not result in any obvious change in the starch structure; however, in rice a mutation in this enzyme has been reported to lead to subtle changes in the amylopectin chain length distribution (20).

The high amylose (also called amylose extender) phenoptype in maize occurs when the SBE IIb isoform is absent or down-regulated. Amylose contents in amylose extender maize were first reported to be increased from about 30% to 65%, depending on the cultivar used (21), further breeding has accumulated genes that can raise the amylose content further. In rice, the absence of SBE IIb leads to increases in the proportion of amylose from about 16% to 26% in Japonica type rices (17).

One focus of our work was to determine which enzyme needed to be targeted in order to generate a high amylose phenotype in wheat. Genetic engineering was used in order to address this question for several reasons. Firstly, it makes possible modifications that are found difficult through conventional breeding. Secondly, it provides a quicker means of modifying traits, especially in crops with complex genomes such as wheat. Wheat is a hexaploid species, production of a knock out mutation of a particular gene requires the identification of single null mutation in each of the three genomes followed by combining them through hybridization. Recent developments in gene modification such as RNA interference (RNAi) permits efficient silencing of target genes, allowing the study of the resultant phenotype. In this research, we used RNAi technology to modify starch properties through altering the expression of SBE IIa and SBE IIb in wheat. This technique involves the use of a gene construct designed to express single stranded self complementary (hairpin) RNA aimed at producing a duplex RNA endogenously that can induce post-transcriptional gene silencing (PTGS) of endogenous gene that has sequence similarity to that present in the transgene (26). With the aim of specifically silencing SBE IIa and SBE IIb in wheat, we generated RNAi constructs by cloning fragments of wheat SBE IIa and SBE IIb cDNAs in inverted repeats separated by an intron from the respective genes (19). Since our intention was to silence the expression exclusively in the endosperm, we used an endosperm-specific high molecular glutenin promoter to target the expression of the transgene in the endosperm alone and not in other parts of the plant. Transforming wheat plants with SBE IIa and SBE IIb constructs using *Agrobacterium*-mediated transformation led to the identification of plants with reduced expression (<1 to 10% of wild type expression) of SBE IIa and SBE IIb, respectively. Western blot analysis using antibodies that are specific for SBE IIa and SBE IIb was conducted to identify such plants. Interestingly, it was noticed that the level of expression of SBE IIb was also reduced in the lines in which SBE IIa was reduced. This phenomenon of cross suppression was not observed in lines in which SBE IIb was reduced; SBE IIa expression was near-wild type in such lines. Starch characteristics of selected SBE IIa targeted (hp-SBE IIa) and SBE IIb targeted (hp-SBE IIb) lines were

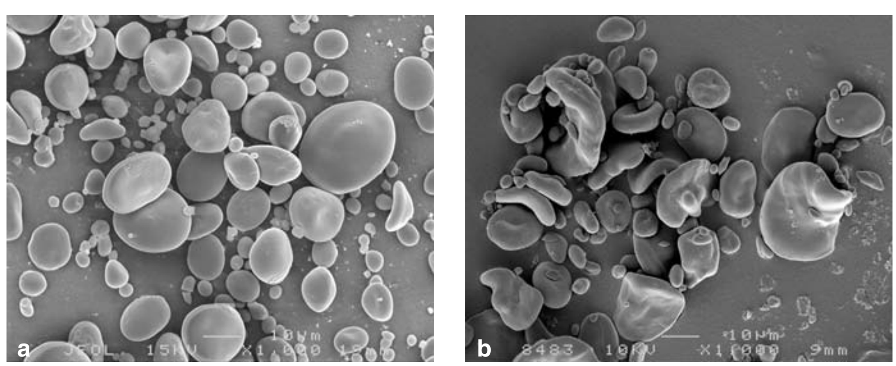

Fig. 2. Scanning electron micrographs of starch granules from wheat (19). Nontransformed control wheat (A) and hp-SBE IIa wheat (B).

analyzed using a range of techniques (18). Significant morphological alterations in starch granules were revealed by scanning electron micrography in hp-SBE IIa lines (Figure 2), where the granules appeared to have similar morphology as wild type hp-SBE IIb lines. Two techniques, iodometry and gel permeation chromatography, were used to estimate the amylose content. Using both the techniques, hp-SBE IIa starch recorded a high amylose content of >70% while hp-SBE IIb starch remained statistically on par with the wild type. Amylose content of hp-SBE IIb and hp-SBE IIa lines determined by gel chromatography is shown in Figure 3. Other structural changes in starch were also observed in hp-SBE IIa lines, such as a significant decrease in the proportion of shorter chains of degree of polymerization 6-12. In contrast to rice and maize, we have found that it is necessary to down regulate the expression of SBE IIa, rather than SBEIIb, in order to obtain a high amylose phenotype in wheat (18). The explanation for this difference in control of amylose content may lie in differences in the expression levels of these enzymes between species. In maize and rice, the major isoform in the endosperm is SBE IIb, whereas in wheat SBE IIa is the predominant form (19).

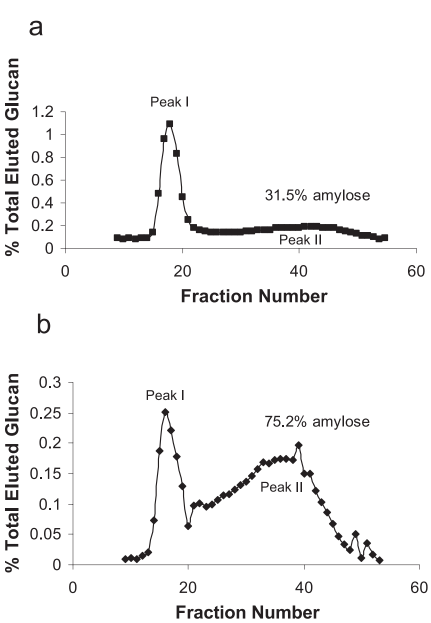

Fig. 3. Amylose content of wheat starch determined by Sepharose CL-2B gel permeation chromatography. The starch content of the eluted fractions was assayed by using a starch assay kit (Sigma). Amylose content (second peak) estimated as a percentage of total starch is shown on respective graphs. The hp-SBE IIa starch (B) recorded a high amylose content of >70%, while the hp-SBE IIb starch (A) remained statistically on par with the wild type.

Clearly, it is essential to establish that any change in grain composition translates to a nutritionally beneficial outcome. A rat trial confirmed that the high amylose wheat has the potential to deliver nutritionally beneficial outcomes. Rats fed a diet containing the high amylose wheat had improved indices of bowel health compared to those fed the normal wheat. Biomarkers including large bowel digesta weight (both wet and dry) and pH were altered favorably. Most importantly, caecal SCFA pools (including butyrate) were raised substantially in rats fed the new wheat, supporting the successful delivery of RS (18).

BARLEYmax—the High Fiber High Amylose Cereal

BARLEYmax (also described in the scientific literature as *Himalaya 292*) is a barley that contains a single nucleotide change in starch synthase IIa (SS IIa), selected originally from a mutant population of a hull-less barley variety "Himalaya" following sodium azide treatment. The loss of the enzyme activity of SS IIa leads to a shrunken grain with a lower amylopectin and relatively higher amylose in grain than normal barley (approximately 70% of seed weight) (15). The loss of SSIIa activity also changes other grain components leading to a novel grain composition (Table I). BARLEYmax not only contains high amylose but also contains high beta-glucan, high NSP, increased protein, and increased lipid. Animal trials showed clearly that BARLEYmax delivers high RS with significantly more starch in the large bowel than standard barley or oats (5,6). These trials also showed that consumption of BARLEYmax is associated with reduction of plasma cholesterol and improved indices of bowel health including increased large bowel content of short chain fatty acids (SCFA) and lower pH value. Human trials revealed a lower glycemic index in human volunteers consuming foods containing processed BARLEYmax as the whole grain (25).

Table I. Grain composition at maturity of wild type Himalaya and BARLEYmax

	Himalaya (mg/100 g grain)	BARLEYmax (mg/100g grain)
Starch	55.0	29.1
% amylose	25.8	73.5
% amylopectin	74.2	26.5
Protein	11.7	17.6
NSP	12.1	20.3
Lipid	3.2	6.9
Ash	2.4	2.2
Beta-glucan	5.8	9.9
Total fiber	15.6	25.3

Although barley is a major cereal crop worldwide, it is mainly used for brewing and also as an animal feed. Its potential for use in human consumption is not well exploited (see CEREAL FOODS WORLD, v. 51, no. 1). BARLEYmax opens a new opportunity to use barley as a component of functional food. Research work has been done to incorporate this new barley (as a whole grain) into wheat flour for bread baking (13). Other types of food are also being produced including cookies and breakfast cereals. In order to increase the production of BARLEYmax and adapt it for various growing conditions, the SSIIa mutation has been introduced into a number of barley backgrounds for production in the United States, Europe, and Australia using marker-assistant breeding.

Conclusion

The increased incidence of the lifestyle- and diet-related diseases, such as cardiovascular disease, type II diabetes, colorectal cancer, and obesity, has focused attention on the development of foods that can form a component of the diets of a broad cross section of the community, and reduce the risk of developing the disease state or assist in attenuating its impact. Cereal carbohydrates have attracted negative press over recent years because of perceptions driven by phenomena such as the Atkins diet that associate consumption of cereals with problems in controlling body weight. While there has been a reevaluation of those perceptions and a rallying of support for consumption of cereals and cereal-carbohydrates, cereals represent an important platform for the delivery of nutritional benefit through their widespread consumption and versatility in foods. In the research described in this article, we demonstrate that it is possible to exploit fundamental knowledge of the processes controlling the synthesis of grain carbohydrates to generate novel cereals with high amylose contents, with the potential to develop foods with high RS levels and reduced glycemic impact. In wheat, rat trials have substantiated the presence of RS and positive impacts on a range of indices of bowel health. In barley, we have extensively substantiated positive nutritional outcomes through animal and human trials. These examples indicate that there is a strong future for harnessing the power of genetic technologies to provide new cereals for the food industry that can deliver powerful benefits for consumers.

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Ahmed Regina leads a research group investigating the control of starch biosynthesis and structure in cereals, particularly high amylose starches. Regina can be reached at Regina.Regina@csiro.au.

Anthony R. Bird is a principal research scientist at CSIRO Human Nutrition. His research focuses on the role played by dietary carbohydrates, especially starch and fiber, in promoting health and reducing the risk of chronic diseases such as bowel cancer and diabetes. A current research priority is the development of improved techniques for measuring resistant starch and predicting glycemic potency of carbohydrate-based foods. Bird can be reached at Tony.Bird@csiro.au.

Zhongyi Li is a molecular biologist at CSIRO Plant Industry and works on the manipulation of cereal starch through genetic manipulation and molecular technology to produce high resistant starch and low glycemic index cereal foods. Li can be reached at zhongyi.li@csiro.au.

Sadequr Rahman has been involved in the characterization of genes implicated in starch biosynthesis. Rahman can be reached at Sadequr.Rahman@csiro.au.

Gülay Mann is the head of Nutrition and Food Technology Capability of Defence Science and Technology Organisation (DSTO), which provides scientific and technical support to the Australian Defence Force (ADF). Mann is providing scientific leadership for DSTO's research programs in Nutrition and Dietetics and Food Science and Technology, and also oversees the management of the freeze-dried meal production line. Previously Mann worked in CSIRO Plant Industry with a research focus on the application of molecular and genetic analyses to investigate the genes responsible for complex dough attributes and the processing of novel cereal grains for maximum health benefit.

Elisabeth Chanliaud is a plant biochemist who has been working for the Limagrain Group for six years. She is research coordinator for the cereal ingredient business of this group. Chinliaud can be reached at elisabeth.chanliaud@limagrain.com.

Pierre Berbezy, currently a project leader in ULICE, is a plant biochemist working on starch metabolism.

David Topping is a metabolic biochemist with a long standing interest in the application of basic science to produce food products that promote public health and assist in the management of conditions such as diabetes. Topping can be reached at David.Topping@csiro.au.

Matthew K. Morell is theme leader in CSIRO, responsible for a portfolio of research on high value grains and oilseeds. Morell can be reached at Matthew.Morell@csiro.au.