Food and Energy Security
Open Access
REVIEW

Molecular and environmental factors determining grain quality in rice

Yi Chen[1], Mei Wang[1,2] & Pieter B. F. Ouwerkerk[1]

[1]Sylvius Laboratory, Department of Molecular and Developmental Genetics, Institute of Biology, Leiden University, Sylviusweg 72, PO Box 9505, 2300 RA, Leiden, The Netherlands
[2]SU BioMedicine-TNO, Utrechtseweg 48, 3704 HE Zeist, PO Box 360, 3700 AJ, Zeist, The Netherlands

Keywords
Environment, genetic, grain, molecular, quality, rice

Correspondence
Pieter B. F. Ouwerkerk, Sylvius Laboratory, Department of Molecular and Developmental Genetics, Institute of Biology, Leiden University, Sylviusweg 72, PO Box 9505, 2300 RA Leiden, The Netherlands.
E-mail: p.b.f.ouwerkerk.2@gmail.com

Funding Information
This research was supported by the Programme for Strategic Scientific Alliances (04-PSA-BD-04) between China and the Netherlands of the Royal Netherlands Academy of Arts and Sciences (KNAW) for MW and PBFO and the KNAW China Exchange Programme (KNAW-CEP 04CDP022, 06CDO033, 07CDP005, 08CDP042) for Y. C.

Received: 2 July 2012; Revised: 24 October 2012; Accepted: 25 October 2012

Food and Energy Security **2012; 1(2): 111–132**

doi: 10.1002/fes3.11

Abstract

Rice among other cereals is key to food security for at least half the world population. Since the 1960s, productivity of rice has largely been improved during the Green Revolution, which included development of new cultivars, irrigation infrastructure, new management techniques, and synthetic fertilizers and pesticides. Nowadays, scientists and breeders are more and more focused on improving the quality of rice for different purposes and markets. For instance, people in the Far East prefer sticky and soft rice, while in India, a non-sticky type is preferred. Consumers from developed countries ask mainly for grain with good cooking quality and eating characteristics, but in many developing regions, nutritional value is crucial as rice is the most consumed staple food. Grain quality is a general concept which covers many characteristics ranging from physical to biochemical and physiological properties. Starch and protein are the two main components of rice endosperm and therefore are key to quality. The knowledge of how starch and protein are synthesized, sorted, and stored in starch granules and protein bodies (PB) is important for rice breeding. Besides that, grain quality has been shown to be affected significantly by growing and environmental conditions, such as water availability, temperature, fertilizer application, drought, and salinity stresses. However, the signal transduction pathways controlling grain quality still remain largely unclear. In the following sections, we first briefly review the four main aspects of grain quality, followed by a discussion of the molecular and genetic basis of starch and seed-storage protein biosynthesis and the effects of environmental factors. Obviously, rice grain is also an important source of mineral micronutrients, as well as important vitamins. Storage of these also plays crucial roles in grain quality and nutritional value, but we will only discuss these aspects briefly in this review.

Grain Quality Traits in Rice

Major traits of grain quality

The traits and parameters used to evaluate grain quality in rice vary across countries. However, four main quality traits (Yu et al. 2008) are widely used to assess quality, namely milling properties, appearance, nutritional value, and cooking quality. After harvesting, rice seeds are milled to remove the outer husk and bran layers in order to produce different types of edible rice based on the requirements of the consumers. Thus, milling quality of the grain determines the final yield and the broken kernel rate of the milled rice, which is of concern for both breeders and farmers. Appearance is one of the crucial properties appealing consumers after milling. Cooking quality determines the easiness of cooking, as well as the firmness and stickiness of the cooked rice which is associated with eating properties. As one of the most important staple food in the world, nutritional value is also valued by consumers.

Some criteria for the main quality traits are widely shared among the different rice-consuming regions. The

brown rice rate, milled rice rate, and head rice rate are the three main parameters used to evaluate the quality and efficiency of the milling process. These rates represent the ratios of grains produced out of different grades of milling. Brown rice is just de-husked to remove the palea and lemma. Removing all of the bran (hard outer layer of the rice grain consisting of the aleurone and pericarp) and germ or embryo results in white milled rice. Head rice is a standard criterium telling the weight percentage of rice grains having a length greater or equal to 75% of the average length of the whole grain. After milling, the appearance of the grain is associated with size, shape (long vs. round), chalkiness, and translucency. Cooking quality is mainly associated with the amylose content, which is one of the two starch types in the grain, as well as by protein content, gelatinization temperature, and gel consistency. Gelatinization is the process in which the intermolecular bonds of starch molecules are broken down in the presence of water and heat, thereby dissolving the starch granules in the endosperm. The gelatinization temperature determines the time required for cooking. Gel consistency was developed as a parameter to index the tendency of cooked rice to harden on cooling and is normally classified as hard, medium, and soft. Protein content is also an important parameter determining nutritional value.

Grain qualities are mainly associated with the storage starch and protein in endosperm

All main quality traits listed above, milling properties, appearance, nutritional value, and cooking quality, are related to the content and composition of protein and starch in the endosperm. The nutritional value of rice grain is determined by the seed-storage protein content which can make up to 5–12% (Villareal and Juliano 1978). Cooking quality is mainly determined by the content and composition of starch and storage protein in the endosperm, of which two types of starch, amylose, and amylopectin are the main factors. Amylose content (He and Suzuki 1987; Sowbhagya et al. 1987; Rani and Bhattacharay 1989; Ong and Blanshard 1995; Singh et al. 2003; Cameron and Wang 2005; Allahgholipour et al. 2006; Kibanda and Luzi-kihupi 2007) and the chain structure of amylopectin (Ong and Blanshard 1995; Ramesh et al. 1999; Mizukami and Takeda 2000; Cameron and Wang 2005; Nakamura et al. 2006) were found to be significantly correlated with hardiness and stickiness of the rice after cooking. The protein content was also suggested to be involved in cooking quality in such that protein–starch interactions can impede starch gelatinization, and disruption of the structure of proteins during cooking can increase viscosity of the rice meal (Hamaker et al. 1991; Hamaker and Griffin 1993; Cameron and Wang 2005; Derycke et al. 2005; Yu et al. 2008). Starch composition also has a decisive role in the appearance of the milled rice. For instance, rice grain can look opaque or chalky. Chalkiness is caused by incomplete filling of the grain which may due to adverse weather conditions and is in fact related to the shape, size, and packing of amyloplasts which are organelles responsible for the synthesis and storage of starch granules within the endosperm. Translucent rice kernels have significantly higher amylose content than chalky grains (Rani and Bhattacharay 1989; Lisle et al. 2000; Singh et al. 2003). The appearance of the rice grain is also altered by milling as this process increases whiteness due to removing the bran which makes the grain more attractive for the consumer, yet at the cost of losing the protein content of the outer part of the brown rice grain. On the other hand, a higher storage protein content in the rice grain was reported to prevent breakage during milling (Leesawatwong et al. 2004). Milling also helps cooking in that it decreases the gelatinization temperature (Champagne et al. 1990; Muramatsu et al. 2006) and improves flavour, hardiness, chewiness, and adhesiveness of cooked rice (Park et al. 2001; Saleh and Meullenet 2007).

Importance of minerals and vitamins

Besides starch and storage proteins, mineral micronutrients such as zinc (Zn), iron (Fe), manganese (Mn), and selenium (Se) are also essential for human health and thus important for the nutritional value of rice grain. However, the content of metal ions in rice grain is usually poor (Heinemann et al. 2005). Minerals in rice grain are also related to cooking traits. A recent study showed that mineral element contents had obvious correlations with amino acid and protein contents of the grain. Furthermore, gel consistency was significantly correlated with potassium (K), copper (Cu), and Mn contents of the grains, and amylose content was significantly associated with K, sodium (Na), magnesium (Mg), Cu, and Mn contents. The alkali spreading value has a positive association with calcium (Ca), Mg, and Mn contents (Jiang et al. 2007). The uptake and distribution of ions in rice is very complicated and seriously affected by environmental factors like soil status, fertilizer appliance, and climate change. Much progress has been made through studying the model plant Arabidopsis. As a result, many interesting reviews have been published concerning uptake and transport of mineral micronutrients in plants (Pittman 2005; Sors et al. 2005; Grotz and Guerinot 2006; Kim and Guerinot 2007; Curie et al. 2009; Palmer and Guerinot 2009; Puig and Penarrubia 2009).

Brown rice is also well known as a good source of the vitamin B complex. For instance, 100-g medium-grain brown rice (raw) contains substantial amounts of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), and vitamin B6 (http://ndb.nal.usda.gov/). Compared with the vitamin B complex, the content of other groups of vitamins in rice grain is relatively poor. The biosynthesis of vitamins B in plants is summarized in several papers (Coxon et al. 2005; Tambasco-Studart et al. 2005; Roje 2007; Asensi-Fabado and Munne-Bosch 2010). Thus, we will not discuss vitamins as well as the mineral micronutrients in the following sections as they are not the main quality traits concerned in this article.

Molecular Basis of Grain Quality in Rice

Comparison of quality traits revealed significant variation among cultivars grown in the same environment (Adu-Kwarteng et al. 2003; Cameron and Wang 2005; Kang et al. 2006; Vidal et al. 2007), which indicates that the decisive factors controlling starch and protein contents and thus grain quality lie in the rice genome itself, that is, in the loci encoding starch synthetases and seed-storage proteins as well as their *cis*- and *trans*-acting regulators. Hereafter, we will discuss genes relevant in starch and seed-storage protein biosynthesis. A schematic overview of genes and proteins and their roles and effects on determining grain quality in rice is shown in Figure 1.

Starch synthetases determine the content and characteristics of amylose and amylopectin in the endosperm

Starch in the endosperm of rice is the dominant form of carbohydrate reserves in grain. There are two different types of storage starches: amylose and amylopectin. Amylose is a linear polymer of D-glucose units, and amylopectin is a highly branched polymer of glucose. A series of enzymes is involved in the biosynthesis of starch in rice grain. Most of these enzymes in rice are represented by different isoforms and are encoded by multiple genes, leading to a highly complex biosynthesis and accumulation process.

Biosynthesis of starch in the developing rice grain starts from conversion of sucrose into glucose and fructose, the main transported form of carbohydrate assimilates in rice. Sucrose synthase (SUS) is responsible for this step by catalyzing the cleaving of sucrose into UDP-glucose and D-fructose. The native form of this enzyme is a tetramer composed of subunits with a molecular mass of about 90 kDa. In rice, there are four different isozymes expressed in different tissues (Chan et al. 1990). Three genes (*RSus1*, *2*, and *3*) have been cloned, and their expression patterns were characterized (Wang et al. 1992a,b, 1999; Yu et al. 1992; Huang et al. 1996; Odegard et al. 1996). It appears that *RSus2* is ubiquitously expressed in both vegetative tissues and developing grains. *RSus3* is highly specific to the grain, and the levels of *RSus1* and *RSus3* in developing grains are closely complementing each other, both spatially and temporary (Huang et al. 1996; Wang et al. 1999). Till now, no mutants of these genes have been reported in rice, which may be because loss-of-function of *RSus2* is lethal for the plants and knocking-out of the other two genes may be compensated by the function of *RSus2*.

After degrading sucrose into glucose and fructose, four types of enzymes are playing key roles in synthesis of starch in cereals: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) (more details on this pathway are reviewed in James et al. 2003 and Jeon et al. 2010). AGPase produces the activated glucosyl donor ADP-glucose that is the primer of the starch chain and is regarded as the rate-limiting enzyme in starch biosynthesis. AGPase in rice has large and small subunits which are encoded by four and two genes, respectively (Jeon et al. 2010). Mutants of *OsAGPL2* and *OsAGPS2b*, which are genes encoding the large and small subunits, respectively, display a shrunken endosperm due to a severe reduction in starch synthesis (Lee et al. 2007).

SS is composed of the so-called soluble (SSS) and granule-bound (GBSS) isoforms and catalyzes the chain-elongation reaction of α-1,4-glucosidic linkage by transferring a glucose moiety from ADP-glucose to the nonreducing end of the linkage of the preexisting starch molecules that act as primers. GBSS and SSS are responsible for the synthesis of amylose and amylopectin, respectively. GBSS in rice has two isoforms expressed in storage (GBSSI) and non-storage (GBSSII) tissues. GBSSI is also called Waxy (Wx), and its role in biosynthesis of amylose is very well defined. The storage starch in nonwaxy varieties is composed of 15–30% amylose and 70–85% amylopectin (Umeda et al. 1991); however, the *wx* mutant endosperm contains almost exclusively amylopectin. *Wx* genes have been cloned from *Oryza sativa* (Okagaki and Wessler 1988; Wang et al. 1990; Hirano and Sano 1991; Okagaki 1992) and *O. glaberrima* (Umeda et al. 1991). Two wild-type alleles, *Wxa* and *Wxb*, were found at the *waxy* locus in cultivated rice. *Wxa* is characteristic for indica rice, and *Wxb* is found mainly in japonica rice (Sano 1984; Sano et al. 1986). A naturally occurring single-base mutation at the 5′ splicing site of the first intron differing *Wxa* and *Wxb* alleles is the direct cause for the expression polymorphism of *Wx* between indica and japonica rice cultivars (Hirano et al. 1998; Isshiki et al. 1998).

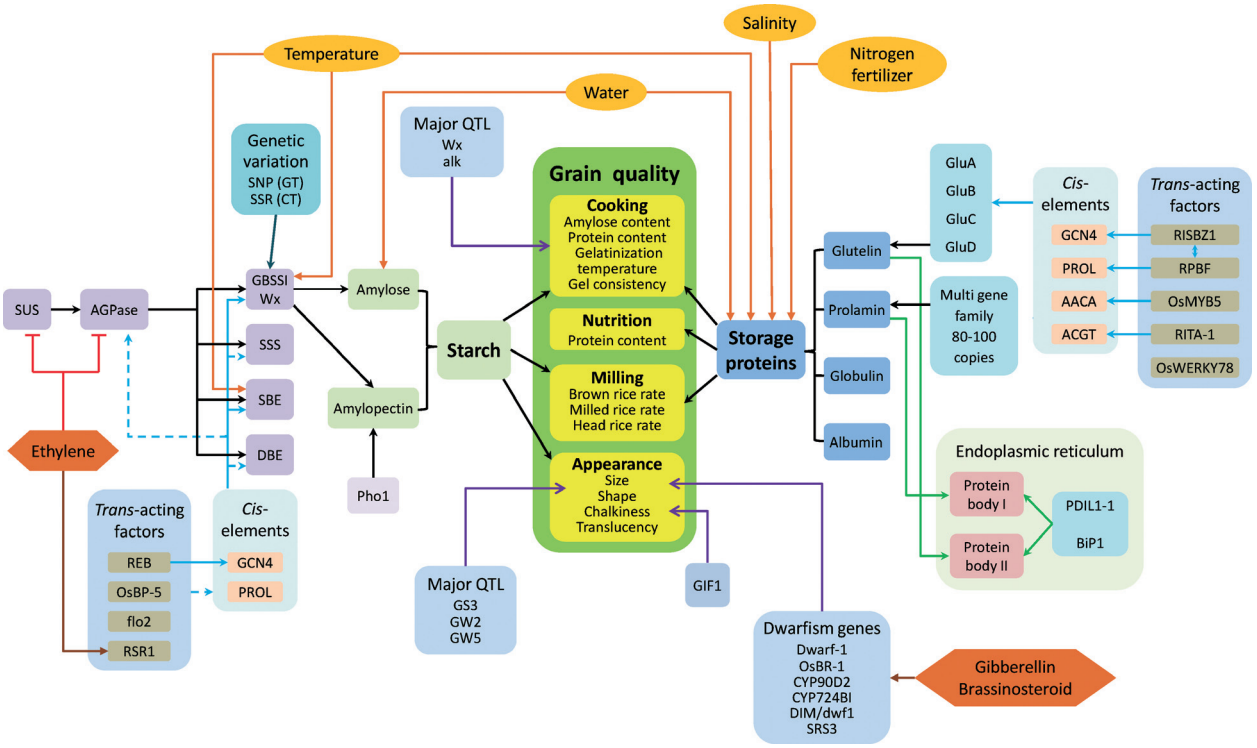

Figure 1. Schematic representation of molecular and environmental factors (orange boxes) influencing the four main aspects (yellow boxes) of grain quality in rice. Enzymes involved in starch (green boxes) metabolism (left side) are in purple boxes. Seed-storage proteins are in blue boxes (right side). Gene and protein codes are referred to and explained in the text.

Synthesis of amylopectin is more complicated than amylose and involves all four types of enzymes including AGPase, SSS, SBE, and DBE (more detailed metabolic systems were reviewed in Nakamura 2002 and Jeon et al. 2010). SSS in rice has eight isoforms, namely SSI, SSIIa (SSII-3), SSIIb (SSII-2), SSIIc (SSII-1), SSIIIa (SSIII-2), SSIIIb (SSIII-1), SSIVa (SSIV-1), and SSIVb (SSIV-2) (Hirose and Terao 2004). SBE generates α-1,6 linkages by cleaving internal α-1,4 bonds and transferring the released reducing ends to C6 hydroxyls, and thus forms branches on polymers. DBE is capable of removing some of these branches. SBE contains three isoforms (BEI, BEIIa, and BEIIb) in rice. Physiochemical analysis of the mutants revealed the various roles of SSI (Fujita et al. 2006), SSIIa (Umemoto et al. 2002, 2004; Nakamura et al. 2005), SSIIIa (Fujita et al. 2007), BEI (Satoh et al. 2003), BEIIa (Nakamura 2002), BEIIb (Nishi et al. 2001; Tanaka et al. 2004) in the synthesis of different chains of amylopectin in recent years. DBE has two forms, isoamylase and pullulanase, which were both cloned (Nakamura et al. 1996; Fujita et al. 1999). Although the role of DBE used to be restricted to the debranching of amylopectin during complete hydrolysis of starch, studies on the mutants *sugary 1* and isoamylase antisense transgenics during the last two decades revealed the involvement of isoamylase and pullulanase in the synthesis of amylopectin in rice (Nakamura et al. 1996, 1997; Kubo et al. 1999; Fujita et al. 2003, 2009). Besides these enzymes, Wx also bears the potential capability in formation of extra-long unit chains (ELCs) of amylopectin in the SSIIIa-deficient mutant, which has a high level of ELC amylopectin and pleiotropically increased Wx in the endosperm (Fujita et al. 2007). This function was verified using transgenic plants by transforming the *Wx* gene into the null-mutant *wx* rice (Hanashiro et al. 2008).

Alteration of amylopectin content and structure has significant impact on the morphology of starch granules and eventually affects cooking and consumption traits. It has been reported that silencing of *SSIIIa* (Fujita et al. 2007; Ryoo et al. 2007), *BEI* (Satoh et al. 2003), *BEIIb* (Nishi et al. 2001), isoamylase gene *OsISA1* (Fujita et al. 2003) changes the gelatinization properties of the starch endosperm. In another study, the indica *SSIIa* gene, which carries two variations in amino acids and thus affects the activity of the enzyme, was introduced into japonica rice which enabled japonica rice cultivars to synthesize indica-type amylopectin. The starch in the transformed japonica rice exhibited gelatinization-resistant properties that are characteristic of starch from indica rice (Nakamura et al. 2005).

Besides starch synthetases, plastidial starch phosphorylase Pho1 also has an important role in amylopectin synthesis. Loss of *Pho1* function caused smaller starch granules to accumulate and modified the amylopectin structure. The size of mature seeds and the starch content in *pho1* mutants showed considerable variation, ranging from shrunken to pseudo-normal (Satoh et al. 2008).

Protein composition of rice grain is mainly determined by glutelins and prolamins

The seed-storage proteins are not only the crucial nitrogen source during germination and initial development of seedlings but also play important roles for the structure of the endosperm. Based on their solubility, storage proteins can be grouped into four types, that is, water-soluble albumin, salt-soluble globulin, alkaline-soluble glutelin, and alcohol-soluble prolamin. Unlike the other crops, up to 80% of the total storage protein in rice is glutelin (Yamagata et al. 1982) and prolamin takes only maximum 20–30%. Although four storage proteins are all initially synthesized on the ER membrane and translocated into the ER lumen, they are stored in two different PB, named PB-I and PB-II. Both PB-I and PB-II are mainly stored in the starchy peripheral part of the endosperm.

Glutelin in rice is synthesized as a 57-kDa precursor and then cleaved into a 37- to 39-kDa acidic subunit and a 22- to 23-kDa basic subunit (Yamagata et al. 1982). Encoded by about 15 genes per haploid genome, glutelin genes can be classified into four subfamilies: GluA, B, C, and D. GluA has four members, whereas GluB has the highest with eight members. Thus far, only two members of GluC and one member of the GluD subfamily have been identified (Takaiwa 1987; Okita et al. 1989; Takaiwa et al. 1991; Mitsukawa et al. 1998; Kusaba et al. 2003; Katsube-Tanaka et al. 2004; Kawakatsu et al. 2008). As the second most abundant protein in rice endosperm, the prolamins have molecular masses ranging from 12 to 17 kDa. The rice prolamins are encoded by a complex multigene family with 80–100 copies per haploid genome (Kim and Okita 1988a,b; Masumura et al. 1989; Barbier and Ishihama 1990; Feng et al. 1990; Masumura et al. 1990; Shyur and Chen 1990; Horikoshi et al. 1991; Shyur et al. 1992; Yamagata et al. 1992; Wen et al. 1993; Mitsukawa et al. 1999).

Because of the redundancy of glutelin and prolamin genes in rice, it is difficult to identify naturally occurring cultivars with obviously altered storage protein levels. The only report so far is a selection of 19 cultivars among 1400 accessions, from Russia, Northern China, and North Korea, showing an increased content of the 57-kDa glutelin precursor with markedly decreased content of acidic and basic subunits (Satoh et al. 1995). However, uncovering the molecular basis behind this qualitative trait is challenging and time consuming, as it will require development of mapping populations and cloning of candidate genes. Gamma-ray irradiation and chemical treatment provide alternative mutagens and have been used to create mutants with variable glutelin and prolamin compositions (Kumamaru et al. 1988; Iida et al. 1993, 1997). A mutant line *NM67* obtained from a collection of 1433 mutated lines of cultivar "Nihonmasari" treated with 0.2% ethyleneimine was found to have a lowered content of glutelin and a higher content of prolamine. Genetic analysis revealed that low-glutelin content was always accompanied by high prolamine, and this phenomenon seemed to be manifested by a single dominant gene. An improved low glutelin and high prolamine line (named LGC-1) has been developed from a backcross between *NM67* and its original cultivar (Iida et al. 1993). Another nine mutant lines lacking glutelin subunits were selected from M_2 seeds of about 10,000 M_1 plants mutagenized with gamma rays or ethylmethanesulfonate (EMS) and from 1400 mutant lines selected originally for morphological characters. Three types of mutants were identified. One line lacks the largest subunit among four minor bands of glutelin acidic subunits. Five lines lack the second-largest subunit band, and three lines do not have the third-largest subunit band. Genetic analysis of the mutated genes showed that these phenotypical characteristics were controlled by single recessive genes named *glu-1*, *glu-2*, and *glu-3*, respectively (Iida et al. 1997). LGC-1 and *glu-1* exhibited the same phenotype, indicating they may have a similar genetic basis. Further research confirmed this hypothesis. In LGC-1 homozygotes, there is a 3.5-kb deletion between two highly similar and tandem-repeated glutelin genes *GluB-5* and *GluB-4* that forms a tail-to-tail inverted repeat, which may produce a double-stranded RNA molecule that will induce RNA silencing like in an RNAi approach (Kusaba et al. 2003). On the other hand, *glu-1* harbors a 129.7-kb deletion, which eliminates the entire *GluB-5* and *GluB-4* gene except half of the first exon of *GluB-5* (Morita et al. 2007). Comparison of LGC-1 with cultivars with normal glutelin content by light microscopy and confocal laser scanning microscopy revealed that low-glutelin rice differs from other cultivars not only in composition of major storage proteins but also in the distribution of PB in the endosperm. The LGC-1 line showed more presence of PB-I in the inner part of the endosperm, whereas in other cultivars examined, PB-I is more present at the outer layer of the endosperm (Furukawa et al. 2003).

Overlapping expression patterns of starch synthetase and seed-storage protein genes

Most of the starch synthetase genes expressed during grain filling of rice follow a highly similar expression style. The mRNA transcripts increase with the development of seeds and peak at either six or ten DAF (days after flowering), and then decline gradually toward maturation. The only exception is the expression of *Waxy* which is initiated at three DAF. After reaching a peak activity at six DAF, the expression of *Waxy* declines toward ten DAF but rises again and peaks at 15 DAF before declining to practically zero at 20 DAF (Duan and Sun 2005). The expression pattern of *Waxy* in rice grain was shown to be cultivar dependent (Wang et al. 1995).

Rice glutelin and prolamin genes are exclusively expressed during seed development. Similar to starch synthetases, the majority of the seed-storage protein genes in rice are developmentally regulated. A recent transcriptional profiling study of six members from the GluA and GluB subfamilies showed that none of them had any detectable expression level at three DAF but with a peak activity at 10 DAF before declining toward maturation (20 DAF) (Duan and Sun 2005). This common pattern was also discovered in other studies on GluA, GluB, and GluC subfamily members (Okita et al. 1989; Takaiwa and Oono 1990, 1991; Mitsukawa et al. 1998). *GluD-1*, however, is different from the other glutelin genes. It is detectable in seed tissue at five DAF with increasing intensity till 30 DAF (Kawakatsu et al. 2008). As described in the previous section, rice prolamins are encoded by 80–100 gene copies. Among them, a 10-kDa prolamine gene and three members of the 13-kDa prolamine family followed a largely identical expression pattern compared with glutelin genes. However, the other two genes from the 13-kDa subfamily showed a distinct expression pattern, in that they were expressed increasingly, but at low level, toward seed maturation (20 DAF) (Duan and Sun 2005). The transcripts of two 16-kDa prolamin genes also showed a gradual increase in expression starting to accumulate from eight DAF onwards to the maturation of grains till 26–28 DAF (Shyur et al. 1992; Mitsukawa et al. 1999).

Common *cis*-regulatory elements exist in regulatory regions of starch synthetase and seed-storage protein genes

All seed-storage protein and starch synthetase genes are strongly expressed during grain development, indicating they may share a similar regulation mechanism on the transcription level. Indeed several universal *cis*-regulatory elements have been identified in the 5′ upstream region of these genes, not only in rice but also in other crops. Comparisons of the promoter sequences of cereal prolamin genes have identified a conserved region at around ~300-bp upstream of the transcriptional start. This so-called bifactorial endosperm box is composed of two closely located motifs, a prolamine box class endosperm motif and a GCN4-like motif (Kreis et al. 1985). GCN4 and prolamin boxes have been discovered in promoters of seed-storage protein genes in wheat, maize, sorghum, barley, wheat, rye, and oats (Colot et al. 1987; Albani et al. 1997; Vicente-Carbajosa et al. 1997; Marzábal et al. 1998; Norre et al. 2002). Two other common motifs are the AACA and ACGT boxes. GCN4, AACA, and the prolamin box were found in GluA, GluB, and GluD family members in rice. The ACGT box also exists in these genes except for *GluA-1* (Takaiwa et al. 1996; Yoshihara and Takaiwa 1996; Yoshihara et al. 1996; Washida et al. 1999; Wu et al. 2000; Kawakatsu et al. 2008; Qu et al. 2008). However, the GluC promoter does not contain any of the fore-mentioned *cis*-elements which means this gene has its own unique way of regulation (Qu et al. 2008). The prolamin and GCN4 boxes are also identified in rice prolamin genes (Zhou and Fan 1993; Wu et al. 1998; Su et al. 2001). Unfortunately, compared with seed-storage protein genes, studies on the promoter regions of starch synthetases are limited. However, the GCN4 and prolamin boxes are also found in regulatory regions of the *Waxy* gene in rice (Cheng et al. 2002) and in the gene for the SBE *sbeIIa* in wheat (Miao et al. 2004). Considering the equivalent expression pattern of starch synthetase genes (Duan and Sun 2005), elements like the GCN4 box and others may also be localized within the 5′ regulatory region of other starch synthetase genes.

Transcriptional regulators of starch synthetases and seed-storage proteins

Despite the increasing number of studies and reports on the activities of different classes of *cis*-elements controlling seed-specific gene expression, identification of the corresponding *trans*-acting factors in rice and other cereals is still limited. One of the earliest known transcription factors specifically involved in grain development is Opaque2 from maize (Schmidt et al. 1992). In rice, a basic leucine zipper family (bZip) protein named RITA-1 was identified first to be able to bind to the ACGT element and activate reporter gene expression in transient assays (Izawa et al. 1994). Another bZip protein named REB was found later to interact specifically with the GCCACGT(c/a)AG sequence in the α-globulin promoter (Nakase et al. 1997). In more recent studies, five different bZip proteins named RISBZ1 to RISBZ5, two of them, RISBZ2 and RISBZ3 were completely identical with RITA-1 and REB, were

identified in a cDNA library derived from rice seeds. These factors were able to bind to the GCN4 box from the rice *GluB-1* promoter, but only RISBZ1 is capable of *trans*-activating the expression of a reporter gene preceded by a minimal promoter fused to a pentamer of the GCN4 box (Onodera et al. 2001; Kawakatsu et al. 2008). The AACA sequence in glutelin promoters is the target site for the Myb domain factor OsMYB5 (Suzuki et al. 1998). The Dof (DNA binding with one finger) prolamin box-binding factor (RPBF) is able to recognize AAAG/CTTT motifs in the *GluB-1* promoter. Both RISBZ1 and RPBF can *trans*-activate GUS activity driven by promoters of different storage protein genes in transient assays, such as *GluA-1*, *GluA-2*, *GluA-3*, *GluB-1*, *GluD-1, 10-kDa Prolamin*, *13-kDa Prolamin*, *16-kDa Prolamin*, and α-*Globulin*. Synergistic interactions between RISBZ1 and RPBF were also discovered in transient assays (Yamamoto et al. 2006; Kawakatsu et al. 2008). However, much of the evidence so far for any biological functions of these transcription factors is from gain-of-function experiments in transient expression studies without phenotypical data on, for example, grain protein content or other yield parameters. Our research group tried to identify T-DNA or transposon-tagged mutants for several transcription factors (Zhang et al. In press). Unfortunately, no obvious phenotype and alteration of grain starch and storage protein content have been discovered. This may be due to the redundancy of the transcription factors and the multi-copy number of the starch synthetase and storage protein genes which makes any future research on this topic challenging. Till now, only rice transgenics in which RISBZ1 and RPBF expression was down-regulated showed alteration in target gene expression and protein accumulation (Kawakatsu et al. 2009).

For starch synthetase genes, there are even less transcription factor genes known regulating their expression than for the seed-storage protein genes. The rice bZip protein REB, which was first isolated to be able to interact with the promoter of α-globulin (Nakase et al. 1997), can also interact with the GCN4 motif in the promoter of the *Wx* gene (Cheng et al. 2002). OsBP-5, a Myc protein can bind specifically to a 31-bp sequence in the 5′ upstream region of the *Wx* gene and can *trans*-activate expression synergistically with the EREBP family protein OsEBP-89 by forming a heterodimer (Zhu et al. 2003). The recessive *floury-2* (*flo-2*) loci of rice causes a strong reduction not only in *Wx* and AGPase expression but also in expression in developing seeds of the genes encoding two isoforms of SBE (Kawasaki et al. 1996). This indicates that coregulation by a common *trans*-acting regulator does occur for different starch synthetases genes. Rice Starch Regulator-1 (RSR1) is an APETALA2/ethylene-responsive element-binding transcription factor, which has been identified as a novel regulator of starch biosynthesis (Fu and Xue 2010). Deficiency of RSR1 results in enhanced expression of starch synthesis genes during grain development. As a consequence, grains of the knockout mutant *rsr1* show an increased amylose content and altered fine structure of amylopectin and consequently form round and loosely packed starch granules, resulting in a decreased gelatinization temperature. It was also found that the lower expression of RISBZ-1 and RPBF, which were identified as transcription factors regulating seed-storage proteins, also caused a reduction in seed starch accumulation (Kawakatsu et al. 2009). This indicates that starch synthetase and storage protein genes share not only common *cis*-elements but also the same transcription factors.

Recently, a WRKY transcription factor named OsWRKY78 has been reported to be involved in regulation of grain size in rice (Zhang et al. 2011). However, starch composition and structure studies showed that this protein was not involved in regulation of starch metabolism, and any downstream target genes need to be further identified and studied.

Post-transcriptional regulation of genes involved in grain development

Regulation of starch synthetase and seed-storage protein genes does not only occur on the transcriptional level but also occur on the post-transcriptional level. Analysis of a collection of 31 rice cultivars for levels of *Wx* transcript, Wx protein, and amylose revealed that the levels are correlated with the ability to excise intron 1 from the leader sequence of the *Wx* mRNA transcript. Comparison of three groups of cultivars which have high, intermediate, or no amylose content revealed that the nucleotide sequences of the *Wx* intron 1 differ among groups. These altered nucleotides contribute to the improper splicing and incomplete splicing of intron 1. As a consequence, the total amount of translatable *Wx* mRNA, and therefore the Wx protein and consequently amylose content, are reduced (Wang et al. 1995; Cai et al. 1998). Later studies further confirmed the importance of specific sequences in *Wx* intron 1 associated to the correct splicing of the pre-mRNA (Ayres et al. 1997; Hirano et al. 1998; Larkin and Park 1999; Isshiki et al. 2000; Mikami et al. 2000; Larkin and Park 2003; Bao et al. 2006a; Prathepha 2007).

Although approximately four-fold more abundant than the prolamines on a weight basis due to their higher molecular mass in mature seeds, glutelins are only slightly more abundant than prolamines on a molar basis. Both proteins are first detected in 10 DAF seeds and their amounts steadily increase throughout seed development. However, the molar ratio of glutelin to prolamine proteins decreased from 1.7 at 10 DAF to 1.2 at 25 DAF,

while the amount of glutelin and prolamine transcripts increased from equal at 5 and 10 DAF to a 40% excess of prolamine transcripts during grain maturation (Kim et al. 1993; Li and Okita 1993). This phenomenon indicates that the expression of the glutelin and prolamin multigene families are differentially regulated, not only on the transcriptional but also on the post-transcriptional level.

Moreover, the ER is an important intracellular structure where a lot of regulation takes place affecting storage protein synthesis and storage. After synthesized in the ER, glutelins are transported to the protein storage vacuole via the Golgi apparatus or vesicles derived directly from the rough ER (Takahashi et al., 2005) and form PB-II. Prolamines, on the other hand, are assembled and deposited within the lumen of the rough ER to form PB-I (Ogawa et al. 1987; Li et al. 1993b; Muntz 1998). In the cells of the developing endosperm, the mRNAs of prolamins and glutelins have been shown to be associated with different areas of the rough ER. Glutelin mRNAs are enriched more on the rough cisternal ER membranes, whereas the prolamine mRNAs are more abundant on the rough ER membranes that surround the PB-Is (Li et al. 1993a). This process might very well represent the first step in the sorting of the two different classes of seed-storage proteins. After transfer into the ER lumen, seed-storage proteins form disulfide bonds and fold with the assistance of foldases and molecular chaperons. *esp2* mutants contain higher levels of the 57-kDa polypeptide and correspondingly lower levels of acidic and basic glutelin subunits. Electron microscopic observations revealed that *esp2* contained a normal PB-II content, but lacked PB-I. Instead, numerous small ER-derived new PBs that contained the 57-kDa glutelin precursor and prolamin polypeptides were observed. These proteins form glutelin–prolamin aggregates via interchain disulfide bonds within the ER lumen (Takemoto et al. 2002). *Esp2* is the structural gene for protein disulfide isomerase-like gene *PDIL1-1*, and it is asymmetrically distributed within the cortical ER and largely restricted to the cisternal ER (Satoh-Cruz et al. 2010). There were also data showing that the interaction of prolamin with the ER chaperone BiP (binding proteins) is important in the formation of ER-derived PBs (Kawagoe et al., 2005; Saito et al. 2009). Severe suppression or overexpression of *BiP1* not only reduced seed-storage protein content and starch accumulation but also changed the composition of the storage proteins (Yasuda et al. 2009; Kawakatsu et al. 2010b; Wakasa et al. 2011). The 57-kDa glutelin precursors are sorted into protein storage vacuoles via Golgi-derived dense vesicles and ER-derived precursor-accumulating vesicles (Takahashi et al., 2005), where they are further processed into acidic and basic subunits. Recent work demonstrated that processing of pro-glutelin by a vacuolar processing enzyme in rice is essential for proper protein storage vacuole structure and compartmentalization of storage proteins (Wang et al. 2009; Kumamaru et al. 2010).

The cytoskeleton is also known to be involved in the localization of seed-storage proteins. Biochemical studies have shown that prolamine mRNAs may be anchored to the surface of type one prolamine PBs via the cytoskeleton (Muench et al. 1998). Confocal microscopy of endosperm cells further revealed that unlike the glutelin PBs, the developing prolamine PBs are not randomly distributed within the endosperm cell, but instead are often enriched in the cortical region of the cell only a few micrometers beneath the plasma membrane. In addition, the peripherally localized prolamine PBs are in very close juxtaposition with the cortical microtubule and actin filament networks (Muench et al. 2000).

Genetic diversity in starch synthetase genes is related to the physiochemical properties of rice grain

Besides the functions of *cis*-acting elements and *trans*-acting factors in regulation of grain quality genes, genetic diversity within the genomic regions of starch synthetase genes also showed clear effects on quality traits in rice. The most well-studied molecular marker in the first intron of *Wx* gene is a single-nucleotide polymorphism (SNP), which is involved in the proper splicing of *Wx* mRNA. This SNP represents a naturally occurring G to T change resulting into two alleles named *Wxa* and *Wxb* which determine a significantly different amylose content resulting in the change of cooking properties and taste of rice (Wang et al. 1995; Ayres et al. 1997; Cai et al. 1998; Hirano et al. 1998; Mikami et al. 2000; Larkin and Park 2003; Han et al. 2004; Bao et al. 2006a; Prathepha 2007; Chen et al. 2008a,b). Besides this, also other SNPs were found in the *Wx* gene which are associated with variations in amylose content and viscosity characteristics (Sato et al. 2002; Larkin and Park 2003; Chen et al. 2008a,b; Tran et al. 2011). Another intensively studied polymorphism is a microsatellite (SSR) locus of $(CT)_n$ repeats identified in the 5′-UTR of the *Wx* gene (Bligh et al. 1995). Various alleles of this locus were identified in nonwaxy cultivars which associate with variation in amylose levels (Ayres et al. 1997; Bergman et al. 2001; Tan and Zhang 2001; McClung et al. 2005; Bao et al. 2006a; Jayamani et al. 2007). Four alleles of this locus, indicated as $(CT)_{16}$, $(CT)_{17}$, $(CT)_{18}$, and $(CT)_{19}$, respectively, were also detected in 56 *waxy*-type cultivars, and class $(CT)_{19}$ is associated with high gelatinization temperature (Bao et al. 2002b). The G to T SNP and CT SSR have already been utilized in marker-assisted crosses and selection of

improved cultivars (Bergman et al. 2001; Ramalingam et al. 2002; Zhang et al. 2005; Jayamani et al. 2007). Several other polymorphisms in starch synthetase genes have been described that are associated with physicochemical properties of grain, such as SSRs in SBE1 and SSSI, SNPs in SBE1, SBE3, and SSIIa, a sequence-tagged site (STS) in SBE1 and one insertion/deletion (InDel) in SSIIa (Bao et al. 2002b; Umemoto et al. 2004; Bao et al. 2006a,b; Sun et al. 2011). Compared with the research on starch synthetases, there are only few studies on genetic diversity of seed-storage protein genes in rice. In the glutelin gene *GluD-1*, a total of 28 SNPs were detected in the sequences of four japonica and indica cultivars (Kawakatsu et al. 2008). However, these SNPs have not yet been reported to be associated with any variation of seed-storage protein content in rice or other quality traits.

Identification of important genes affecting different quality traits by mapping-based approaches

Dwarfism in plants is long known to be an attractive trait in crop breeding because it increases the harvest index. Although the most obvious phenotypes of dwarf rice are reduced plant height and erect leaves, many mutants also bear small and round grains. However, the molecular bases of such phenotypes have not been elucidated well till the rapid development of genetic approaches such as map-based cloning in the last two decades became possible. The *Dwarf-1 (D1)* gene has been mapped on chromosome 5 as the α-subunit of a GTP-binding protein (G protein) (Cho et al. 1994; Fujisawa et al. 1999). The *d1* mutant was shown to be insensitive to both gibberellins (GA) (Mitsunaga et al. 1994; Ueguchi-Tanaka et al. 2000; Bethke et al. 2006) and brassinosteroids (BR) (Wang et al. 2006; Oki et al. 2009), suggesting that the G protein plays a role in both pathways. The cell number in *d1* leaf sheath, internode, root, and lemma was reduced (Izawa et al. 2010), which might also be the reason for the smaller seeds. Studies on other dwarf mutants have identified four more genes as the molecular bases of dwarfism in those mutants. *OsBR1* is a putative BR receptor kinase mapped on chromosome 1 in the study of *d61* (Yamamuro et al. 2000). *CYP90D2*, *CYP724BI*, and *DIM/dwf1* encode three BR biosynthesis enzymes which were mapped on chromosome 1, 4, and 10, respectively, in the studies on *d2* (Hong et al. 2003), *d11* (Tanabe et al. 2005), and *brd2* (Hong et al. 2005), respectively. Another mutant exhibiting small and round seeds as well as shortened panicles and internodes is named *srs3* and is caused by a defect in a novel kinesin 13 protein gene (*SRS3*) which was mapped on chromosome 5. The cell length of grains in the longitudinal direction in *srs3* is shorter than that in the wild type (Kitagawa et al. 2010). A gene regulating grain filling, *GIF1*, was mapped on chromosome 4 and encodes a cell-wall invertase required for carbon partitioning during early grain filling. A GIF1 loss-of-function mutant showed more grain chalkiness due to the abnormal development and arrangement of starch granules (Wang et al. 2008).

Quantitative trait loci (QTL) analysis revealed that many grain quality traits are not controlled by single genes, but involve multiple regions in the rice genome. In doubled haploid and other inbred lines, two major QTLs were identified which are responsible for the main variance of amylose content and gelatinization temperature traits, respectively (He et al. 1999; Tan et al. 1999; Li et al. 2003; Bao et al. 2004b; Fan et al. 2005; Takeuchi et al. 2007), that correspond to the *Wx* gene and the alkali degeneration gene (*alk*) gene on chromosome 6, respectively. In addition, the *Wx* locus also showed a major effect on gel consistency (Fan et al. 2005). The results of the genetics studies further substantiated the important roles of the two genes and the QTL regions in determining cooking quality of rice. In addition, various other QTL studies have identified three other major genes, *GS3* for grain length (Huang et al. 1997; Redoña and Mackill 1998; Tan et al. 2000; Kubo et al. 2001; Thomson et al. 2003; Aluko et al. 2004; Fan et al. 2006), *GW2* (Song et al. 2007; Guo et al. 2009) and *GW5* (*qSW5*) (Shomura et al. 2008; Weng et al. 2008) for grain width, which are localized on chromosome 3, 2, and 5, respectively. *GS3* encodes a protein with a putative PEBP-like domain, a trans-membrane region, a putative TNFR/NGFR family cysteine-rich domain, and a VWFC module. *GW2* encodes a RING-type protein with E3 ubiquitin ligase activity and *GW5* encodes a novel protein without significant homology to any proteins with known biochemical functions. Furthermore, several other QTLs responsible for minor variations in traits of all the main quality aspects have been mapped on different other loci (Huang et al. 1997; Redoña and Mackill 1998; Bao et al. 2000; Tan et al. 2000; Bao et al. 2002a; Aluko et al. 2004; Wan et al. 2004; Tanaka et al. 2006; Wada et al. 2006; Yoon et al. 2006; Tabata et al. 2007; Takeuchi et al. 2007; Kobayashi and Tomita 2008; Kobayashi et al. 2008; Zhang et al. 2008a; Sabouri 2009; Kwon et al. 2011).

Hormonal regulation

Besides GA and BR which regulate the dwarfism genes and thus control grain shape and size, other plant hormones also showed effects on grain quality traits though the molecular basis is yet to be clarified. Ethylene is a plant hormone regulating fruit ripening by coordinating the expression of genes that are responsible for a variety

of processes, including an increase in respiration, autocatalytic ethylene production, and changes in color, texture, aroma, and flavor. In rice, ethylene evolution rate was significant and negatively correlated with grain-filling rate (Liu et al. 2008), resulting in an inverse correlation with chalky kernel percentage and chalkiness (Yang et al. 2007; Zhang et al. 2009a). Cultivars with a low 1-aminocyclopropane-1-carboxylic acid (ACC) concentration, one of the key components in ethylene biosynthesis pathway, in grains exhibited a close amyloplast arrangement and little space between starch granules, whereas those with a high ACC concentration in grains showed a loose arrangement and wide space between the granules (Yang et al. 2007). Application of ACC to panicles at early (Zhang et al. 2009a), mid, and late (Yang et al. 2007) grain-filling stages significantly increased chalky kernel percentage, chalky area, and chalkiness. The results were reversed when amino-ethoxyvinylglycine, an inhibitor of ACC activity, was applied to panicles. These effects of ethylene on the appearance of the grain may largely relate to the activities of the key enzymes involved in sucrose to starch conversion in the grains. AGPase and SUS activities were found to be negatively correlate with ethylene concentration (Mohapatra et al. 2009). Application of aminoethoxyvinylglycine to panicles at the early grain-filling stage significantly increased activities of SUS, AGPase, and soluble SS (Zhang et al. 2009b). In addition, the effects of ethylene depend on the positions of spikelets on the panicles, with stronger influences on grain filling and quality of the kernels of the basal spikelets (Naik and Mohapatra 2000). Furthermore, several studies revealed that the interaction between abscisic acid (ABA) and ethylene may be involved in mediating the post-anthesis development of spikelets (Yang et al. 2004, 2006; Zhang et al. 2009b). Applications of GA and cytokinin have been also reported to enhance development of spikelets, while indole-3-acetic acid (IAA) increased spikelet growth and development in the distal branches but suppressed them in the proximal branches (Patel and Mohapatra 1992). Alteration of ethylene, ABA, and GA levels in rice is often related to water deficit and drought stress which affects grain-filling rate and eventually changes yield and grain quality (Yang et al. 2001, 2004; Liu et al. 2008; Zhang et al. 2009b).

Environmental and Culture System Effects on Grain Quality Traits

Grain quality is determined by a variety of developmental processes which are affected not only by the genetic constitution but also by environmental conditions. Significant genotype × environment effects were found for several quality traits in rice cultivars grown at different locations and also during different seasons (Bao et al. 2004a; Cameron et al. 2008; Sharifi et al. 2009). Figure 1 shows a schematic overview of several environmental factors and stresses on grain quality of rice and the interactions with genes and proteins involved in starch metabolism and seed-storage deposition.

Effects of water shortage and drought on grain quality

Water is one of the most essential inputs for production of crops, and rice among all the cereals consumes the most water for irrigation. In many systems, production of 1-kg rice takes 5000 L of water although this can be reduced to about 2000 L (Lal 2007). The water status of the soil has a dramatic effect on yield, and it also affects grain quality (Dingkuhn and Le Gal 1996). Traditionally, rice is planted under submerged condition; however, nonflooded plastic film mulching (PM) and nonflooded wheat straw mulching (SM) are being considered new water-saving techniques in rice production. Different water management treatments, namely PM, water-saving irrigation, and conventional irrigation, significantly affected brown rice rate, head rice rate, chalky grain rate, amylose content, and protein content in a cultivar and grain position dependent manner. Of all variables, water treatment had the strongest effect on protein content (Cheng et al. 2003b). SM was found to significantly increase milling quality and reduced the percentage of chalky grain, chalky size, and chalkiness, while PM showed opposite effects. Gel consistency was found decreased under PM (Zhang et al. 2008b). Rice is the only crop which can survive periods of submergence. However, flooding just before harvest brought visible changes to the physical appearance of grains. The kernels in flood-affected samples became soft and developed fissures which contributed to low head rice recoveries, and the milled rice had lower kernel weight and protein content, but showed higher amylose and ash content (Singh et al. 1990).

Growing rice in an upland nonflooded environment also affects grain quality. A recent study involved several cooking and nutrient quality traits, including amylose content, gel consistency, gelatinization temperature, and protein content, in the same populations grown under upland and lowland conditions. The phenotypic values of all four traits were significantly higher under upland environment than lowland environment (Guo et al. 2007).

Fertilizer application

Nitrogen level in the soil strongly affects yield and grain quality. The current hypothesis is that yield is related to the nitrogen supplying capacity of soil, which in turn

determines grain protein content (Perez et al. 1996). Application of nitrogen fertilizer at different stages from panicle initiation, heading, flowering to grain-filling stages, all has been shown to strongly increase seed-storage protein content (Nangju and De Datta 1970; Taira 1970; Seetanun and De Datta 1973; Nagarajah et al. 1975; Vaughan et al. 1980; Perez et al. 1990, 1996; Souza et al. 1999; Leesawatwong et al. 2004, 2005), as well as to improve protein content-related traits like the milled rice rate, head rice rate (Wopereis-Pura et al. 2002; Leesawatwong et al. 2005), and translucency (Perez et al. 1990, 1996). In addition, it was also reported that application of nitrogen increased gel consistency and decreased amylose content of rice kernels, while this treatment did not significantly affect gelatinization temperature and protein content, thus apparently genetic factors also play a role in determining protein content in response to nitrogen fertilizer (Bahmaniar and Ranjbar 2007). Besides nitrogen, potassium is another essential fertilizing element for rice production (Fageria et al. 1990a,b). The effects of potassium on grain quality were not well understood till a recent discovery showing that application of potassium fertilizer increased gel consistency and grain protein content, but had no significant effect on gelatinization temperature and amylose content (Bahmaniar and Ranjbar 2007).

Soil salinity

Salinity is another important condition of the soil which can strongly affect grain quality. A comparison of rice cultivars grown in low salinity and high salinity regions showed that an increased level of salinity significantly lowered protein content in seven of nine cultivars, but had no effect on amylose content and the alkali spreading value (Juliano and El-Shirbeeny 1981). Another study indicated that grains of both saline tolerant and susceptible cultivars grown on saline soils have higher storage protein content, but less translucent grain, lower starch and amylose content than grown on normal soil. Thus, these differences were not related to salinity tolerance (Siscar-Lee et al. 1990).

Seasonal effects and temperature

Rice is largely grown as a transplanted crop. Delay of sowing and transplanting date may affect grain quality due to the differences in temperature and solar radiation. Late planting of the rice delays flowering time and results in partial filling of the spikelets, thus lowering the milling yield and head rice recovery during processing (Shahi et al. 1975; Dhaliwal et al. 1986). The grain dimensions (length/width) were also affected by late sowing and transplanting, but resulted in higher protein content. However, under these conditions, the amylose content decreased (Dhaliwal et al. 1986). Reduction of amylose may be to some extent due to the higher temperature during grain filling because of the late sowing and transplanting. It was reported that *Wx* gene expression was increased in response to low temperature (18°C). The longer rice plants were exposed to low temperature, the higher the levels of Wx protein, and the greater the accumulation of amylose (Hirano and Sano 1998). The G-T polymorphism within the first intron of *Wx* gene has been reported to be related to the different efficiency of RNA splicing and processing when treated with low and high temperatures during grain development (Larkin and Park 1999). This could be one of the crucial reasons behind the different level of *Wx* transcripts under different temperature regimes. Besides *Wx* transcripts, SBEs especially BEIIb were reported down-regulated due to high temperature, whereas α-amylases were up-regulated (Yamakawa et al. 2007). Apart from the effects on transcription, the regulation of temperature is also reflected by its influences on the activities of key enzymes for the biosynthesis of starch (Cheng et al. 2003a; Jiang et al. 2003; Satoh et al. 2008). Thus, the structure and composition of amylose and amylopectin are affected by temperature. For instance, temperature change during grain filling affects the chain length distribution of amylopectin (Umemoto et al. 1999; Yamakawa et al. 2007). High temperature also causes reduction in the amount of large mature amyloplasts and increase in the number of small immature amyloplasts containing small single starch granules (Zakaria et al. 2002). Besides starch, storage protein content is also affected by temperature. Elevated temperature decreases accumulation of prolamin, which is consistent with diminished expression of prolamin genes (Yamakawa et al. 2007; Ma et al. 2009). On the other hand, the relative content of glutelin is not changed in response to high-temperature stress, but the relative content of two glutelin subunits was reduced and the amount of glutelin precursor was improved (Ma et al. 2009). High-temperature stress results in a severe chalky appearance which is due to changes in starch and protein composition. Recently, a study on gene expression in combination with metabolite measurements in rice endosperm revealed several possible key steps for the inhibition of starch accumulation and the accumulation of amino acids in the developing caryopsis exposed to high temperature. For instance, the import of sucrose into endosperm cells might be impaired, but the supply of certain amino acids was enhanced due to the high temperature, which may eventually affect the starch and protein biosynthesis. It provided us more comprehensive knowledge of the mechanism behind high temperature

stress on determination of the grain quality (Yamakawa and Hakata 2010).

Conclusion and Future Perspectives

Seeds or grains from cereals such as rice, maize, wheat, barley, and sorghum are the main resource for human nutrition and animal feed throughout the world. Grains consist of several types of tissue and their development is a highly complicated and well-regulated developmental process. From a nutritional aspect, the seed-storage proteins and starch are the two main components. Accumulation of storage starch and protein is coordinated with cellular differentiation and development of different tissues in the caryopsis, which is the dry, indehiscent fruit in which the grain is formed. This suggests that understanding of grain development and in particular the communication between different cell types will also be crucial for understanding grain quality in addition to understanding regulation of starch synthetase and seed-storage protein genes. The composition of the grain and its quality also depends on the carbon and nitrogen metabolism in source tissues, as well as the translocation of photo-assimilates and amino acids to the sink tissue, the kernels, which implicates that holistic studies on the whole plant level are critical. Last but not least, the molecular basis and the signaling pathways responding to environmental factors which can have strong and adverse effects on grain quality are far from clear yet. Large advances have been made in understanding the molecular and genetic basis of grain quality in rice. Seed-storage protein genes and the genes underlying starch biosynthesis have been cloned, and their functions in grain quality were described. The regulation of these genes on the levels of transcription and post-transcription, during development and in response to environmental conditions, is yet far from being understood. Many important *cis*- and *trans*-acting factors have been identified. However, most transcription regulators were identified through artificial *in vitro* systems which need further confirmation with genetic studies. Last decade many transposon and T-DNA mutant resources have been developed which will help understanding gene functions (Krishnan et al. 2009).

Scientists have attempted to develop and improve grain quality through classical breeding as well as biotechnology. A nice example is the down-regulation of ALK in rice using an RNAi approach which results in changes in gelatinization temperature and gel consistency (Gao et al. 2011). However, extra care has to be taken as down-regulation or up-regulation of the expression of a single gene might result in unpredictable and complex biochemical and physiological changes. It has been reported that modification of a single starch synthesis gene led to changes in all traits related to cooking quality (Tian et al. 2009) and a reduction of one or several storage proteins could be compensated for by increases in other proteins (Kawakatsu et al. 2010a).

Although significant efforts have been made, future research on grain quality in rice will continue to study functions of genes including regulators of seed-storage proteins and starch synthesis, as well as identifying regulators of transporters involved in assimilate uptake, and screening for receptors and components of relevant developmental and environmental stress pathways.

Meanwhile, newly developed technologies, such as next-generation sequencing which allows resequencing and genotyping of large collections of rice cultivars and the use of the data in Genome-Wide Association Studies (GWAS) (e.g., Huang et al. 2010), have provided us with advanced molecular and genetic approaches to identify gene and marker-trait associations. Integration of proteomic, transcriptomic, metabolomic, and phenotypical information will help scientists to describe a comprehensive model on the development of the grain and a better understanding of how the composition of the storage materials in the endosperm is linked and determined by endogenous developmental and exogenous signals and factors.

Conflict of Interest

None declared.

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