Chapter 10
Genomics of Cereal-Based Functional Foods

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10.1 Introduction

Functional foods have been defined by the Food and Nutrition Board (FNB) of the National Academy of Sciences, USA as "any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrients it contains". Japan was the first country to promote the concept of functional foods as Food for Specific Health Use (FOSHU) endorsed by the Japanese Ministry of Health (Arai 1996). With the increase in public awareness about nutrition and health, functional foods or "foods with a purpose" have gained increased popularity (Verbeke et al. 2009).

Fruits, nuts, berries and vegetables are the most widely known sources of bioactive compounds, whereas cereals, with an annual consumption of 332 kg/person (estimation for 2015, FAO Corporate Documentary Repository, http://www.fao.org/docrep/005/Y4252E/y4252e05.htm, accessed on May 16, 2012), have often been marginalized as functional foods. Recent findings about the health

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benefits of whole-grain cereals and cereal products (Behall et al. 2006; Fardet et al. 2008; He et al. 2010) have renewed interest in the potential of cereals as functional foods. Whole grain cereals have greater nutritional value than the refined or polished cereals, because the bran and germ portion have high fiber content and the majority of bioactive compounds (Champ 2008; Fardet et al. 2008; He et al. 2010). Cereal-based foods have functional food properties due to their carbohydrate constituents (β-glucans, arabinoxylans, inulin), and bioactive compounds such as phenolics (flavones, chalcones, alkylresorcinols, ferulic acid, anthocyanins), carotenoids (β-carotene, xanthophylls), and vitamin E. The physical location of functional components present in the various parts of grains of common cereals is summarized in Table 10.1.

Cereals can be a good source of both probiotic and prebiotic foods because of their diverse carbohydrate composition (Charalampoulos et al. 2002). Probiotic foods contain microorganisms that benefit the consumer's health by improving their intestinal microbial balance (Fuller 1989). Prebiotic food on the other hand, is not digested in the upper gastrointestinal tract but beneficially affects the host health by selectively stimulating the growth and/or activity of useful bacteria in the colon (Gibson and Roberfroid 1995). Table 10.2 summarizes the content of carbohydrate-based functional components in common cereals. Bioactive compounds present in cereals, like phenolic acids, flavonoids, carotenoids, and tocopherols have useful antioxidant properties. They help reduce oxidative stress in the cells and quench the damaging free-radicals, thereby protecting cells from ageing, degeneration, and carcinogenesis (Astorg 1997; He et al. 2010). In fact, bioactive compounds like tocotrienols even reduce the bad cholesterol levels in blood thereby playing protective role against cardiovascular diseases (Das et al. 2008).

The genome sequencing and gene annotation of cereals such as rice (http://rice.plantbiology.msu.edu/), maize (http://magi.plantgenomics.iastate.edu/), barley (International Barley Sequencing Consortium, 2012) and sorghum (Paterson et al. 2009), and the ongoing genome sequencing of wheat (http://www.wheatgenome.org/), have provided a wealth of information about the genes related to bioactive components. Genetic variation studies indicated high heritability for arabinoxylan fiber, carotenoids, and other bioactive compounds, but, significant genotype x environment interactions make it difficult to identify breeding lines with consistently high bioactive compounds across environments and years (Shewry et al. 2010). Here we summarize recent advances in the genomics of various functional food components of common cereals based on their carbohydrate components (Class I) and bioactive components (Class II).

10.2 Carbohydrate-Based Functional Food Components (Class I)

10.2.1 Beta Glucans

The cell walls of grasses are characterized by the presence of (1,3: 1,4)-β-D-glucans composed of unsubstituted, unbranched polysaccharide containing

Table 10.1 Tissue distribution of major functional food components in different cereal grains. Modified from Champ (2008)

Part of grain	Food component	Cereals	Functional properties	References
Pericarp and testa	Arabinoxylans	Wheat, barley, rice, rye	Increase fecal biomass, improves gut health and lipid metabolism	Glei et al. (2006); Neyrinck et al. (2011)
	Phenolic acids	All	Improve redox state, anticancer	Rhodes and Price (1997)
	Flavonoids	All	Antioxidant, anticancer, antiaging, hepatoprotective	Hertog et al. (1993); Middleton et al. (2000)
Aleurone	Minerals	All	Mg maintains cardiac health, fights muscle fatigue; Fe, Zn and Cu maintain proper blood circulation, growth, development and other bodily functions; Ca is essential for good bone health	Cox et al. (1991); Prasad (1998); Haas and Brownlie (2001); Institute of medicine, food and nutrition board (2001)
	Arabinoxylans	As described in pericarp and testa	Described above	As described in pericarp and testa
	Inulin	Wheat, barley, rye	Prebiotic effect, improves gut health, slows glycemic response	Schneeman (1999)
Endosperm	β-glucans	Oat, barley, rye	Reduce glycemic index, Prebiotic effect	Keegstra and Walton (2006); Pennisi (2009)
	Resistant starch	All	Slows glycemic response, prebiotic effect	Topping and Clifton (2001)
Germ	Lipids, unsaturated fatty acids	All	May decrease oxidative stress in patients with hypercholesteromia	Simopoulos (1991)
	Vitamins : E, B6, Folate, Carotenoids	All	Reduce oxidative stress, protect against macular degeneration due to ageing, anticancer	Kohlmeier and Hastings (1995); Astorg (1997); Sen et al. (2006)
	Minerals	All	As described in aleurone	As described in aleurone

Table 10.2 Carbohydrate based functional food components (percent dry grain weight) of common cereals

Cereal	β-glucan (%)	Arabinoxylans (%)	Amylose (%)	Fructan (%)	References
Wheat	0.4–1.4	5.8	23–28	0.9–1.8	Henry (1987); Shelton and Lee (2000); Fretzdorff and Welge (2003); Izydorczyk and Dexter (2008)
Rice	0.4	2.6	16–33	–	Shelton and Lee (2000); Dermibas (2005); Izydorczyk and Dexter (2008)
Maize	0.5	3.5–6.1	24	0.1	Caimi et al. (1996); Shelton and Lee (2000); Dermibas (2005); Izydorczyk and Dexter (2008)
Barley	2.5–11.3	3.50–6.05	22–26	0.6–1	Shelton and Lee (2000); Jones (2007); Izydorczyk and Dexter (2008)
Oat	2.2–7.8	2.7–3.5	16–27	–	Shelton and Lee (2000); Izydorczyk and Dexter (2008)
Rye	1.2–2	7.6–12	24–31	0.6–1	Shelton and Lee (2000); Jones (2007); Izydorczyk and Dexter (2008)
Millets	0.5	–	17	–	Shelton and Lee (2000); Dermibas (2005)

β-D-glucopyranosoyl monomers linked through C(O)3 and C(O)4 atoms with the (1,4)-linkage being more abundant (Burton and Fincher 2009; Burton et al. 2010). Generally, the degree of polymerization (DP) of (1,3: 1,4)-β-D-glucans may vary up to 1,000-fold or more in most grasses (Fincher 2009). Among the cereals, barley has the highest content of (1,3: 1,4)-β-d-glucan (2.5–11.3 %), followed by oat (2.2–7.8 %), rye (1.2–2 %) and wheat (0.4–1.4 %) (Izydorczyk and Dexter 2008). Beta-glucans have been reported to lower serum cholesterol, improve lipid metabolism, reduce glycemic index and even reduce the risk of colorectal cancer (Keegstra and Walton 2006; Pennisi 2009). Beta-glucans are excellent prebiotic components of functional foods because they selectively promote the growth of lactobacilli and bifidobacteria in vivo (Snart et al. 2006) and in vitro (Jaskari et al. 1993).

Genetic and environmental variation of β-glucan content in barley has been investigated by various workers (Stuart et al. 1988; Kenn et al. 1993; Fastnaught et al. 1996). Although the genetic variation exists for breeding high β-glucan barley lines, environmental variation strongly impacts the β-glucan content and hence breeding for consistently high β-glucan content (Shewry 2008). Manickavelu et al. (2011) mapped four quantitative trait loci (QTL) on chromosomes 3A, 1B, 5B and 6D in a wheat recombinant inbred population contributing up to 43 % of variation in β-glucan content.

The synthesis of (1,3: 1,4)-β-D-glucan is mediated by *cellulose synthase-like* (*Csl*) genes that share a superfamily with *cellulose synthase* (*CesA*) genes. The Csl proteins are predicted to be integral membrane proteins having a "DDDQXXRW" motif (Hazen et al. 2002). Thirty-seven *Csl* genes are known in rice which belong to six families, *CslA*, *CslC*, *CslD*, *CslE*, *CslF*, and *CslH*, having 10, 9, 4, 5, 8 and 2 genes, respectively.

Burton et al. (2006) used a comparative genomics approach to clone the *CslF* group of genes on rice chromosome 7 that correspond to a highly significant QTL on barley chromosome 2H affecting (1,3: 1,4)-β-D-glucan content in mature barley grain (Han et al. 1995). Burton et al. (2006) identified six genes (*OsCslF1*, *OsCslF2*, *OsCslF3*, *OsCslF4, OsCslF8* and *OsCslF9*) located on a 118 kb interval on chromosome 7 in rice. These genes when mobilized into *Arabidopsis* resulted in (1,3: 1,4)-β-D-glucan synthesis in cell walls, which is lacking in wild type plants. The other two genes of this family, *OsCslF6* and *OsCslF7*, are located on rice chromosomes 8 and 10, respectively (Burton et al. 2006).

Burton et al. (2008) identified and mapped seven genes of the *HvCslF* family in barley. Of these seven genes, *HvCslF3*, *HvCslF4*, *HvCslF8* and *HvCslF10* were located in the centromeric region of chromosome 2H; *HvCslF6* near the centromere on 7H; *HvCslF7* on 5H long arm and *HvCslF9* on 1H short arm near the centromere. Transcript profiles of the *HvCslF* family members showed individual patterns of abundance in different tissues, with the exception of *HvCslF6*, which showed consistently higher expression in many of the tissues examined (Burton et al. 2008). Later, Burton et al. (2010) reported that over-expression of barley *HvCslF6* under the control of endosperm specific oat globulin promoter resulted in more than 80 % increase in (1,3: 1,4)-β-D-glucan content in transgenic barley grains.

Nemeth et al. (2010) used microarray analysis to identify potential candidate genes involved in (1,3: 1,4)-β-D-glucan synthesis in wheat using cDNA isolated from whole caryopses and fractions enriched with starchy endosperm tissue, during various stages of development. They found that *TaCslF6*, an ortholog of barley gene *HvCslF6*, had high expression in wheat endosperm and, moreover, its down regulation by RNAi resulted in decreased (1,3: 1,4)-β-D-glucan content in the endosperm. In oat, Chawade et al. (2010) used Targeting induced Local Lesions in Genome (TILLING) to identify mutants in the *AsCslF6* gene that affected (1,3: 1,4)-β-D-glucan content. Comparative genomics, expression profiling, mutant selection and gene knockouts are providing better understanding of the enzymes and genes regulating cell wall synthesis and it will be possible to manipulate cell wall composition of cereal grains in the near future to meet the dietary and industrial requirements.

10.2.2 Arabinoxylans

Arabinoxylans are linear chain backbone consisting of β-D-xylopyranosyl (Xyl*p*) residues linked through (1 → 4) glycosidic linkages. Some of the Xyl*p* residues have α-L-arabinofuranosyl (Ara*f*) residues attached to them, leading to four structural elements in the molecules of arabinoxylans viz., monosubstituted Xyl*p* at O-2 or O-3, di-substituted Xyl*p* at O-3, and unsubsituted Xyl*p*. The relative ratios of these structural elements vary across species (Izydorczyk and Dexter 2008). Arabinoxylans (AX) improve gut health, by promoting growth of useful bifidobacteria (Glei et al. 2006; Neyrinck et al. 2011). Neyrinck et al. 2012 found that wheat-derived arabinoxylans increased satietogenic gut peptides and reduced metabolic endotoxemia in diet-induced obese mice. The genes for the assembly of arabinoxylans are not well characterized, although the genes of cellulose synthase-like (*Csl*) and Glycosyl trasferases (*GT*) families have been reported to play important roles in synthesis and feruloylation of arabinoxylans (Urahara et al. 2004; Mitchell et al. 2007). Mitchell et al. (2007) used a bioinformatics approach with differential expression of orthologous genes between *Arabidopsis* and rice, to identify genes involved in AX synthesis and feruloylation, assuming that AX synthesis genes will be expressed more in grasses than in dicots. Genes of families *GT43*, *GT47* and *GT61* and proteins containing the PF02458 domain, which are expressed at higher levels in grasses and are integral membrane proteins, were reported to be the candidates for AX synthesis (Mitchell et al. 2007). They reported that genes in GT43 family coded β, 1 → 4 xylan synthase, GT47 family encoded xylan α-1,2 or α-1,3 arabinosyl transferases and genes in GT61 family encoded feruloyl-AX-β-1,2 Xylosyl transferases. Oikawa et al. (2010), using plant protein family information-based predictor for endomembrane (PFANTOM) reported that GT43 and GT47 family genes play important role in xylan synthesis in rice. Bosch et al. (2011) while studying cell wall biogenesis in maize elongating and non-elongating internodes found maize ortholgoues of rice GT61, GT43

and GT47 to be the most promising candidates for xylan synthesis. More studies are needed to develop better understanding of cell wall biosynthesis in cereals to manipulate the levels of AX to be nutritionally beneficial (Bosch et al. 2011).

10.2.3 Resistant Starch

Starch is composed of two structural components, amylose and amylopectin. Amylose is a long, essentially linear, polymer of glucose monomers with α-1,4 linkages whereas amylopectin is more complex with α-1,6 branching in addition to the α-1,4 bonds. Generally reserve starches contain amylose and amylopectin in the ratio of about 1:3 (Rahman et al. 2007). High amylose content is associated with starch resistant to digestion by the amylolytic enzymes present in the upper digestive tract and acts as a substrate for fermentation by the microflora inhabiting the large intestine (Bird and Topping 2001; Ito et al. 1999). Short-chain fatty acids produced as a result have been reported to benefit gut health (Topping and Clifton 2001). Figure 10.1 shows the amylose biosynthesis pathway in cereals. Enzymes significantly affecting amylose content in cereals are Granule Bound Starch Synthase-I (GBSS-I), Starch Synthase (SS) I–IV and Starch Branching Enzymes (SBE) I-II.

GBSS-I (Wx) is essential for amylose biosynthesis in wheat, rice and maize, and the absence of GBSS-I leads to waxy endosperm with no amylose (Shannon and Garwood 1984; Kirubuchi-Otobe et al. 1997). Nakamura et al. (1995) combined the null alleles of *GBSS-I* homoeoloci of wheat to produce waxy or amylose-free wheat. Slade et al. (2005) used TILLING to identify mutations in all the three homoeoloci of *GBSS-I* in hexaploid and tetraploid wheat cultivars and combined all the mutant homoeoalleles to produce a waxy phenotype with a significantly reduced level of amylose content. Amylopectin branching or content does not appear to be affected by the absence of GBSS-I (Rahman et al. 2007). Itoh et al. (2003) showed overexpression of *Wx* to increase amylose content in rice, but more studies are needed to propose this as a method of choice.

Yamamori et al. (2000) demonstrated *SSIIa* to be more important in determining the structure of amylopectin. Mutation in this gene in wheat resulted in shorter chain starch molecules and about 35 % higher amylose content over wild type (Yamamori et al. 2000). The amount of resistant starch in the high amylose SSIIa mutant increased by more than 10-fold after autoclaving as compared to wild type wheat in the native state (Yamamori et al. 2006). In barley, mutation in *SSIIa* leads to even higher increase (65 %) in amylose content compared to the wild type (Morell et al. 2003). In maize, Zhang et al. (2004) demonstrated that an insertion in *SSIIa* leads to a sugary-2 mutation with a simultaneous increase of 26–40 % in amylose content. *SSIa* mutants did not affect amylose content in rice (Fujita et al. 2006). Mutants for the *SSIa* gene have not been reported in other cereals (Rahman et al. 2007).

OsSSIIIa may play an important role in generating long chains of starch molecules in rice (Ryoo et al. 2007). Null mutants of rice *SSIIIa*, generated by T-DNA insertions, had smaller and rounder starch granules that were loosely packed in

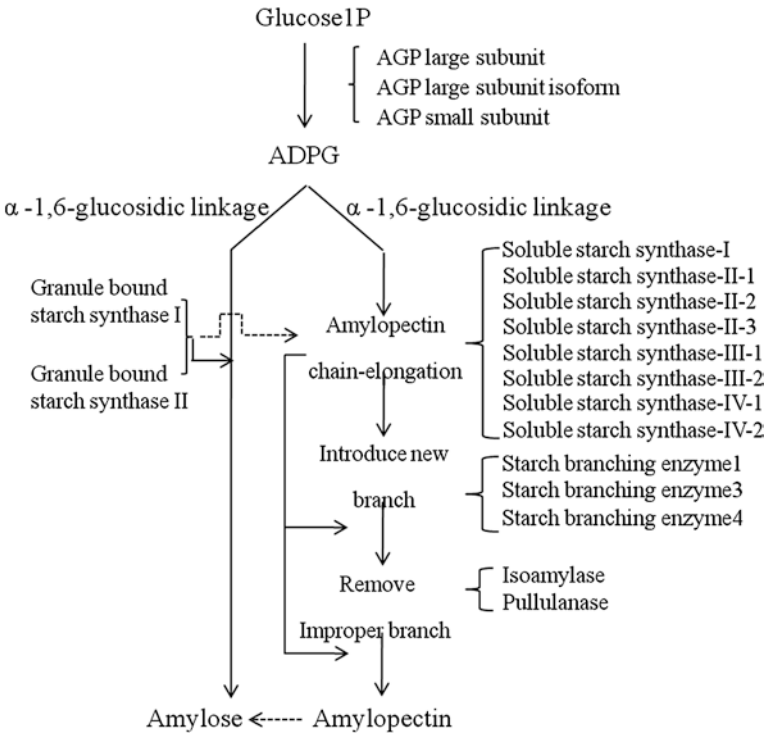

Fig. 10.1 Starch synthesis in cereals and the role of various enzymes in different steps of starch synthesis. Modified from Tian et al. (2009)

the endosperm. Hence, the *Oryza sativa SSIIIa* (*OsSSIIIa*) mutations were named *white-core floury endosperm* 5–1 (*flo5–1*) and *flo5–2* and had reduced content of long chains having DP 30 or above. The loss in *SSIII* of maize led to the dull-I phenotype (Gao et al. 2001) and only moderate increase in amylose content.

SBE induces 1,6 branching in starch and thus is important for amylopectin formation. SBE-I, SBE-IIa and SBE-IIb are the three isoforms of the enzyme. Loss of SBE activity leads to an increased level of amylose. In maize, loss of *SBEIIa* leads to nearly 80 % higher amylose content and has been commercially exploited as amylose extender to produce Hi-maize (Brown 2004). A similar mutation in rice led to an increase in amylose content of only 25–35 % (Nishi et al. 2001). In wheat, down-regulation of *SBEIIa* and *SBEIIb* by RNAi increased amylose content by 80 % (Regina et al. 2006). The high amylose wheat lines in rat feeding trials showed the benefits of resistant starch on gut health (Regina et al. 2006). Likewise, in durum wheat silencing of *SBEIIa* by RNAi led to an increase of amylose content up by 75 % (Sestili et al. 2010). However, a similar approach with *SBEIIb* did not increase amylose content. In barley, simultaneous down-regulation of both *SBEIIa and SBEIIb* by RNAi by more than 80 % produced a high amylose phenotype (>70 %) whereas a reduction in the expression of either

of these isoforms alone had only a minor impact on amylose content (Regina et al. 2010). Thus, increasing the expression of *GBSS* and decreasing *SSII* and *SBEII* activities have been successfully used to increase resistant starch in cereal grains.

10.2.4 Inulin

Inulin is a member of fructan group of polysaccharides having chains of β (2–1) linked fructose units (Degree of polymerization, DP: 2–60) attached to a sucrose molecule. It is highly water soluble alternative storage form of carbohydrate and occurs in the cell vacuoles of about 15 % of the species of the flowering plants (Hellwege et al. 2000). The most common dietary sources of inulin are wheat, onion, garlic, banana and leek. Because of the β-configuration of the anomeric C2 in its fructose monomers, inulin resists hydrolysis by the human small intestine digestive enzymes which specifically hydrolyze α-glycosidic bonds (Roberfroid 2007). In the colon, inulin supports growth of useful bacteria that are beneficial in preventing colon cancer (Reddy et al. 1997; Poulsen et al. 2002). As an ideal dietary fiber, inulin increases fecal biomass and regularizes bowel habits (Gibson et al. 1995; Kleessen et al. 1997). It is also known to enhance bioavailability of minerals in the diet (Abrams et al. 2007) and to improve body defense mechanisms (Guarner 2005).

The inulin biosynthesis model was first proposed by Edelman and Jefford (1968) in *Helianthus tuberosus*. Of the two enzymes, sucrose: sucrose 1-fructosyltransferase (1-SST) and fructan: fructan 1-fructosyltransferase (1-FFT), 1-SST catalyzes the synthesis of the trisaccharide 1-kestose from two molecules of sucrose. Subsequently, 1-FFT transfers fructosyl residues reversibly from one fructan to another, producing a mixture of fructans with variable chain lengths. Some modifications have been reported in this generalized model (Duchateau et al. 1995). In vitro synthesis of inulin using 1-SST from *H. tuberosus* (Lüscher et al. 1996) and 1-FFT from *Chicorium intybus* (Van den Ende and Laere 1996) yielded fructans with DP less than 25. In the modified model, enzyme 6-fructosyltransferase (6-FST) introduces new fructosyl units in the elongating fructan chain (Nagaraj et al. 2004). Furthermore, enzymes such as fructan exohydrolases (FEHs) can modify the structure of synthesized fructan by specific trimming of fructosyl chains.

Sprenger et al. (1995) were the first to clone a gene for a plant enzyme for fructan biosynthesis, *6-FST*, from barley. Transformation of *Nicotiana plumbaginifolia*, lacking fructans, with barley *6-FST* led to fructan production (Sprenger et al. 1995). Kawakami and Yoshida (2002) cloned *6-FST* and *1-SST* from wheat. Functional characterization was done in the methylotrophic yeast *Pichia pastoris*, which showed fructosyltransferase activity upon transformation. Kawakami and Yoshida (2005) cloned *1-FFT* gene from wheat and studied its function by overexpressing it in *P. pastoris*. Their results indicated that *1-FFT* is essential for biosynthesis of fructans accumulating in frost-tolerant wheat. Fructan accumulates in wheat stems during growth and anthesis, from where it is mobilized to grains by fructan 1-exohydrolase (1-FEH) activity during grain filling. Van den Ende et al. (2003) cloned two

isoforms of *1-FEH* in wheat and showed that they play important role in trimming fructans not only during grain filling but also during active fructan synthesis. Van Riet et al. (2006) cloned *fructan* 6-*exohydrolase* (6-*FEH*) from wheat and found that it plays an important role in the trimming of the fructans in conjunction with 1-*FEH*.

Huynh et al. (2008) mapped five QTL for fructan accumulation on wheat chromosomes 2B, 3B, 5A, 6D and 7A. The QTL on 6D and 7A contributed to the largest phenotypic variance of 17 and 27 %, respectively. Zhang et al. (2008) determined the intron–exon structure of 1-*FEH* genes in wheat, mapped them on chromosomes 6A, 6B and 6D and verified their postulated role in fructan accumulation in grains.

Long-chain inulin molecules are desirable for foodstuffs such as ice-cream, milkshakes, yogurt, cookies, cakes, pudding, breakfast cereal, and as a neutral base in cosmetic applications and pharmaceuticals. Jenkins et al. (2011) reported recently that long-chain inulin molecules (with DP>15) beneficially modulate microbial growth in the gut that yield healthy short chain fatty acids (SCFAs). The processes for accumulating long chain inulin molecules rather than crude mixtures of long and short chain inulin molecules in root extracts of artichoke have been developed (Hellwege et al. 2008). Manipulating the trimming enzymes of the inulin biosynthesis pathway (FEH) may be a feasible approach to accumulate long-chain inulin molecules, preferentially in the cereal grains. Bird et al. (2004a, b) reported a mutant (*M292*) in in a hull-less barley variety 'Himalaya' that lowered plasma cholesterol and enhanced short-chain fatty acids in the guts of rats and pigs. Clarke et al. (2008) reported that M292 had a mutation in *Starch synthase* (*SSIIa*) gene which, in addition to enhancing free sugars, β-glucans and arabinoxylans also increased inulin content by 42-fold compared to the wild type variety. The wild type variety 'Himalaya' had 0.1 mg/kg inulin in the grains, whereas the mutant M292 had 4.2 mg/kg grain inulin content (Clarke et al. 2008). More studies are needed to validate the role of *SSIIa* in increasing grain inulin content.

10.3 Bioactive Compounds (Class II)

10.3.1 Polyphenols

Polyphenols are compounds bearing one or more aromatic rings with one or more hydroxyl groups (Liu 2007). Though termed secondary metabolites, polyphenols play an essential role in protecting plants from UV radiation (Stalikas 2007), inhibiting pathogens (Abdel-Aal et al. 2001) and providing structural integrity to the cell wall (Klepacka and Fornal 2006). Cereals contain high levels of polyphenols that contribute in the prevention of degenerative diseases such as cancer and cardiovascular diseases (Liu 2007; He et al. 2010). The health effects of phenolic compounds depend on the amount consumed and on their bioavailability (Manach et al. 2004).

Cereals contain a variety of polyphenols including phenolic acids, flavonoids (flavonols, flavones, flavonones, isoflavones and anthocyanins),

proanthocyanidins, condensed tannins, catechins and lignans. The majority of phenolics in cereals are present in the bran fraction as insoluble and bound compounds in the form of ester and ether linkages with polysaccharides such as arabinoxylan and lignin in the cell wall (Liyana-Pathirana and Shahidi 2006; Fernandez-Orozco et al. 2010).

Genetic variation for polyphenol accumulation and composition has been documented among different cereals (Adom et al. 2003; Menga et al. 2010; Shewry et al. 2010). Significant correlations between the contents of bioactive components and environmental factors were found and even highly heritable components differed in amount over different years and sites (Fernandez-Orozco et al. 2010; Shewry et al. 2010). Bound phenolics, which comprise the greatest proportion of the total phenolics, resulted in the most heritable compounds compared to the free and conjugated forms (Fernandez-Orozco et al. 2010). Higher levels of total phenolics, ferulic acid and flavonoids were detected in Emmer wheat compared to Einkorn and bread wheat species (Li et al. 2008; Serpen et al. 2008), but further studies are needed on a larger sample of wheats with various ploidy levels.

The biosynthesis of phenolics is initiated by the shikimic acid pathway (Heldt 2005) which produces phenylalanine, the first substrate of the phenyl propanoid pathway and proceeds with the synthesis of different classes of compounds, including phenolic acids and flavonoids (Fig. 10.2). The pathway is known to be strongly affected by various stimuli including light, pathogens and wounding

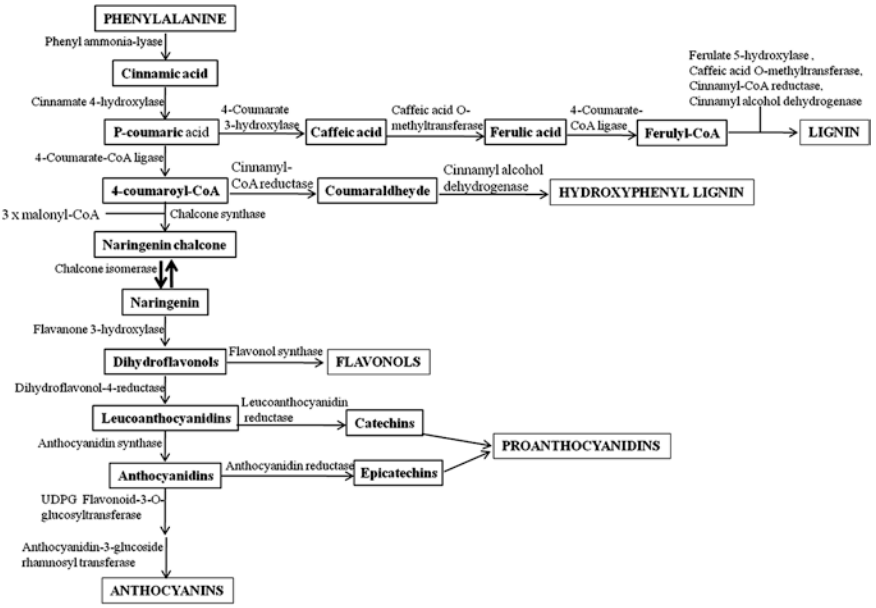

Fig. 10.2 Schematic representation of the general phenylpropanoid pathway in plants, leading to the synthesis of phenolic acids, lignin, different classes of flavonoids and proanthocyanidins. Modified from Deluc et al. (2006)

(Weaver and Herrmann 1997). Possible strategies to enhance the biosynthesis of specific phenolics include over-expression of structural genes involved in rate-limiting steps, and the manipulation of transcription factors that simultaneously activate several genes in one pathway (Grotewold 2008).

Phenolic Acids

Phenolic acids represent the most common form of phenolic compounds found in whole grains. Among these, the most abundant are derivatives of hydroxycinnamic acids (Sosulski et al. 1982). The biosynthesis of hydroxycinnamic acids begins with the deamination of phenylalanine to produce cinnamic acid by the enzyme phenylalanine ammonia-lyase (Fig. 10.2). Further enzymatic reactions include hydroxylation of the aromatic ring, methylation of selected phenolic hydroxyl groups, activation of the cinnamic acids to cinnamoyl-CoA esters, and reduction of these esters to cinnamaldehydes and cinnamyl alcohols.

In most plants, the enzyme phenylalanine ammonia-lyase (PAL) is encoded by a small gene family (Wanner et al. 1995; Zhu et al. 1995). In monocotyledons, genes involved in the synthesis of PAL were isolated from DNA libraries in rice (Minami and Tanaka 1993; Zhu et al. 1995) and wheat (Li and Liao 2003). Kervinen et al. (1998) isolated five different genes in barley encoding PAL from a root cDNA library that were highly similar to the wheat and rice *PAL* sequences. Similar approaches were used to clone other key genes involved in the biosynthesis of phenolic acids in maize (Collazo et al. 1992), wheat (Ma et al. 2002) and rice (Yang et al. 2005).

Only a few attempts have been made to specifically increase the content of phenolic acids in cereal crops. Dias and Grotewold (2003) reported higher content of ferulic, chlorogenic and other phenolic acids in cultured maize cells transformed by the transcription factor *ZmMyb-IF35*. Mao et al. (2007) studied secondary metabolism in maize lines transformed with the wheat oxalate oxidase (*OxO*) gene. In leaves of the *OxO* maize lines, the amount of phenolic acids significantly increased while synthesis of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), a naturally occurring hydroxamic acid insecticide was reduced. Ferulic acid exhibited the largest increase and accounted for 80.4 % of the total soluble phenolics. These results depend on a diversion in the shikimate pathway leading to production of phenolic and hydroxamic acids. More studies are needed to manipulate phenolic acid synthesis pathway in a nutritionally applicable way.

Flavonoids

Flavonoids represent a large family of low-molecular-weight phenolics involved in a wide range of functions (Dixon and Paiva 1995). In cereals, dozens of different flavonoids have been identified mostly conjugated to various sugar moieties (Dykes and Rooney 2007). Variation in flavonoid synthesis depends upon the

enzymatic function/activity of genes in either the core or side branches of the flavonoid pathway (Fig. 10.2). Multiple copies of genes and specific regulatory factors are responsible for the variation in flavonoids in different tissues and organs of plants (Dias and Grotewold 2003; Zhou et al. 2010).

The biosynthesis of flavonoids is initiated by the step catalysed by the enzyme chalcone synthase (CHS) which produces the aglycone flavonoid naringenin chalcone from malonyl-CoA and coumaroyl-CoA precursors (Heller and Forkmann 1994). In maize, CHS is encoded by a duplicated genetic locus (Wienand et al. 1986; Franken et al. 1991). In the majority of plants including cereals, chalcones are not the end-products. The pathway proceeds with several enzymatic steps to flavanones, dihydroflavonols and, finally, to the anthocyanins, the major water soluble pigments in flowers and fruits (Grotewold and Peterson 1994; Deboo et al. 1995). The synthesis of isoflavones, aurones, flavones, proanthocyanidins and flavonols is well documented in maize and more than 20 structural and regulatory genes have been identified (Mol et al. 1998; Grotewold 2006). However, little is known about the final transfer of anthocyanins into the vacuole (Marrs et al. 1995; Alfenito et al. 1998).

Most of the structural genes involved in the flavonoid pathway have been identified, characterized and mapped in wheat (Munkvold et al. 2004; Himi and Noda 2004, 2005; Himi et al. 2011). Khlestkina et al. (2008a) identified four distinct copies of *Flavanone 3-hydroxylase* (*F3H*) gene in bread wheat by PCR-based cloning. In barley, a cDNA library screened with a probe from *Antirrhinum majus* was used to clone the gene encoding flavanone-3-hydroxylase (Meldgaard 1992). Some of the genes involved in the synthesis of flavonoids in cereals have also been mapped. In wheat, *CHS* was found to map to chromosomes of homoeologous groups 1 and 2 (Li et al. 1999), *CHI* to homoeologous group 5 and 7D (Li et al. 1999), *F3H* and *DFR* to homoeologous groups 2 (Khlestkina et al. 2008b) and 3 (Himi and Noda 2004), respectively.

The regulation of flavonoid metabolism is achieved mainly through transcriptional regulation of genes involved in biosynthetic pathway (Martin et al. 2001; Davies and Schwinn 2003). A number of regulatory genes required for anthocyanin regulation have been identified, cloned, and characterized in several species. These transcription factors belong to two classes, MYB superfamily and basic-Helix-Loop-Helix (bHLH), and together with a WD40 protein, are thought to regulate the anthocyanin biosynthetic genes co-operatively (Koes et al. 2005).

Regulatory genes controlling the tissue specificity of structural genes were identified by mutant analysis in maize (Paz-Ares et al. 1986; Cone et al. 1993a, b; Pilu et al. 2003), *Arabidopsis* (Paz-Ares et al. 1987; Vom Endt et al. 2002), *Antirhinum* (snapdragon; Martin et al. 1991), *Petunia* (Quattrocchio et al. 1993), *Vitis vinifera* (grape; Deluc et al. 2008) and wheat (Himi et al. 2011). Two types of transcription factors grouped as the *R*/*B* family (basic helix–loop–helix, bHLH-type) and the *C1*/*Pl* family (Myb-type) were shown to upregulate the structural genes required for the production of anthocyanin (Consonni et al. 1993; Pilu et al. 2003). In addition, transcription factors *P*, *TT2*, *TT8* and *Del* also regulate part of the flavonoid biosynthesis (Martin et al 1991; Vom Endt et al. 2002).

The enzymes that direct the splitting of flavonoid synthesis pathway from the phenylpropanoid pathway are critical for the increased production of various flavonoids. Shin et al. (2006) obtained the novel synthesis of several classes of flavonoids in the endosperm of rice by expressing two maize regulatory genes (*C1* and *R-S*) using an endosperm-specific promoter. *C1*, when transferred to wheat induced anthocyanin pigmentation in otherwise non-pigmented wheat coleoptiles (Ahmed et al. 2003). In addition, the *R* and *Rc-1* genes were shown to upregulate key genes of the flavonoid pathway in wheat (Hartmann et al. 2005; Himi and Noda 2005; Himi et al. 2011).

10.3.2 Carotenoids

Carotenoids are pigments conferring the characteristic yellow to red color to fruits and flowers. Structurally, they are isoprenoid compounds having generally eight isoprene units and long polyene chains with 3–15 conjugated double bonds (Weedon and Moss 1995). More than 600 carotenoids have been identified in plants including α-carotene, β-carotene, lycopene, lutein, zeaxanthin, cryptoxanthin, citroxanthin and violaxanthin, etc., (Kahlon and Keagy 2003). The most famous member of the carotenoids is β-carotene, which is a precursor of vitamin A; its deficiency leads to xerophthalmia and also cataracts and macular degeneration with ageing. Carotenoids may also have protective effects in cardiovascular diseases and cancer (Kohlmeier and Hastings 1995; Astorg 1997).

Carotenoid synthesis starts in the plastids of higher plants by the action of IPP isomerase and GGPP synthase converting four molecules of isopentyl diphosphate (IPP), to geranyl geranyl diphosphate (GGPP) (Giuliano et al. 2008). Phytoene synthase subsequently condenses two molecules of GGPP to form 15-*cis*-phytoene, which is the first dedicated step in the carotenoid biosynthesis (Beyer et al. 1985). Figure 10.3 gives a schematic representation of the carotenoid biosynthesis pathway in plants.

Ye et al. (2000) produced golden rice with increased β-carotene content by introducing the *phytoene synthase* (*psy*) gene from daffodil together with a bacterial phytoene desaturase (*crtI*) gene from *Erwinia uredovora* placed under control of the endosperm-specific glutelin (Gt1) and the constitutive cauliflower mosaic virus (CaMV) 35*S* promoters, respectively. Paine et al. (2005) developed golden rice-2 with 23-fold higher total carotenoid accumulation by introducing the maize *psy* gene compared to the original golden rice (Ye et al. 2000). Giuliano et al. (2008) estimated that 100 % of the recommended dietary allowance (RDA) of vitamin A for children and 38 % for adults can be obtained with 60 g/day consumption of golden rice-2.

Wong et al. (2004) reported QTL mapping of β-carotene synthesis pathway genes in maize. The β-carotene biosynthetic pathway in maize was also studied using loss-of-function mutants (Buckner et al. 1990, 1996; Li et al. 2007; Zhu et al. 2008). A mutant of *phytoene synthase* (*y1*) of maize has white endosperm

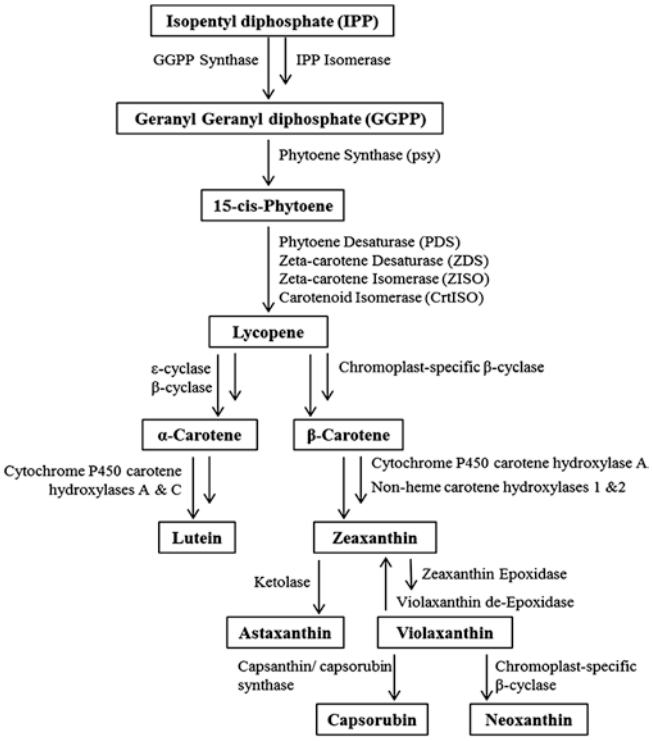

Fig. 10.3 Carotenoid biosynthesis pathway in plants. Modified from Giuliano et al. (2008)

and very low carotenoid levels. Phytoene desaturase is the second enzyme and is responsible for a two-step desaturation of phytoene to ζ (Zeta)- carotene which is then further desaturated to other forms of carotenoids such as lycopene and β-carotene. Yan et al. (2010) reported cloning of gene β-carotene hydroxylase-1 (*crtRB1*) in maize and further demonstrated a rare genetic variation in *crtRB1* to enhance β-carotene levels in maize.

Hexaploid bread wheat (*T. aestivum*) has low carotenoid levels (1.94 μg/g), whereas diploid einkorn wheat and tetraploid emmer wheat have relatively higher carotenoid content (9.62 and 6.27 μg/g, respectively), which is however, lower than that of corn (35.11 μg/g) (Panfili et al. 2004; Abdel-Aal et al. 2002, 2007). Lutein is the predominant carotenoid in wheat and comprises 80–90 % of the total carotenoid content, the remaining being zeaxanthin, β-carotene, and lutein esters (Abdel-Aal et al. 2002). Lutein content has been reported to be higher in the flour than the bran portion in all the wheat species analyzed (Abdel-Aal et al. 2002). Zhang et al. (2005) transferred yellow pigment gene (*Y*) from *Lophopyrum ponticum* to wheat cultivars. They proposed *Y* gene to be either an efficient enzyme in early steps of carotenoid biosynthetic pathway or a regulatory factor that affects several steps of the carotenoid biosynthetic pathway (Zhang et al. 2005).

Pozniak et al. (2007) mapped genes *psy1* and *psy2* on group-7 and -5 chromosomes, respectively, in durum wheat, of which *psy1* had a strong association with yellow pigment content of endosperm (Pozniak et al. 2007; Singh et al. 2009). A similar association is known in maize endosperm yellow pigment and maize *psy1* gene (Gallagher et al. 2004). Zhang and Dubcovsky (2008) isolated the *psy1-A* and *psy1-B1* genes from two durum cultivars, which was followed by the development of functional markers for flour color in wheat by He et al. (2009). Wang et al. (2009) cloned and made a phylogenetic analysis of the *psy1* gene in common wheat and related species. All the genes had six exons and five introns. Sequence divergence due to single nucleotide polymorphisms (SNPs) and insertion deletions (InDels) were present among the different clusters. Cong et al. (2010) cloned cDNA and made an expression analysis of the wheat *phytoene desaturase* (*PDS*) and ζ-carotene desaturase (*ZDS*) genes and found them to have high homology with those of other higher plant species.

10.3.3 Tocopherols and Tocotrienols (Vitamin E)

Vitamin E is a family of fat-soluble antioxidants consisting of α-, β-, γ-, and δ-tocopherols and the corresponding α-, β-, γ-, and δ- tocotrienols. Alpha-tocopherol is the form of vitamin E that is preferentially absorbed and accumulated in humans (Rigotti 2007). Compared to tocopherols, tocotrienols have been less investigated, although they show higher antioxidant potential (Sen et al. 2006). This is due to widespread occurrence of tocopherols in plants as the principal vitamin E components of leaves and seeds in most dicot species (Padley et al. 1994). On the other hand, tocotrienols typically account for the majority of the total vitamin E content in the seeds of monocots, such as rice, wheat and oats (Peterson and Qureshi 1993; Padley et al. 1994). From the human health point of view, tocotrienols have been shown to have specialized roles in protecting neurons from damage (Sen et al. 2006) and in cholesterol reduction (Das et al. 2008). Tocopherol compounds, in both durum and bread wheat are mostly present in the germ fraction (Panfili et al. 2003; Borrelli et al. 2008). Table 10.3 summarizes the content of various components of vitamin E in the grains of common cereals.

The tocopherol biosynthetic pathway in plants has been extensively studied for over 30 years (Whistance and Threlfall 1970; Grusak and DellaPenna 1999) and the enzymes and genes of the pathway have been isolated (DellaPenna 2005). With the exception of Vitamin-E-defective (VTE3) (Cheng et al. 2003), tocopherol biosynthetic enzymes share significant homology between plants and cyanobacteria, underscoring the evolutionary relationship between these organisms.

The first step in tocopherol synthesis involves the production of the aromatic head group, homogentisic acid (HGA), from p-hydroxyphenylpyruvic acid (HPP) by the enzyme p-hydroxyphenylpyruvic acid dioxygenase (HPPD), as reviewed by DellaPenna (2005). Cahoon et al. (2003) isolated *HPT* from tocotrienol-accumulating seeds of barley, wheat and rice and expressed barley *HPT* in tobacco calli

Table 10.3 Tocopherol and Tocotrienol content of major cereal flour, milling products and selected products (modified from Piironen et al. 1986)

Cereal grain	Tocopherols (T) and Tocotrienols (T3) in mg/g of fresh product							Vitamin E
	α-T	**α-T3**	**β-T**	**β-T3**	**γT**	**γT3**	**δT**	**(mg of α-T eq)**
Wheat flour	1.6	0.3	0.8	1.7	–	–	–	2.1
Wheat bran	1.6	1.5	0.8	5.6	–	–	–	2.7
Wheat germ	22.1	0.3	8.6	1.0	–	–	<0.1	25.7
Rye flour	0.6	0.4	0.3	0.6	–	–	–	0.8
Rye meal	1.0	1.4	0.3	1.1	–	–	–	1.6
Barley meal	0.3	1.6	<0.1	0.6	0.1	0.6	<0.1	0.8
Rice, brown	0.6	0.4	0.1	<0.01	0.1	0.7	<0.1	0.8
Rice, polished	<0.1	0.1	<0.1	<0.1	<0.1	0.3	<0.1	0.1
Millet, whole grain	0.1	<0.1	0.1	–	1.7	<0.1	0.6	0.3
Oats, rolled	0.8	2.0	0.1	0.3	–	<0.1	–	1.5
Oats, puffed	0.8	1.4	0.1	0.3	–	<0.1	<0.1	1.3
Semolina	0.15	0.11	0.08	1.33	–	–	–	0.28
Macaroni	0.1	0.1	0.1	0.8	–	–	–	0.2
Corn flake	<0.1	0.2	–	–	0.1	0.4	<0.1	0.1
Popcorn	0.35	0.27	0.03	–	2.61	0.23	0.15	0.71

The components present in trace amounts are shown by a '–'

using the CaMV 35S promoter. Barley *HGGT* was expressed in *Arabidopsis thaliana* leaves, which accumulated large amounts of tocotrienols upon transformation. High tocotrienol corn was designed by expressing barley *HGGT* in maize, under the control of embryo specific promoter for corn *oleosin* gene, showing that a single metabolic step was sufficient to enhance the effective level of vitamin E six-fold (Cahoon et al. 2003).

10.4 Future Perspectives

Functional food components vary across the cereal crops and within different tissues of the grain. Knowledge of the genetics, biochemistry and genomics of functional food components also differs among crop plants and is more advanced in rice and corn than in wheat, barley and oats. Moreover, large genome size of wheat, barley and oats, together with polyploidy in wheat and oats further complicate genetic and genomic analysis. High-quality sequences of wheat genome and genes are urgently needed and will greatly accelerate functional food component research.

The next challenge will be to elucidate metabolic pathways and structural and regulatory genes for functional food components. As the reviewed literature reveals, this work is already in progress and needs to be continued at an accelerated pace. Comparative genomics and bioinformatics-based approaches will be useful in leveraging information from model organisms, rice and maize to other cereal crops. However, many genes are crop-specific, so that functional genomics tools must be developed in each cereal crop plant. In this regard, TILLING appears to be a versatile tool for crops such as wheat and barley where other functional tools are not that well developed. TILLING will be useful for mining novel alleles of genes of metabolic pathways, increasing diversity in the trait of interest, as demonstrated by the directed search of specific mutants for high amylose starch. However, TILLING may not be feasible for multigene families where techniques such as RNAi may be more appropriate for knocking down specific gene activity. A transgenic approach was used to produce golden rice but public acceptance has been problematic. TILLING is a promising strategy for the targeted breeding for genes of interest with no biosafety issues because it is an entirely non-transgenic approach. Genetics, breeding and transgenic approaches have been and can be used to design cereal crops with optimum expression of functional food compounds such as β-glucan, amylose, inulin, phenolics, flavonoids, carotenoids, and vitamin E.

Wild germplasm is another untapped resource of useful genetic variation in the functional food compounds. In the past, related wild species have been used as sources of many useful genes for resistance against biotic and abiotic stresses, but they have not been used so far in improvement of cereals for their use as functional foods. Evaluating natural variation in the wild relatives of crop plants for functional food components and molecular breeding of those traits for increasing the functional food value of cereal crops should be fully explored.

10.5 Summary and Outlook

Cereals are major components of the human diet, and the content of compounds that are beneficial to human health has become a fascinating and important subject of research. With increasing knowledge of the biosynthetic pathways of functional food components, the exact roles played by the various genes involved and the factors affecting the end product, it is becoming increasingly possible to design cereal crops as functional foods, with nutritional role beyond use as a source of calories.

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