Rice

Genes and Their Molecular Functions Determining Seed Structure, Components, and Quality of Rice

Pei Li[1†], Yu-Hao Chen[1†], Jun Lu[1], Chang-Quan Zhang[1,2], Qiao-Quan Liu[1,2*] and Qian-Feng Li[1,2*]

Abstract

With the improvement of people's living standards and rice trade worldwide, the demand for high-quality rice is increasing. Therefore, breeding high quality rice is critical to meet the market demand. However, progress in improving rice grain quality lags far behind that of rice yield. This might be because of the complexity of rice grain quality research, and the lack of consensus definition and evaluation standards for high quality rice. In general, the main components of rice grain quality are milling quality (MQ), appearance quality (AQ), eating and cooking quality (ECQ), and nutritional quality (NQ). Importantly, all these quality traits are determined directly or indirectly by the structure and composition of the rice seeds. Structurally, rice seeds mainly comprise the spikelet hull, seed coat, aleurone layer, embryo, and endosperm. Among them, the size of spikelet hull is the key determinant of rice grain size, which usually affects rice AQ, MQ, and ECQ. The endosperm, mainly composed of starch and protein, is the major edible part of the rice seed. Therefore, the content, constitution, and physicochemical properties of starch and protein are crucial for multiple rice grain quality traits. Moreover, the other substances, such as lipids, minerals, vitamins, and phytochemicals, included in different parts of the rice seed, also contribute significantly to rice grain quality, especially the NQ. Rice seed growth and development are precisely controlled by many genes; therefore, cloning and dissecting these quality-related genes will enhance our knowledge of rice grain quality and will assist with the breeding of high quality rice. This review focuses on summarizing the recent progress on cloning key genes and their functions in regulating rice seed structure and composition, and their corresponding contributions to rice grain quality. This information will facilitate and advance future high quality rice breeding programs.

Keywords: Rice grain quality, Seed structure, Seed size, Grain component, Starch, Protein, Gene cloning, Molecular function, Regulatory network

Introduction

Rice is the major food crop for more than half of the world's population. Breeding elite rice with high yield and quality are the major goals of crop geneticists and rice breeders. In recent decades, benefiting from the discovery and application of "green revolution gene" and "heterosis", rice yield has improved greatly (Evenson and Gollin [2003](); Pingali [2012](); Chen et al. [2019](); Liu et al. [2020](); Wu et al. [2020]()). However, the progress of rice quality-related research and breeding practices lag far behind that of rice yield. This might reflect the emphasis placed on rice yield in the past, the complexity of rice quality studies, and the lack of consensus definitions and evaluation standards of rice quality. As people's living standards and the worldwide rice trade improve, the demand for high-quality rice is increasing. Therefore, breeding high quality rice is critical to meet market demand. A

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*Correspondence: qqliu@yzu.edu.cn; qfli@yzu.edu.cn
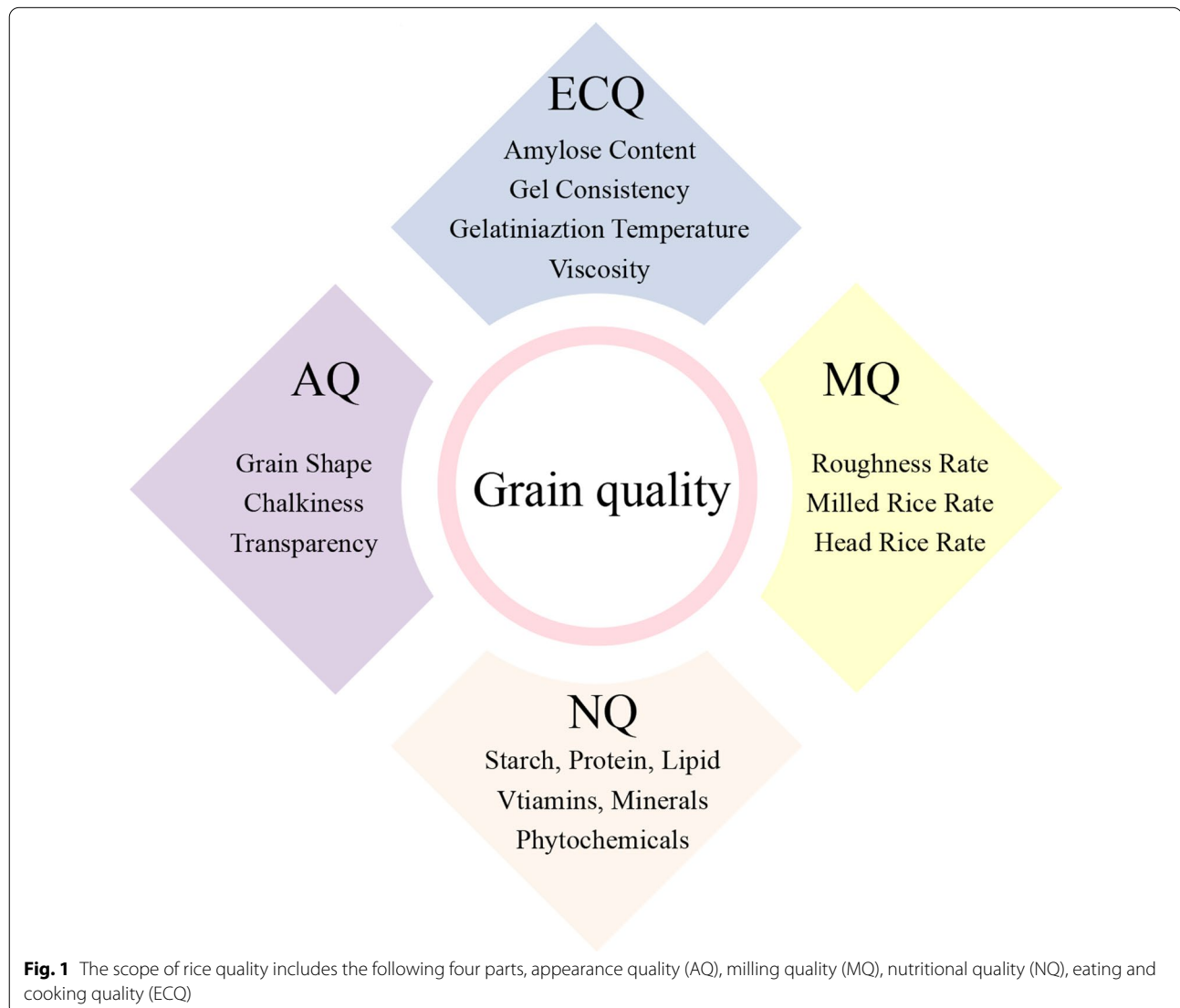[†]Pei Li and Yu-Hao Chen have contributed equally to this work
[1] Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding/ Jiangsu Key Laboratory of Crop Genetics and Physiology/State Key Laboratory of Hybrid Rice, College of Agriculture, Yangzhou University, Yangzhou 225009, Jiangsu, China
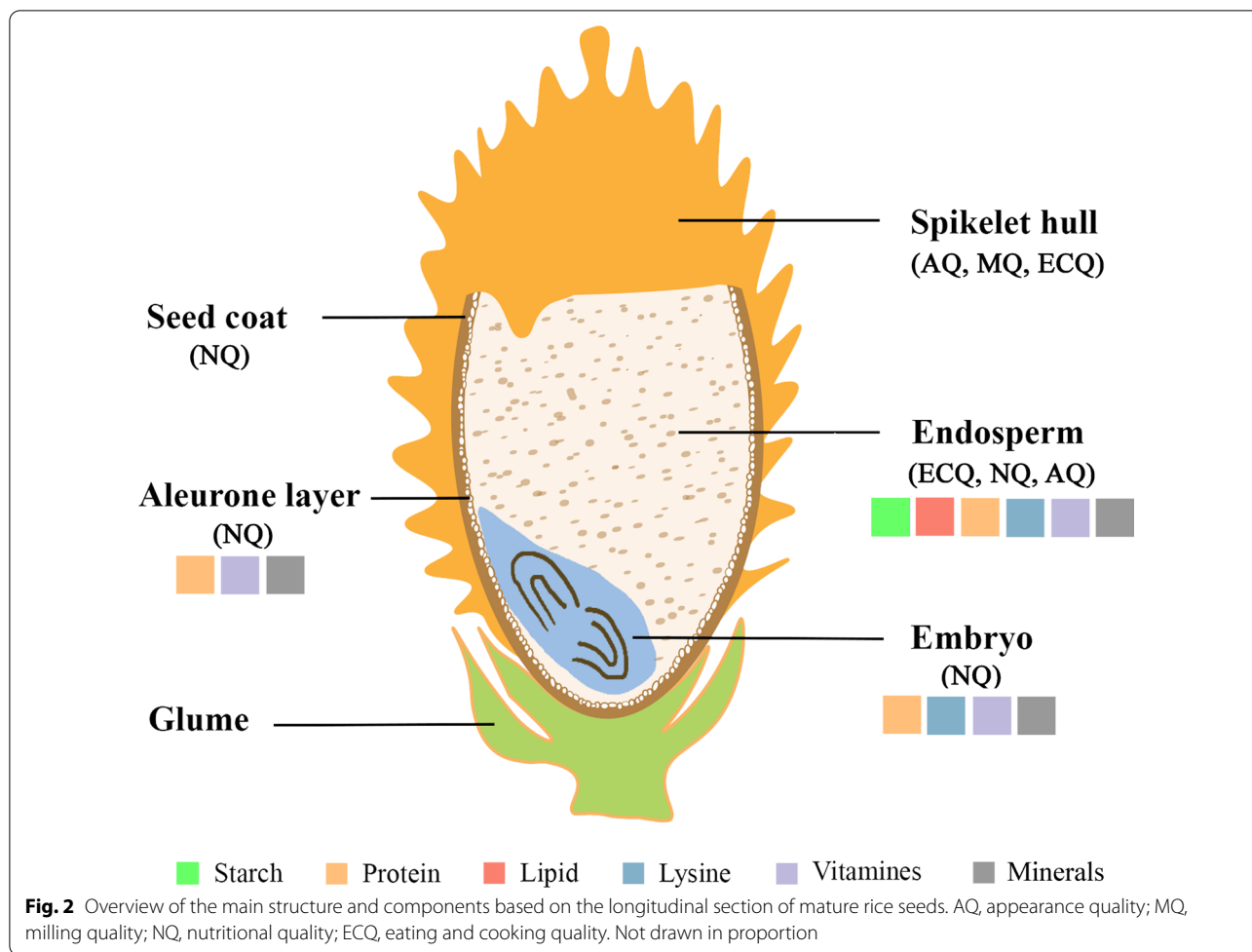Full list of author information is available at the end of the article

series of physical and chemical indexes are used for the comprehensive evaluation of rice quality during the rice processing and cooking. In general, rice quality is divided into four main sections: milling quality (MQ), appearance quality (AQ), nutritional quality (NQ), and eating and cooking quality (ECQ) (Fig. 1) (Bao 2014). Rice MQ refers to the integrity of rice during processing, including the roughness rate, milled rice rate, and head rice rate. AQ usually includes grain shape, chalkiness, transparency, and other indicators. Rice NQ is influenced by the quantity and quality of starch, protein, vitamins, minerals, and other phytochemicals that are beneficial to human health. ECQ mainly reflects the characteristics and palatability of cooked rice. Amylose content (AC), gel consistency (GC), gelatinization temperature (GT), and the results gained using a rapid viscosity analyzer (RVA)

are usually used as indirect indicators to estimate rice ECQ (Zhang et al. 2016).

In general, rice grain quality should be closely correlated with rice seed characteristics. In brief, the rice seed structure mainly includes the spikelet hull, seed coat, aleurone layer, embryo, and endosperm (Fig. 2). The size, composition, and quality of these seed structures affect rice quality markedly. For example, in most cases, the size of the spikelet hull determines the grain size. Grain size is an important rice agronomic trait, which is not only a key element of rice yield, but also is a direct index for rice quality. Rice grain size includes grain length, width, thickness, and the ratio of length to width, which usually affects rice AQ, MQ, and ECQ. Recently, a series of grain size-related genes have been cloned successfully and functionally dissected (Li et al.

Fig. 1 The scope of rice quality includes the following four parts, appearance quality (AQ), milling quality (MQ), nutritional quality (NQ), eating and cooking quality (ECQ)

Fig. 2 Overview of the main structure and components based on the longitudinal section of mature rice seeds. AQ, appearance quality; MQ, milling quality; NQ, nutritional quality; ECQ, eating and cooking quality. Not drawn in proportion

2019). The endosperm is the major edible part of rice seed. Therefore, the composition and proportion of endosperm components are the most important determinants of rice quality. In general, the rice endosperm includes starch, storage protein, lipids, minerals, and other trace elements. Among them, starch and protein are two major components of rice endosperm, accounting for approximately 80% and 10%, respectively, of the weight of milled rice (Wang et al. 2020a). As the largest constituent of rice endosperm, starch consists of two glucose polymers, amylose and amylopectin. The amylose content (AC) and amylopectin structure correlate tightly with rice ECQ (Leng et al. 2014). In addition, the arrangement of starch granules will affect rice AQ. For example, a looser arrangement of starch grains will form cavities inside or between the starch granules, thus decreasing the transparency of the rice grain and resulting in the so-called opaque or chalky endosperm (Zhang et al. 2017). As for proteins, the second largest component of rice endosperm, their content and amino acid composition will affect almost all aspects of

rice quality (Duan and Sun 2005). Moreover, other substances in the endosperm, including lipids, free amino acids, minerals, vitamins, and other phytochemicals, albeit present in lower in quantities, are crucial for rice NQ. The other structures of rice seeds, including the embryo, seed coat, and aleurone layer, are rich in protein, fat, vitamins, and minerals. For example, functional metabolites, such as gamma aminobutyric acid (GABA), are enriched in embryo and aleurone layer compared with that in the endosperm (Pereira et al. 2021).

Each structure of the rice seed correlates with various rice quality traits, and rice grain development is directly controlled by many genes; hence, cloning and dissecting these quality-genes will aid the breeding of high quality rice. Therefore, this review focused on summarizing the recently cloned key genes involved in regulating rice seed structure and composition, and their roles in modulation of rice qualities (Table 1), which will facilitate and advance future high quality rice breeding programs.

Table 1 List of genes involved in controlling rice seed structures and grain qualities

Gene ID	Gene name	Protein category	Effect the quality	References
Spikelet hull & grain size & grain quality				
G protein pathway				
Os03g0407400	GS3	Gγ subunit	Grain length (AQ)	Fan et al. (2006) Mao et al. (2010) Sun et al. (2018a)
Os09g0441900	DEP1/qPE9	Gγ subunit	Grain length (AQ)	Zhou et al. (2009) Huang et al. (2009)
Os03g0635100	RGG1	Gγ subunit	Grain length (AQ)	Tao et al. (2020)
Os02g0137800	RGG2	Gγ subunit	Grain length (AQ)	Miao et al. (2019)
Os08g0456600	GGC2	Gγ subunit	Grain length (AQ)	Sun et al. (2018a)
Os05g0333200	D1/RGA1	Gα subunit	Grain length (AQ)	Fujisawa et al. (1999) Ashikari et al. (1999) Sun et al. (2018a)
Os03g0669200	RGB1	Gβ subunit	Grain length (AQ)	Utsunomiya et al. (2011) Zhang et al. (2021b)
The ubiquitin–proteasome pathway				
Os02g0244100	GW2	E3 ubiquitin ligase	Grain width (AQ)	Song et al. (2007)
Os02g0244300	LG1/OsUBP15	Ubiquitin specific protease	Grain width (AQ)	Shi et al. (2019)
Os03g0232600	TUD1	U-box E3 ubiquitin ligase	Grain length (AQ)	Hu et al. (2013)
Os02g0512400	OsGRX8/WG1	CC-type glutaredoxin	Grain length, grain width (AQ)	Hao et al. (2021)
Os06g0265400	OsbZIP47	bZIP transcription factor	Grain width (AQ)	Hao et al. (2021)
Os08g0537800	WTG1/OsOTUB1	Deubiquitinating enzyme	Grain width, grain thickness (AQ)	Huang et al. (2017)
Os06g0650300	GW6a/OsIHAT1	GNAT-like Protein	Grain length (AQ)	Song et al. (2015) Gao et al. (2021)
Os05g0551000	CLG1/OsHRZ2	RING E3 ubiquitin ligase	Grain length (AQ)	Yang et al. (2021b)
Mitogen-activated protein kinase (MAPK) signaling				
Os02g0787300	OsMKK4/SMG1	Mitogen-activated protein kinase kinase	Grain length, grain width (AQ)	Duan et al. (2014) Guo et al. (2018)
Os06g0154500	OsMAPK6/DSG1	Mitogen activated protein kinase	Grain length, grain width (AQ)	Liu et al. (2015b)
Os04g0559800	SMG2/OsMKKK10	Mitogen activated protein kinase kinase kinase	Grain length, grain width (AQ)	Xu et al. (2018)
Os01g0699500	OsMKKK70	Mitogen activated protein kinase kinase kinase	Grain length, grain width (AQ)	Liu et al. (2021)
Os01g0699600	OsMKKK62	Mitogen activated protein kinase kinase kinase	Grain length, grain width (AQ)	Liu et al. (2021)
Os05g0115800	OsMKP1/GSN1	MAPK phosphatase	Grain length, grain width (AQ)	Guo et al. (2018) Xu et al. (2018)
Phytohormone perception and homeostasis				
Brassinosteroids				
Os03g0602300	BRD1/OsDWARF	Brassinosteroid biosynthetic enzyme	Grain length (AQ)	Hong et al. (2002) Mori et al. (2002)
Os10g0397400	BRD2	Brassinosteroid biosynthetic enzyme	Grain length (AQ)	Hong et al. (2005)
Os04g0469800	D11/ CYP724B1	Brassinosteroid biosynthetic enzyme	Grain length (AQ)	Tanabe et al. (2005) Zhu et al. (2015) Wu et al. (2016b) Zhou et al. (2017c)
Os05g0187500	GW5/GSE5/qGW5	Calmodulin binding protein	Grain width, chalkiness (AQ) Milled rice rate (MQ)	Weng et al. (2008) Shomura et al. (2008) Liu et al. (2017) Duan et al. (2017)
Os05g0207500	OsGSK2	GSK3/SHAGGY-like kinase	Grain length (AQ)	Tong et al. (2012)
Os02g0236200	OsGSK3	GSK3/SHAGGY-like kinase	Grain length (AQ)	Gao et al. (2019)

Table 1 (continued)

Gene ID	Gene name	Protein category	Effect the quality	References
Os07g0580500	OsBZR1	Transcription factor in BR pathway	Grain length, grain width, grain thickness (AQ)	Zhu et al. (2015)
Os02g0517531	LARGE1/ OML4	MEI2-like protein	Grain length, grain width (AQ)	Lyu et al. (2020)
Os06g0127800	DLT/D62/GS6	GRAS family protein	Grain width (AQ)	Tong et al. (2009) Sun et al. (2013)
Os05g0158500	GS5	Putative serine carboxypeptidase	Grain width (AQ)	Li et al. (2011) Xu et al. (2015)
Os05g0343400	OsWRKY53	WRKY transcription factor	Grain length, grain width (AQ)	Tian et al. (2017)
Os08g0174700	OsBAK1	BRI1-associated receptor kinase	Grain length, grain width (AQ)	Li et al. (2009)
Os03g0646900	GL3.1/GL3-1/qGL3/OsPPKL1	Ser/Thr phosphatase	Grain width (AQ)	Hu et al. (2012) Qi et al. (2012) Zhang et al. (2012a) Gao et al. (2019)
Os04g0674500	OsmiR396d	MicroRNA	Grain length, grain width (AQ)	Miao et al. (2020)
Os02g0701300	GS2/GL2/OsGRF4/PT2/ LGS1	Growth-regulating factor	Grain length, grain width (AQ)	Che et al. (2015) Duan et al. (2015) Hu et al. (2015) Sun et al. (2016) Li et al. (2016) Sun et al. (2016)
Os05g0458600	OsLAC	Laccase-like protein	Grain length, grain width (AQ)	Zhang et al. (2013) Zhong et al. (2020)
Os02g0169400	OsAGO17	Argonaute protein	Grain length, grain width (AQ)	Zhong et al. (2020)
Os09g0448500	GS9	Transcriptional activator	Grain width, chalkiness (AQ)	Zhao et al. (2018)
Os01g0718300	OsBRI1/D61	BR receptor kinase	Grain length, grain width (AQ)	Yamamuro et al. (2000)
Os01g0226700	OFP1	OVATE family protein	Grain length, grain width (AQ)	Xiao et al. (2017)
Os01g0732300	OFP3	OVATE family protein	Grain length, grain width (AQ)	Xiao et al. (2020)
Os01g0864000	OFP8	OVATE family protein	Grain length, grain width (AQ)	Yang et al. (2016a, b)
Os05g0324600	OFP19	OVATE family protein	Grain length (AQ)	Yang et al. (2018a)
Os05g0477200	OFP22	OVATE family protein	Grain length, grain width (AQ)	Chen et al. (2021)
Os10g0515400	GW10	Cytochrome P450 subfamily protein	Grain length, grain width (AQ)	Zhan et al. (2021)
Os07g0175100	POW1	Homeodomain-like protein	Grain length, grain width (AQ)	Zhang et al. (2021c)
Auxin				
Os06g0623700	TGW6	Protein with IAA-glucose hydrolase activity	Grain length, grain width (AQ)	Ishimaru et al. (2013) Akabane et al. (2021) Kabir and Nonhebel (2021)
Os03g0175800	BG1	Positive regulator of auxin response and transport	Grain length, grain width (AQ)	Liu et al. (2015a)
Os03g0841800	qTGW3/GL3.3/qGL6	GSK3/SHAGGY-like kinase	Grain length, grain width, grain thickness (AQ)	Hu et al. (2018) Xia et al. (2018) Ying et al. (2018)
Os02g0164900	OsARF6	Auxin response factor	Grain length (AQ)	Qiao et al. (2021)
Os05g0447200	OsAUX3/qGL5	Auxin influx carrier	Grain length (AQ)	Qiao et al. (2021)
GA				
Os06g0266800	OsGSR1/GW6/OsGASR7	GA-stimulated protein	Grain length, grain width (AQ)	Shi et al. (2020)
Cytokinin				
Os01g0680200	OsPUP4/ BG3	Purine permease	Grain length, grain width, grain thickness (AQ)	Xiao et al. (2019) Yin et al. (2020)
Os04g0615700	OsAGO2	AGO family protein	Grain length (AQ)	Yin et al. (2020)
Transcriptional regulatory factors				
SQUAMOSA promoter binding protein-like (SPL) family				
Os07g0505200	OsSPL13/GLW7	Squamosa promoter-binding-like protein	Grain length, grain width (AQ)	Si et al. (2016) Segami et al. (2017)

Table 1 (continued)

Gene ID	Gene name	Protein category	Effect the quality	References
Os11g0247300	*SRS5/TID1*	Alpha-tubulin protein	Grain length, grain width (AQ)	Sunohara et al. (2009) Segami et al. (2012) Segami et al. (2017)
Os08g0531600	*qGW8/OsSPL16*	Squamosa promoter binding-like protein	AC, GC (ECQ)) Protein content (NQ)	Wang et al. (2012) Wang et al. (2015a)
Os07g0603300	*GL7/GW7*	TRM-containing protein	Grain length, grain width, chalkiness (AQ) AC, GC (ECQ)) Protein content (NQ)	Wang et al. (2015c)
APETALA2-type (AP2) transcription factors				
Os05g0389000	*SMOS1/SHB*	AP2-type transcription factor	Grain length, grain width (AQ)	Aya et al. (2014)
Os07g0235800	*SSH1*	AP2-like transcription factor	Grain length, grain width (AQ)	Jiang et al. (2019)
Basic helix-loop-helix (bHLH) family				
Os04g0350700	*An-1*	Basic helix-loop-helix protein	Grain length (AQ)	Luo et al. (2013)
Os03g0171300	*PGL1*	Atypical non-DNA-binding bHLH protein	Grain length (AQ)	Heang and Sassa (2012)
Other transcription factors				
Os06g0666100	*GL6/SG6*	PLATZ transcription factor	Grain length (AQ)	Wang et al. (2019) Zhou and Xue (2020)
Os03g0215400	*OsMADS1/qLGY3*	MADS-domain transcription factor	Grain length (AQ)	Liu et al. (2018b, a) Yu et al. (2018)
Os03g0333200	*FLR1*	Receptor-like kinase	Grain width, chalkiness (AQ)	Wang et al. (2021)
Os01g0769700	*FLR2*	Receptor-like kinase	Grain length (AQ)	Wang et al. (2021)
Other functional proteins				
Os08g0485500	*GAD1*	Secretory signal peptide	Grain length (AQ)	Jin et al. (2016)
ORGLA04G0254300	*GL4*	Myb like protein similar to SH4/SHA1	Grain length (AQ)	Wu et al. (2017)
Os07g0214300	*RAG2*	16-kDa α-amylase/trypsin inhibitor	Grain length, grain width (AQ) Protein content, lipid content (NQ)	Zhou et al. (2017b)
Endosperm components and grain quality				
Starch				
Os06g0133000	*Wx*	Granule-bound starch synthase	AC, GC, GT (ECQ) Transparency, chalkiness (AQ) Digestion, RS (NQ)	Wang et al. (2010) Zhang et al. (2019a) Huang et al. (2020) Zhang et al. (2021a) Zhou et al. (2021a)
Os02g0744700	*OsSSIIb/SSII-2*	Soluble starch synthase	AC, GC, GT (ECQ) Transparency (AQ) Protein content (NQ)	Li et al. (2018a, b) Xu et al. (2020)
Os06g0229800	*OsSSIIa/SSII-3/ALK*	Soluble starch synthase	GT (ECQ)	Umemoto et al. (2002) Gao et al. (2003) Chen et al. (2020) Zhang et al. (2020)
Os08g0191433	*OsSSIIIa/FLO5*	Soluble starch synthase	Chalkiness (AQ) AC, GC (ECQ) Digestion, RS (NQ)	Fujita et al. (2007) Zhou et al. (2016)
Os02g0528200	*OsSBEIIb/SBE3*	Starch branching enzyme	Chalkiness (AQ) AC, GC (ECQ) Digestion, RS (NQ)	Zhu et al. (2012) Baysal et al. (2020) Miura et al. (2021)
Os07g0182000	*OsbZIP58/ RISBZ1*	bZIP transcription factor	Chalkiness (AQ) AC (ECQ)	Yamamoto et al. (2006) Kawakatsu et al. (2009) Wang et al. (2013)
Os02g0725900	*OsNF-YB1*	Component of the NF-Y/HAP transcription factor complex	Grain length, grain width, chalkiness (AQ) AC (ECQ) Protein content, lipid content (NQ)	Bello et al. (2019) Xu et al. (2021)

Table 1 (continued)

Gene ID	Gene name	Protein category	Effect the quality	References
Os10g0191900	*OsNF-YC12/OsNF-YC11*	Nuclear transcription factor Y subunit C	Grain length, grain width, chalkiness (AQ) AC (ECQ) Protein content, lipid content (NQ)	Bello et al. (2019) Xiong et al. (2019)
Os12g0189500	*OsYUC11*	Flavin-containing monooxygenase	Grain length, grain width, chalkiness (AQ) Protein content, lipid content (NQ)	Xu et al. (2021)
Os02g0682200	*OsMADS6/AFG1*	MADS-box protein	Grain length, grain width (AQ) AC, GC (ECQ) Protein content (NQ)	Zhang et al. (2010)
Os02g0170300	*OsMADS29*	MADS-box protein	Shrunken seeds (AQ) AC (ECQ)	Yin and Xue (2012) Nayar et al. (2013)
Os08g0531700	*OsMADS7/OsMADS45*	MADS-box protein	AC (ECQ)	Zhang et al. (2018a)
Os01g0104500	*OsNAC20/ONAC020*	NAC transcription factor	Grain width, grain thickness (AQ) Starch content (ECQ) Protein content (NQ)	Wang et al. (2020a)
Os01g0393100	*OsNAC26/ONAC026*	NAC transcription factor	Grain width, grain thickness (AQ) Starch content (ECQ) Protein content (NQ)	Wang et al. (2020a)
Os03g0686900	*FLO6*	CBM48 domain-containing protein	Transparency, chalkiness (AQ) Starch content (ECQ) Protein content, lipid content (NQ)	Peng et al. (2014)
Os07g0688100	*FLO18*	PPR protein	Transparency, chalkiness (AQ) AC, starch content (ECQ) Protein content, lipid content(NQ)	Yu et al. (2021)
Os03g0168400	*FLO10*	PPR protein	Transparency (AQ) Protein content, lipid content (NQ)	Wu et al. (2019)
Os08g0290000	*FGR1*	Nuclear-localized PPR protein	Grain thickness, transparency (AQ) AC, starch content (ECQ)	Hao et al. (2019)
Os03g0728200	*FLO14*	Nuclear-localized PPR protein	Chalkiness (AQ)	Xue et al. (2019)
Os07g0181000	*OsPK2/PKpa1*	Plastidic pyruvate kinase	Starch content (ECQ) Protein content, lipid content (NQ)	Cai et al. (2018)
Protein				
Os10g0400200	*OsGluA2/qGPC-10*	Glutelin type-A2 precursor	GC, starch content (ECQ) Protein content (NQ)	Yang et al. (2019)
Os05g0499100	*Glb1*	26 kDa α-gloubulin	Seed storage protein (NQ)	Wu et al. (1998)
Os01g0762500	*GluA1*	Glutelin type-A	Seed storage protein (NQ)	Qu et al. (2008)
Os02g0249800	*GluB1a*	Glutelin type-B	Seed storage protein (NQ)	Wu et al. (1998)
Os02g0249900	*GluB1b*	Glutelin type-B	Seed storage protein (NQ)	Wu et al. (1998)
Os02g0268300	*GluB4*	Glutelin type-B	Seed storage protein (NQ)	Qu and Takaiwa (2004)
Os02g0268100	*GluB5*	Glutelin type-B	Seed storage protein (NQ)	Qu et al. (2008)
Os02g0722400	*OsAAP10*	Amino acid permease	AC, RVA (ECQ) Protein content (NQ)	Wang et al. (2020b)
Os12g0631100	*OsRab5a/gpa1*	Small GTPase	Chalkiness (AQ) Protein content (NQ)	Ren et al. (2020)
Os06g0643000	*OsGPA5*	Rab5a effector	Chalkiness (AQ) Protein content (NQ)	Ren et al. (2020)
Os03g0835800	*GPA3*	Regulator of post-Golgi vesicular Traffic	AC (ECQ) Protein content (NQ)	Ren et al. (2014)
Os08g0127100	*OsHT/OsLHT1*	Amino acid transporter	AC, GC (ECQ) Protein content (NQ)	Guo et al. (2020a) Guo et al. (2020b)
Os02g0252400	*RPBF/OsDof3*	Prolamin box binding factor	Starch content (ECQ) Protein content, lipid content (NQ)	Kawakatsu et al. (2009)
Os07g0668600	*OsGZF1*	CCCH-type zinc-finger transcription factor	Glutelin content (NQ)	Chen et al. (2014)

Table 1 (continued)

Gene ID	Gene name	Protein category	Effect the quality	References
Lipid/fat				
Os02g0716500	OsFAD2-1	Fatty acid desaturase	Lipid content (NQ)	Shi et al. (2012)
Os12g0104400	OsFAD3	Fatty acid desaturase	Lipid content (NQ)	Liu et al. (2012)
Os03g0369100	OsLTP36	Lipid transfer protein	Lipid content (NQ)	Wang et al. (2015b)
Os04g0436100	OsACOT	Acyl-CoA thioesterase	Grain length, grain width (AQ) Lipid content (NQ)	Zhao et al. (2019)
Os08g0110700	FSE1	Phospholipase-like protein	Lipid content (NQ)	Long et al. (2018)
Os01g0172400	OsPLDα1	Phospholipase Dα	Phytic acid content (NQ) AC,GT, setback viscosity, viscosity profiles (ECQ)	Khan et al. (2019) Khan et al. (2020)
Lysine (Amino acids)				
Os01g0927900	AK2	Aspartate kinase	Lysine content (NQ)	Yang et al. (2020)
Os04g0574800	DHPS	Dihydrodipicolinate synthase	Lysine content (NQ)	Yang et al. (2020) Yang et al. (2021a)
Os02g0783700	OsLKR/SDH	Lysine ketoglutarate reductase	Lysine content (NQ)	Yang et al. (2020)
Fe, Zn (Minerals)				
Os08g0207500	OsZIP4	Zinc-regulated transporter	Zinc content (NQ)	Ishimaru et al. (2007b)
Os05g0472700	OsZIP5	Zinc-regulated transporter	Zinc content (NQ)	Lee et al. (2010a)
Os07g0232800	OsZIP8	Zinc-regulated transporter	Zinc content (NQ)	Lee et al. (2010b)
Os03g0667500	OsIRT1	Iron-regulated transporter	Iron content (NQ)	Lee and An (2009)
Os03g0667300	OsIRT2	Iron-regulated transporter	Iron content (NQ)	Nakanishi et al. (2006)
Os11g0106700	OsFER1	Rice ferritin protein	Iron content (NQ)	Stein et al. (2009)
Os12g0106000	OsFER2	Rice ferritin protein	Iron content (NQ)	Stein et al. (2009)
Os03g0307300	OsNAS1	Nicotianamine synthase	Iron content (NQ)	Inoue et al. (2003)
Os03g0307200	OsNAS2	Nicotianamine synthase	Iron content (NQ)	Inoue et al. (2003)
Os07g0689600	OsNAS3	Nicotianamine synthase	Iron content (NQ)	Inoue et al. (2003)
Os04g0542200	OsYSL9	Probable metal-nicotianamine transporter	Iron content (NQ)	Senoura et al. (2017)
Os04g0463400	OsVIT1	Vacuolar membrane transporter	Iron content (NQ)	Zhang et al. (2012b)
Os09g0396900	OsVIT2	Vacuolar membrane transporter	Iron content (NQ)	Zhang et al. (2012b)
Phytochemicals				
Os08g0424500	OsBadh2	Betaine aldehyde dehydrogenasea	2-AP content (ECQ)	Chen et al. (2006) Chen et al. (2008) Kovach et al. (2009) Bradbury et al. (2010) Hui et al. (2021)
Embryo and grain quality				
Os07g0603700	OsGE	Cytochrome P450	Embryo size (NQ)	Nagasawa et al. (2013)
Os04g0447800	OsGAD2	Glutamate decarboxylase	GABA content (NQ)	Akama and Takaiwa (2007) Akama et al. (2009)
Os02g0112900	OsGABA-T	γ-aminobutyrate transaminase	GABA content (NQ)	Shimajiri et al. (2013)
Os03g0236200	OsGAD3	Glutamate decarboxylase	GABA content (NQ)	Akama et al. (2020)
Aleurone layer and grain quality				
Os05g0509700	TA1/OsmtSSB1	Mitochondrion-targeted single-stranded DNA binding protein	Thick aleurone (NQ)	Li et al. (2021)
Os01g0218032	TA2-1/OsROS1	DNA demethylase	Thick aleurone (NQ)	Liu et al. (2018a)
Seed coat and grain quality				
Os07g0211500	Rc	bHLH protein	Anthocyanin content (NQ)	Sweeney et al. (2006)
Os01g0633500	Rd/OsDFR	Dihydroflavonol reductase	Anthocyanin content (NQ)	Furukawa et al. (2007)
Os04g0557500	Kala4/OsB2	bHLH transcription factor	Anthocyanin content (NQ)	Oikawa et al. (2015)
Os06g0205100	OsC1	MYB transcriptional activator	Anthocyanin content (NQ)	Sun et al. (2018b)
Os02g0682500	OsTTG1	WD40 repeat protein	Anthocyanin content (NQ)	Yang et al. (2021c)

Genes Regulating Spikelet Hull Development and Their Roles in Grain Qualities

In general, rice MQ correlates negatively with the grain length, grain length–width ratio, and grain length–thickness ratio, and positively with the grain width, grain thickness, and grain width–thickness ratio. Hence, reducing the length and increasing the width and thickness of rice grains are beneficial to improving rice MQ. Rice AQ is usually related to grain size, chalkiness, and transparency. The chalky grain percentage correlates positively with the 1000-grain weight, grain width, grain thickness, and grain width–thickness ratio. There is a significant positive correlation between the grain width and chalkiness. The grain filling rate of a wide grain is too fast, which leads to a looser arrangement of granules and the subsequent formation of chalkiness. Chalkiness is a negative element of rice quality, the higher the chalkiness, the poorer AQ, MQ, and ECQ of rice (Cheng et al. 2005; Yamakawa et al. 2007). For example, chalky rice breaks more easily during processing, resulting in less head rice and a decreased MQ. In contrast, smaller or slender seeds have less chalkiness because of the short distance for grain filling. Therefore, decreasing the rice grain width could promote rice AQ. Importantly, grain weight loss resulting from reduced grain width could be compensated by increasing the grain length. In conclusion, breeding elite rice with a slender grain shape is a practical strategy to improve rice quality without sacrificing its yield.

Therefore, the study of grain size has become a hot research topic among rice geneticists and breeders. At present, more than 400 quantitative trait loci (QTLs) linked to grain size have been mapped on all 12 chromosomes of rice, and over 80 grain size-related genes have been cloned (Huang et al. 2013; Zuo and Li 2014). Most importantly, several grain shape genes have been used in rice breeding practice, such as *GS3* and *DEP1/qPE9* (Huang et al. 2009; Zhou et al. 2009). This topic is well documented by several review papers (Li and Li 2016; Azizi et al. 2019; Li et al. 2019). Therefore, we have only summarized the functions and regulatory mechanisms of the key cloned genes related to grain size, especially the newly identified genes (Fig. 3).

G Protein Pathways

The heterotrimeric G-protein complex consists of Gα, Gβ, and Gγ subunits. *GS3*, encoding a non-canonical Gγ subunit, is a main QTL controlling grain length and is a negative regulator of grain size (Fan et al. 2006). The *gs3* allele corresponds to long rice grains. The GS3 protein contains four functional domains, and the organ size regulation (OSR) domain is both necessary and sufficient to limit grain size (Mao et al. 2010). *DEP1/qPE9*, encoding another noncanonical Gγ subunit, is a major QTL for rice panicle architecture and grain size (Huang et al. 2009; Zhou et al. 2009). The *dep1/qpe9-1* allele, encoding a truncated protein, leads to erect panicles and smaller grains. Consistently, overexpression of *DEP1* increases the grain size, while knockout of the gene makes the grains smaller (Sun et al. 2018a). *DEP1/qPE9-1* regulates starch accumulation positively, mainly through promoting the expression of starch biosynthesis-related genes, thus prolonging the duration of the grain filling process, which finally affects the grain size (Zhang et al. 2012b).

In addition to GS3 and DEP1, the G protein γ subunits also include RGG1, RGG2 and GGC2. Grain size is regulated positively by GGC2 and negatively by RGG1 and RGG2 (Kato et al. 2004; Sun et al. 2018a; Miao et al. 2019; Xu et al. 2019). Recently, RGG1 was reported to be involved in regulating the cytokinin content, thus forming a G protein-cytokinin module to control rice grain size (Tao et al. 2020). In addition, genetic analysis indicated that these Gγ proteins require Gα (RGA1) and Gβ (RGB1) subunits to control grain length (Sun et al. 2018a). A recent report indicated that RGB1 not only controls the grain size, but also controls the grain filling process by regulating the expression of *OsNF-YB1*, encoding a critical downstream effector of RGB1. In addition, OsNF-YB1 directly interacts with the *OsYUC11* promoter to stimulate its expression, thus altering auxin homeostasis, starch biosynthesis and grain size (Zhang et al. 2021b).

The Ubiquitin–Proteasome Pathway

The ubiquitin proteasome pathway (UPP) is an important system in eukaryotes that regulates protein stability and activity. *GW2*, a main QTL controlling grain width and weight, encodes a nuclear ring E3 ubiquitin ligase (Song et al. 2007). A loss of function allele of *GW2* promotes the proliferation of spikelet shell cells and produces wide grains. Importantly, this mutation notably enlarges the grain size and increases rice yield, but has little effect on rice AQ and ECQ. *Large grain1-D* (*lg1-D*) encodes a ubiquitin-specific protease 15 (OsUBP15). Loss-of-function of *OsUBP15* or suppressing its expression generates narrower rice seeds, while *OsUBP15* overexpression increases rice grain width significantly. Moreover, *OsUBP15* and *GW2* genetically interact with each other to co-regulate grain width (Shi et al. 2019).

WG1 encodes a CC glutaredoxin that promotes grain growth by enhancing cell proliferation, while OsbZIP47 negatively regulates grain width and weight by suppressing cell proliferation. WG1 interacts with and inhibits the transcriptional activity of transcription factor OsbZIP47 by recruiting ASP1, a transcriptional co-repressor.
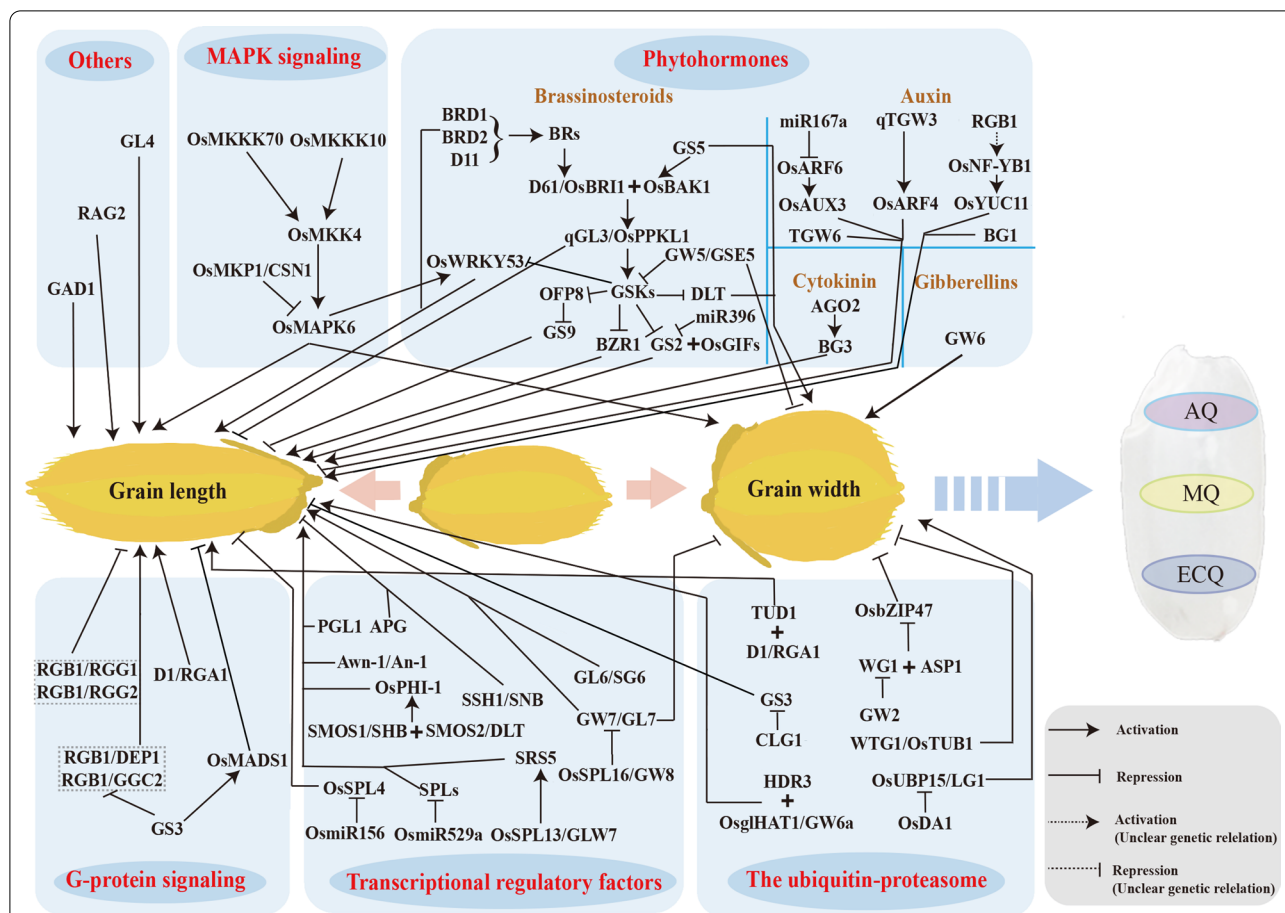
Fig. 3 The cloned genes and the major regulatory networks controlling rice size. Rice seed size is regulated by multiple signaling pathways, including G protein pathways, the ubiquitin–proteasome pathway, MAPK signaling pathways, phytohormones, and transcription regulatory factors. A dotted line indicates that the genetic relationship needs to be further verified. MAPK, Mitogen-Activated Protein Kinase; AQ, appearance quality; MQ, milling quality; ECQ, eating and cooking quality

Moreover, WG1 is ubiquitinated by E3 ubiquitin ligase GW2 and is subsequently degraded. Genetic analysis demonstrated that the three proteins form a GW2-WG1-OsbZIP47 molecular regulatory module to coordinate grain size and weight (Hao et al. 2021).

TUD1 (*Taihu Dwarf1*) encodes a functional U-box E3 ubiquitin ligase, which interacts directly with D1/RGA1 to regulate plant height and grain size. In addition, *TUD1* and *D1* work together to regulate brassinosteroid (BR) signaling and produce short grains by reducing cell division (Hu et al. 2013). *OsTUB1/WIDE AND THICK GRAIN (WTG1)*, encoding a deubiquitinase, controls grain size by affecting cell proliferation. Knockout of *OsTUB1/WTG1* resulted in wider grains (Huang et al. 2017). In addition, two recent studies revealed novel regulators involved in UPP-mediated grain size regulation. One is HOMOLOG OF DA1 ON RICE CHROMOSOME 3 (HDR3), a ubiquitin interacting motif (UIM) type active ubiquitin receptor, can interact with and

stabilize GW6a to slow down its degradation, thus promoting cell division and increasing the grain filling rate, which ultimately regulates grain size positively (Gao et al. 2021). GW6a is a histone acetyltransferase, whose overexpression increases grain weight and yield by increasing the cell number and accelerating grain filling (Gao et al. 2021). The other protein is Chang Li Geng1-1 (CLG1-1), an E3 ubiquitin ligase, which can ubiquitinate and mediate the degradation of GS3, thus changing G protein signaling and regulating the grain length (Yang et al. 2021b).

Mitogen-Activated Protein Kinase (MAPK) Signaling

The MAPK cascade signaling pathway also plays an important role in regulating rice grain size. Typical MAPK pathways are usually composed of MAPKs, MAPK kinases (MKKs) and MKK kinases (MKKKs) (Zhang et al. 2018b). The OsMKKK10-OsMKK4-OsMAPK6 molecular cascade positively regulates rice grain size and weight (Xu et al. 2018). Disruption the

expression of either member leads to smaller rice grains, while overexpression of these genes produces larger rice grains (Duan et al. 2014; Liu et al. 2015b). Furthermore, OsMAPK6 phosphorylates the transcription factor OsWRKY53 and enhances its activity (Tian et al. 2017), while GSK2 directly phosphorylates WRKY53 and lowers its stability (Tian et al. 2021).

In addition, OsMKKK70 also functions through the established OsMKK4–OsMAPK6–OsWRKY53 module (Liu et al. 2021). Overexpression of *OsMKKK70* leads to longer grain length and increased rice leaf angle. Moreover, overexpression of the genes encoding constitutively active OsMKK4, OsMAPK6, and OsWRKY53 in the context of *osmkk62/70* double mutation can partially restore its phenotype of grain size and leaf angle, implying that these elements operate in the same regulatory pathway.

GSN1 encodes the mitogen activated protein kinase phosphatase, OsMKP1. Suppression of *GSN1* expression induces the proliferation of rice glume cells, resulting in larger but fewer rice grains. GSN1 directly interacts with and inactivates OsMAPK6 through dephosphorylation, thus playing an opposite role to OsMKK4 (Guo et al. 2018; Xu et al. 2018). Therefore, the key to controlling rice grain size is to accurately regulate OsMAPK6 activity through reversible phosphorylation.

Phytohormone Perception and Homeostasis

Plant hormones, as central regulators of plant growth and development, not only orchestrating intrinsic developmental programs, but also conveying environmental inputs. Recently, a series of publications reported that some phytohormones, including BR, auxin, gibberellic acid (GA), and cytokinin, also play essential roles in regulating seed size via multiple molecular mechanisms.

BR is Involved in the Regulation of Grain Size

BRs, a group of plant-specific polyhydroxylated steroidal hormones, control a wide range of growth and developmental events, including grain size (Li et al. 2018b). Some BR mutants with defects in both BR biosynthesis or signaling, such as *brd1* (Hong et al. 2002; Mori et al. 2002), *dwaf*2 (Hong et al. 2005), *dwaf11* (Tanabe et al. 2005; Zhu et al. 2015; Wu et al. 2016b; Zhou et al. 2017c), *OsBRI1/D61* (Yamamuro et al. 2000), *OsBAK1* (Li et al. 2009), *GS6/DLT/D62* (Tong et al. 2009; Sun et al. 2013), and *OsBZR1* (Zhu et al. 2015), usually exhibit shorter plants and smaller grains. One exception is GSK2, which, as a GSK3/SHAGGY like kinase homologous to Arabidopsis BIN2 in rice, is a central negative regulator of the BR pathway. Therefore, suppressing *GSK2* expression increased both grain size and leaf angles (Tong et al. 2012). Therefore, BR is a growth-promoting hormone with positive roles in regulating rice grain size.

GW5 is a main QTL for grain width with three haplotypes (Zhou et al. 2017a). GW5 participates in the BR pathway and regulates grain width and weight by inhibiting the function of GSK2 kinase, thus releasing the active forms of OsBZR1 and DLT transcription factors (Liu et al. 2017). Meanwhile, *GW5* also affects rice quality, including the chalkiness rate, brown rice rate, and milled rice rate. *GS5*, encoding a serine carboxypeptidase (Yu et al. 2000), is a QTL controlling rice grain width. Increased expression of *GS5* inhibits the endocytosis of OsBAK1-7 and subsequently increases BR signaling and promotes grain size (Xu et al. 2015).

GL3.1/GL3-1/qGL3/OsPPKl1 encodes a protein phosphatase OsPPKl1, which regulates rice grain length negatively by dephosphorylating OsGSK3 and inhibiting BR signaling (Hu et al. 2012; Qi et al. 2012; Zhang et al. 2012a; Gao et al. 2019). OsmiR396d regulates grain size by repressing the growth regulator GS2/GL2/OsGRF4, and GSK2 plays a similar inhibitory function, thereby affecting cell proliferation and grain shape (Che et al. 2015; Duan et al. 2015; Hu et al. 2015; Li et al. 2016; Sun et al. 2016). A recent study also showed that OsAGO17 forms an RNA-induced silencing complex (RISC) with OsmiR397b, which then affects rice development by inhibiting the expression of *OsLAC*, encoding a negative regulator of both grain size and grain number per panicle (Zhang et al. 2013; Zhong et al. 2020). Moreover, GSK2 interacts with and phosphorylates OML4, a negative regulator of grain size, thus modulating OML4 protein stability. Therefore, GSK2 and OML4 act in the same genetic pathway to regulate rice grain size (Lyu et al. 2020).

The mutation of *GS9*, encoding a novel transcriptional activator, leads to slender rice grains and reduced chalkiness, without affecting other major agronomic traits (Zhao et al. 2018). GS9 directly interacts with OVATE family proteins, thus forming a transcriptional complex to regulate glume cell division. Moreover, OFP8 and OFP14 inhibit the transcriptional activation activity of GS9, while OFP8 is directly suppressed by OsGSK2 in the BR pathway (Yang et al. 2016a). In addition to OFP8 and OFP14, several other OFP proteins also participate in grain size regulation via the BR pathway, including OFP1 (Xiao et al. 2017), OFP3 (Xiao et al. 2020), OFP19 (Yang et al. 2018a), and OFP22 (Chen et al. 2021). Among them, OFP1 is a positive regulator of BR signaling and seed size, while the others are all negative regulators.

Furthermore, two novel BR-related grain size genes were reported recently. One is *GW10*, encoding a P450 subfamily 89A2 homology protein, which plays a positive role in controlling grain size through the BR pathway (Zhan et al. 2021). The other is *POW1* (*Put On Weight 1*), encoding an unknown protein. The *pow1* mutant enhanced the endogenous BR content, leading to an

enlarged leaf angle and grain size. Interestingly, down-regulating the expression of BR biosynthesis or signaling genes could only restore the leaf angle phenotype of *pow1* mutant, not the grain size. Further analysis shows that POW1 regulates grain size by inhibiting the transactivation activity of TAF2, its interacting protein. Hence, two regulatory modules, POW1-TAF2 and POW1-BR, are established that specifically regulate the grain size and leaf angle, respectively (Zhang et al. 2021c).

Auxin

Auxin is an important classical phytohormone with essential roles in many aspects of plant growth and development events, including grain size. *qTGW6*, as a main QTL controlling rice grain weight, encodes indole-3-acetic acid (IAA)-glucose hydrolase to generate free IAA. A loss-of-function *TGW6* allele promoted grain length and weight (Ishimaru et al. 2013). Recent research indicated that *TGW6* is exclusively expressed in pre-emergent inflorescences, suggesting that TGW6 might play important roles in regulating pollen development (Akabane et al. 2021; Kabir and Nonhebel 2021).

BG1, an auxin primary response gene, encodes a protein that participates in the regulation of auxin transport. It affects grain size by promoting cell division and elongation (Liu et al. 2015a). *qTGW3/GL3.3* is a major QTL for grain weight, encoding a SHAGGY-like kinase 41 (OsSK41) (Hu et al. 2018; Xia et al. 2018; Ying et al. 2018). OsSK41 directly interacts with and phosphorylates OsARF4, a transcription repressor in the auxin pathway. Moreover, the OsSK41-OsARF4 module regulates rice grain size negatively by modulating the expression of a number of auxin-response genes. Recently, another transcription factor, OsARF6, was reported to bind directly to the *OsAUX3* promoter to increase its expression (Qiao et al. 2021). OsARF6 and OsAUX3 regulate rice grain length and weight negatively by modulating the auxin content and distribution in glume cells, consequently affecting rice grain longitudinal elongation. miR167a, a positive regulator of grain length and weight, directly silences *OsARF6* mRNA. Hence, a novel miR167a-*OsARF6-OsAUX3* regulatory module is established successfully.

GA

The tetracyclic diterpenoid phytohormone GA has multiple roles in plant growth and development. However, the participation of GA in regulating rice grain size is rarely reported. Recently, Shi et al. (2020) indicated that a QTL for grain size, designated as *GW6* (*GRAIN WIDTH 6*), was successfully cloned. *GW6*, encoding a GA-induced GAST family protein, plays positive roles in regulating grain size and weight. Moreover, knockout of *GW6*

reduced the GA content in young panicles. Importantly, a natural variation in the CAAT-box of the *GW6* promoter determines its transcript abundance, as well as the grain width and weight, thus providing valuable natural genetic resources for rice breeding programs.

Cytokinin

A dominant mutant *big grain 3 (bg3-D)* was isolated, which featured larger rice grains. *BG3*, encoding a purine permease, OsPUP4, regulates grain size positively by controlling both the long-distance transport and local allocation of cytokinin (Xiao et al. 2019). Recently, Yin et al. (2020) reported that ARGONAUTE (AGO) proteins are essential to assemble RNA-induced silencing complexes to silence target genes. Overexpression of *AGO2* boosts both the salt-stress resistance and grain length of rice by modulating the histone methylation level of *BG3*, hence promoting its expression. Salt treatment results in a similar cytokinin distribution pattern to *AGO2* overexpression rice, implying that the cytokinin distribution pattern is critical to regulate stress tolerance and rice grain size.

Transcription Factors

Transcription factors (TFs) play vital roles in regulating plant growth and development by responding to upstream signals and modulating downstream transcriptional networks. Certain TFs are also involved in controlling seed size.

SQUAMOSA Promoter Binding Protein-Like (SPL) Transcription Factor Family

SPL transcription factors are involved in controlling the tiller, panicle configuration, and grain size of rice. A genome-wide association analysis (GWAS) indicated that a major QTL *GLW7*, encoding the TF OsSPL13, is a key element leading to larger grains and more panicles (Si et al. 2016). GLW7 binds directly to the promoter of *SRS5*, a positive regulator of grain length, and activates its expression (Segami et al. 2017). Suppressing the expression of *OsSPL16*/*GW8* produces long grains, and thus decreased chalkiness and improved transparency of rice seeds (Wang et al. 2012). GW8 directly binds to the *GW7/GL7* promoter and inhibits its expression (Wang et al. 2015a). *GW7*, encoding a TONNEAU1-recruiting motif protein, plays a positive role in producing slender grains via differential regulation of cell division in both longitudinal and transverse directions. Importantly, a semi-dominant *GW7* allele enhances rice grain quality without any yield penalty (Wang et al. 2015c). Therefore, the identified OsSPL16/GW8-GW7 regulatory module should be useful in future elite rice breeding programs to improve both rice quality and yield. SPLs are known to be regulated by microRNAs, such as miR156 (Xie et al.

2006). Recently, two regulatory modules, miR529a-SPLs and OsmiR156-SPL4, are revealed and their essential roles in controlling rice grain size are studied (Hu et al. 2021; Yan et al. 2021).

APETALA2-Type (AP) Transcription Factors
SMOS1/SHB encodes an APETALA2 (AP2) transcription factor with an incomplete AP2 domain. The grains and other organs of *smos1* mutants are smaller because of the smaller cells and an abnormal microtubule orientation. SMOS1 directly modulates the transcription of a cell expansion regulator, *phosphorylation inducible protein 1* (*OsPHI-1*) (Aya et al. 2014). SMOS1 interacts with SMOS2/DLT to form a key protein complex to orchestrate BR and auxin signaling, thereby coordinating rice growth and development, including grain size (Hirano et al. 2017). Moreover, *Suppression of Shattering 1* (*SSH1*) is a novel allele of *SUPERNUMERARY BRACT* (*SNB*), encoding an AP2 transcription factor. A point mutation in the ninth intron of *SNB* alters its mRNA splicing and decreases SNB expression, consequently reducing rice shattering and increasing grain size (Jiang et al. 2019).

Basic Helix-Loop-Helix (bHLH) Family
Awn-1(*An-1*) encodes a bHLH protein that regulates awn development, grain size, and grain number in rice. Increased *An-1* expression causes long awns and grains, but decreases the grain number per panicle (Luo et al. 2013). PGL1 is an atypical bHLH protein without DNA binding activity. Overexpression of *PGL1* increases the grain length and weight while, APG exerts an opposite effect. PGL1 interacts directly with APG to regulate grain size antagonistically (Heang and Sassa 2012).

Other Transcription Factors
SHORT GRAIN 6 (SG6)/GL6 encodes a plant specific PLTAZ transcription factor which regulates grain length positively by promoting cell proliferation in young panicles and grains (Wang et al. 2019; Zhou and Xue 2020). GL6 interacts with RPC53 and OsTFC1 to participate in the RNA polymerase III transcription machinery and regulates the expression of genes involved in rice grain development and the cell cycle, thus regulates grain length positively (Wang et al. 2019). FLR family proteins, such as FLR1, FLR2, and FLR8, play negative roles, while FLR15 plays a positive role, in regulating grain size. Although the grains of the *flr8* mutant are larger, their quality remains the same. Moreover, *FLR1* can modulate the number of glume cells and the expression of starch metabolism genes, thus affecting seed size and grain filling (Wang et al. 2021). *qLGY3/OsLG3b*, a QTL for grain length, encodes the MADS-domain transcription factor,
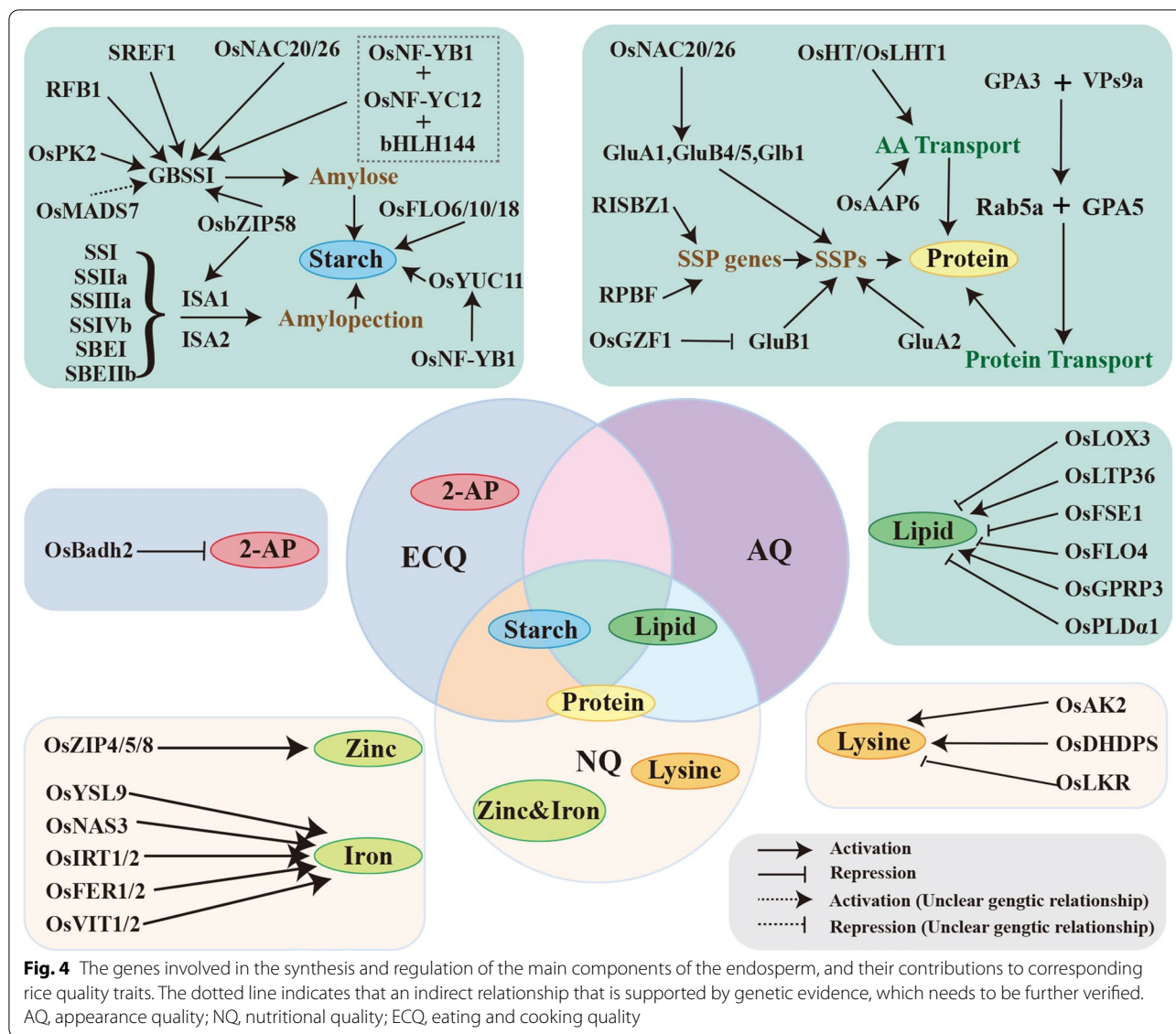
OsMADS1. *qLGY3/ OsLG3b* allele leads to the alternative splicing of *OsMADS1*, which is artificially selected and corresponds to a long grain phenotype (Liu et al. 2018b; Yu et al. 2018). GS3 and DEP1 directly interact with MADS1 to promote its transcriptional activity and hence inhibit grain growth (Liu et al. 2018b).

Other Functional Proteins
GAD1 encodes a small secretary signal peptide and its mutation leads to reduced grain numbers, shorter grains, and awnless rice. Mechanistically, GAD1 regulates the length of the grain and awn by modulating cell numbers (Jin et al. 2016). *GL4*, a QTL for grain length originating from African rice, encodes a Myb-like protein similar to SH4/SHA1. GL4 controls grain length by regulating the elongation of longitudinal cells of both the outer and inner glumes. A single nucleotide polymorphism (SNP) mutation in *GL4* leads to a premature stop codon and consequently a truncated protein, which results in small seeds and the loss of seed shattering during African rice domestication (Wu et al. 2017). In addition, overexpression of *RAG2*, encoding a 16-kDa α-amylase/trypsin inhibitor, significantly increases the grain size and 1000-grain weight, as well as the protein and total lipid contents (Zhou et al. 2017b).

Genes Regulating Endosperm Components and Their Roles in Grain Quality
The components of the rice endosperm include starch, proteins, amino acids, lipids, vitamins, minerals, and other metabolites. Among them, starch and protein account for about 80% and 10% of the dry weight of rice endosperm, respectively (Wang et al. 2020a). Therefore, the constitution and quality of starch and protein contribute majorly to rice grain quality (Fig. 4). In general, starch is the primary determinant of rice ECQ and AQ (Li et al. 2018a). For example, rice AC influences a series of quality related parameters, including hardness, viscosity, and transparency. Proteins are considered as the most important elements of rice NQ. Their content and quality determine rice NQ. Protein quality is mainly evaluated by assessing essential amino acids, such as lysine. In contrast to starch and protein, the content of lipid is low in rice, accounting for only about 0.3–0.6% of rice weight (Morrison 1988). The lipid content, similar to the protein content, correlates negatively with rice ECQ. Nevertheless, lipids have a great influence on the storage, processing, and consumption of rice. In addition to these three components, some micronutrients and phytochemicals are also important in rice quality, such as vitamins and minerals (Buttery et al. 1983; Kovach et al. 2009).

Fig. 4 The genes involved in the synthesis and regulation of the main components of the endosperm, and their contributions to corresponding rice quality traits. The dotted line indicates that an indirect relationship that is supported by genetic evidence, which needs to be further verified. AQ, appearance quality; NQ, nutritional quality; ECQ, eating and cooking quality

Genes Controlling Starch Biosynthesis, Their Transcriptional Regulation, and Their Effects on Rice Quality

Starch Biosynthesis Enzymes

Starch is mainly stored in the form of starch granules in endosperm cells. According to their different glycosidic bond connections, starch is usually classified into two groups, amylose and amylopectin. The amylose content (AC) is the most important effector of rice ECQ (Duan and Sun 2005). According to their different ACs, rice varieties can be divided into waxy (< 2%), very low (3–9%), low (10–19%), intermediate (20–25%), and high (> 25%) amylose types (Zhang et al. 2017). In general, the higher the AC, the harder the texture of the cooked rice. Meanwhile, the AC is also related closely to rice transparency

(Li et al. 2018a). Glutinous rice, lacking almost all amylose, has a completely opaque, waxy endosperm.

To date, almost all the genes encoding key enzymes involved in starch biosynthesis have been cloned and studied, such as ADP-glucose pyrophosphorylases (AGPases), granule-bound starch synthases (GBSSs), soluble starch synthases (SSSs), starch branching enzymes (SBEs), debranching enzymes (DBEs), and starch phosphorylase (PHO) (Zemach et al. 2010; Seung and Smith 2019). Detailed information related to starch biosynthesis is well summarized by some excellent reviews (Huang et al. 2020, 2021a); therefore, we will only focus on several key genes that determine rice quality.

AGPase uses glucose-1-phosphate (Glu-1-P) as a substrate to generate ADPglucose, which is then used to

synthesize amylose via GBSSI, and to generate amylopectin via the cooperation of a series of other enzymes, including SSSs, SBEs, and DBEs (Jeon et al. 2010; Pfister and Zeeman 2016). Among them, GBSSI, encoded by the *Waxy* (*Wx*) gene, is the sole enzyme directly controlling amylose synthesis, and is thus the primary determinant of rice AC, GC, and pasting property (Wang et al. 2010). Therefore, *Wx* has been studied extensively and used widely to improve rice ECQ. Hence, a number of useful natural *Wx* alleles have been cloned and applied in rice breeding practice. Up to now, about ten natural *Wx* alleles have been reported, including the newly cloned Wx^{lv} and Wx^{mp}/Wx^{la} (Zhang et al. 2019a, 2021a; Zhou et al. 2021a). The *wx* allele, a null allele that does not encode a functional GBSSI, exists in glutinous rice, AC < 2% (Wanchana et al. 2003). Wx^a and Wx^b are two major *Wx* alleles that are distributed widely in most indica rice and japonica rice varieties, respectively, corresponding to high and low ACs.

The discovery of the *Wx* ancestor gene, Wx^{lv}, and the differentiation of its functional sites might explain the evolutionary trend of the AC, from high to low during rice domestication (Zhang et al. 2019a). The AC decreased slightly because of the variation in amino acid sequence caused by a SNP mutation in exon 6 of Wx^{in}. Rice with the Wx^{op}/Wx^{hp} (AC ~ 12.8%), Wx^{mp} (AC ~ 10.5%), and Wx^{mq} (AC ~ 10%) alleles are the so called soft rice (Hiroyuki et al. 2002; Mikami et al. 2008; Liu et al. 2009; Zhang et al. 2021a; Zhou et al. 2021a, b), which are famous for their good taste and high ECQ. The rare allele Wx^{mw}/Wx^{la}, derived from the homologous recombination of Wx^{in} and Wx^b, has a low AC, high transparency, good taste, and excellent ECQ (Zhang et al. 2021a; Zhou et al. 2021a). In addition to the identification of natural *Wx* alleles, the CRISPR/Cas9 gene editing strategy was also applied for accurate editing and to generate novel and excellent *Wx* alleles (Huang et al. 2020; Zeng et al. 2020).

The synthesis of amylopectin is complex and is coordinately regulated by several groups of enzymes. Moreover, each group contains several different types of enzymes. For example, SSS comprises SSSI, SSSII, SSSIII, and SSSIV. Except for SSSI, the other type of SSS all contain more than one isoform. Each enzyme isoform plays a distinct role in amylopectin biosynthesis. In general, SSSI prolongs the amylopectin by synthesizing short chains, while SSSII synthesizes medium-length amylopectin. *SSSIIa*/*ALK* is the major gene regulating rice GT (Gao et al. 2003). The different expression levels and allele types of *ALK* are the main cause for the differential amylopectin structure between indica and japonica subspecies (Umemoto et al. 2002). Two SNP-induced amino acid mutations affect the function of SSSIIa, resulting

in a decrease in the branch chain length and GT (Bao et al. 2006). Recently, a detailed analysis of various *ALK* alleles, including a new identified ALK^d allele, was performed, which clarified their roles in regulating rice GT, AC, and general taste values, demonstrating *ALK* is a crucial molecular target to improve rice ECQ (Chen et al. 2020; Zhang et al. 2020). Another SSSII isoform, SSSII-2, was reported to have the potential to improve rice quality. Suppressing *SSSII-2* expression produced a novel soft rice with a low AC, improved taste, and transparent endosperm (Li et al. 2018a). Furthermore, simultaneous modulation of *SSSII-2*, *SSSIIa*, and *Wx* coordinated the biosynthesis of amylose and amylopectin, hence successfully improving rice ECQ (Huang et al. 2021b).

SSSIII synthesizes long amylopectin chains and SSSIIIa is an important target to study amylopectin biosynthesis and breed healthy rice. Resistant starch (RS) can reduce the incidence of type 2 diabetes and reduce the probability of obesity; therefore, high RS rice is considered to be a healthy food. SSIIIa affects the structure of amylopectin, the amylose content, and the physicochemical properties of starch grains in indica rice together with the Wx^a allele, resulting in a higher AC and an increased lipid content, subsequently increasing the amount of amylose–lipid complex and RS starch (Zhou et al.2016). That study suggested that modulating the *SSSIIIa* and *Wx* genes could benefit future breeding of high RS rice (Zhou et al. 2016).

Transcription Factors

The biosynthesis of starch is crucial to both seed development and propagation, and a number of transcription factors are involved in regulating the expression of starch synthesis-related genes (SSRGs). OsbZIP58/RISBZ1 binds specifically to the ACGT motif in the *Wx* gene promoter (Wang et al. 2013), thus enhancing its expression. In addition, OsbZIP58 also binds to the promoters of *AGPL3*, *SSSIIa*, *SBE1*, and *ISA2* to regulate their expression levels. bZIP58 is a core regulator of starch synthesis and its null mutant is chalky and its total starch and AC are decreased (Wang et al. 2013). In addition, OsbZIP58 interacts with RPBF, a Dof family transcription factor, to participate in the synthesis of storage substances, including starch, protein, and lipid, during rice endosperm development (Kawakatsu et al. 2009).

As an NF-Y transcription factor, OsNF-YB1 regulates endosperm sucrose transport and grain filling. Suppression of *OsNF-YB1* expression leads to development defects of rice seeds with increased grain chalkiness and a decreased AC, resulting in a decline in rice quality (Sun et al. 2014). Knockout of *OsNF-YB1* resulted in an increased protein content and decreased grain size and contents of amylose, total starch, crude fiber, and lipid, subsequently altering rice quality (Bello et al. 2019;

Xu et al. 2021). In addition, OsNF-YB1 binds directly to the promoter of *OsYUC11* and activates its expression. As a key element in auxin biosynthesis, OsYUC11 affects grain filling and the accumulation of endosperm storage products in rice (Xu et al. 2021). Another NF-Y member, NF-YC12, coordinates various pathways to regulate endosperm development and the accumulation of seed storage substances in rice. The phenotype of the *osnf-yb12* mutant is similar to that of the *osnf-yb1* mutant, with changed grain weight, starch, and protein accumulations (Bello et al. 2019; Xiong et al. 2019). Furthermore, NF-YB1 combines with NF-YC12 and bHLH144 to form an NF-YB1-YC12-bHLH144 heterotrimeric complex that coordinates grain development and rice quality. Hence, the mutation of any gene in the complex would change starch synthesis in the rice endosperm (Bello et al. 2019).

Several members of MADS box family transcription factors participate in the regulation of starch biosynthesis. OsMADS6 is highly expressed in the endosperm and regulates the expression of SSRGs. Its mutation leads to decreased starch plumpness and abnormal endosperm development (Zhang et al. 2010). Suppression of *OsMADS29* expression caused abnormal seed development, such as shrunken seeds, a low grain-filling rate, and insufficient starch accumulation. Evidences indicates that OsMADS29 modulates the expression of genes related to programmed cell death (PCD), thus affecting the early development of rice seeds (Yin and Xue 2012). Another study revealed that OsMADS29 affects embryo and endosperm development, including starch biosynthesis, by modulating cytokinin signaling and biosynthesis (Nayar et al. 2013). As a high temperature induced gene, *OsMADS7* encodes a protein involved in stabilization of the AC in response to high temperature, mainly by enhancing the expression of GBSSI, the key enzyme controlling amylose biosynthesis. Therefore, OsMADS7 is a valuable molecular target for breeding elite rice with ideal thermal tolerance and ECQ (Zhang et al. 2018a).

NAC transcription factors are plant-specific and participate in various processes of plant development, including biosynthesis of storage substance of the rice endosperm. Mutation of *OsNAC20* or *OsNAC26* alone does not have any effect on rice grains. However, the contents of starch and storage proteins in *osnac20/26* double mutants are decreased. Further evidence demonstrated that OsNAC20 and OsNAC26 can promote the expression of multiple genes involved in starch and storage protein biosynthesis directly, such as those encoding SSSI, Pul, glutelin A1 (GluA1), glutelin B4/5 (GluB4/5), α globulin, and 16 kDa prolamin, thus regulating the synthesis of both starch and storage proteins (Wang et al. 2020a). ONAC127 and ONAC129 are not directly involved in starch synthesis in the rice endosperm. They regulate grain filling and starch accumulation by forming heterodimers, and participating in cytoplasmic transport and heat stress translation (Ren et al. 2021).

Other Proteins

In addition to the above mentioned starch synthesis-related enzymes and transcription factors, other proteins are involved in regulating starch biosynthesis. In general, the mutation of these genes, such as *FLO6* (Peng et al. 2014), *FLO10* (Wu et al. 2019), *FLO14* (Xue et al. 2019), *FLO18* (Yu et al. 2021), *FGR1* (Hao et al. 2019) and *OsPK2* (Cai et al. 2018), lead to defects in starch biosynthesis and the formation of abnormal starch granules, resulting in opaque, chalky, or powdery grains. Interestingly, only FLO6 shows a direct correlation with starch biosynthesis-related enzymes. FLO6 binds directly to starch through a CBM48 domain at the C terminus and to ISA1 through a domain at the N terminus, suggesting its role as a bridge between ISA1 and starch during starch synthesis (Peng et al. 2014).

Storage Proteins, Transporters of Amino Acids and Proteins, and Other Regulators of Proteins

Proteins are the secondary major components of rice endosperm, which could be divided into three categories, storage proteins, structural proteins, and protective proteins. The protein content and its amino acid constitution affect rice NQ directly. Moreover, the protein content is also involved in the regulation of rice ECQ. In general, a negative correlation exists between the protein content and ECQ in rice (Hori et al. 2016).

Storage Proteins

The rice storage proteins (SSPs) can be divided into four categories, albumin, globulin, prolamine, and glutelin. Among them, glutelin, as the most abundant SSP, has the highest nutritional value because of its high digestibility and lysine content (He et al. 2021). There are 15 glutelin encoding genes in the rice genome, which are classified into four subfamilies, GluA, GluB, GluC, and GluD (Kawakatsu and Takaiwa 2010). Glu genes encode 57 kDa pro-glutelin, consisting of a signal peptide, a 37 kDa acidic subunit, and a 20 kDa basic subunit. A SNP in the *GluA2* promoter leads to a difference in the total protein content between indica and japonica rice. Hence, all haplotypes can be divided into two expression types, *OsGlu-A2^{LET}* and *OsGluA2^{HET}*. *OsGluA2^{LET}* mainly exists in japonica rice, with low expression of OsGlu. Meanwhile, *OsGluA2^{HET}* is highly expressed in indica rice. Therefore, the expression of *OsGlu* genes correlate closely with the grain total protein content and NQ (Yang et al. 2019).

Transporters of Amino Acids and Proteins

Efficient amino acid transfer, depending on amino acid transporters (AATs), is essential for protein biosynthesis in rice grains. Lysine-Histidine-type Transporter 1 (OsLHT1) can transport a broad spectrum of amino acids effectively. *OsLHT1* mutation leads to declined root uptake and consequent transfer of amino acids to rice shoots (Guo et al. 2020b). Moreover, *Oslht1* mutant rice has a reduced panicle length, seed setting rate, grain number per panicle, and total grain weight. More N and free amino acids are retained in the flag leaf of the *Oslht1* mutant than in the wild-type at maturity, implying its essential roles in transferring amino acids from leaves to seeds, thus ensuring grain development and rice nutrition quality (Guo et al. 2020a). Furthermore, a number of amino acid permeases (AAPs), a main type of AAT, are responsible for amino acid loading in rice seeds. Mutation of *OsAAP10* decreases the content of both protein and amylose in rice seeds. In addition, the RVA profile of *osaap10* mutant seeds exhibits a higher peak viscosity, disintegration value, and lower recovery value, thus improving the ECQ (Wang et al. 2020b). GPA1/OsRab5a, a small GTPase, regulates the transport of glutelin to protein body II (PBII) and affects the protein content. GPA3 interacts directly with Rab5a and guanine exchange factor VPS9a, forming a regulatory complex with them. These three proteins regulate dense vesicle (DV)-mediated post-Golgi transport synergistically in rice. The *gpa3* mutant showed a powdery endosperm, abnormal accumulation of glutelin precursors, irregularly arranged starch grains, decreased amylose content, and increased levels protein and lipids (Ren et al. 2014). GPA5, an effector of Rab5a, is also required for post-Golgi trafficking of storage proteins (Ren et al. 2020). *GPA5* mutation also leads to a powdery white endosperm, resulting from the abnormal accumulation of glutelin precursors and a reduction in α globulin, consequently forming loosely arranged and round compound starch granules. GPA5 interacts with class C core vacuole/endosome tethering (CORVET) complex and VAMP727-containing soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex to promote the fusion of DVs and protein storage vacuoles to complete glutelin transportation.

Other Regulators

In addition to the above mentioned storage or functional proteins, other regulators also participate in regulating the protein content in rice endosperm. For example, RISBZ1/OsbZIP58 and RPBF regulate the expression of seed storage protein genes and the consequent protein content (Kawakatsu et al. 2009). OsGZF1, a CCCH type zinc finger protein, binds specifically to the core promoter region of *GluB-1*, thereby suppressing its expression and the accumulation of glutelins (Chen et al. 2014). Moreover, transcription factors NAC20 and NAC26 also bind directly to the promoters of *SSP* genes and regulate their expression in rice (Wang et al. 2020a). In addition to transcriptional regulation, control of glutelin mRNA localization is another important mechanism that modulates glutelin accumulation. The zip codes of storage protein mRNAs require assistance from RNA-binding proteins (RBPs) for their correct localization. RBP-A, RBP-P, RBP-L, and Tudor-SN are reported to bind to the mRNAs of both glutelin and prolamin to aid their localization (Wang et al. 2008; Doroshenk et al. 2014; Chou et al. 2017, 2019; Tian et al. 2018; Tian et al. 2019a). A recent study showed that a quaternary protein complex, including RBP-P, RBP-L, Rab5a, and a membrane fusion protein NSF, cooperates to coordinate the transport of glutelin mRNAs in the rice endosperm (Tian et al. 2020).

Lipids

The main fatty acids in rice are palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2). Among them, linoleic acid is relatively good for human health. Lipids not only affect the rice NQ, but also influence the AQ and ECQ. Phospholipids and glycolipids can interact with starch in rice, thus reducing the water absorption and expansibility of starch, and can increase its GT.

The genes involved in carbon flow, lipid biosynthesis, transport, and oxidation affect the quantity and quality of lipids in rice. Pyruvate phosphate dikinase (encoded by *OsPPDKB)* regulates both carbon metabolism and carbon flow for starch and fat biosynthesis during rice filling. Its mutation produces a white powdery endosperm and a significantly increased fat content (Zhao et al. 2017). Another gene *Floury Shrunken Endosperm1* (*FSE1*), encoding a phospholipase-like protein, regulates galactolipid biosynthesis in the rice endosperm. *FSE1* mutation not only reduced the total galactolipid content significantly, but also caused abnormal amyloplast development in the developing endosperm, thus providing a novel connection between lipid metabolism and starch synthesis in rice (Long et al. 2018).

Fatty acid desaturase (*FAD*) genes, including *OsFAD2* and *OsFAD3*, participate directly in different steps of fatty acid synthesis (Liu et al. 2012; Shi et al. 2012; Ding et al. 2015). *OsACOT*, a major target of miR1432-mediated cleavage, encodes acyl-CoA thioesterase, which is involved in the biosynthesis of medium-chain fatty acids. Suppression of miR1432 expression or overexpression of miR1432-resistant form of *OsACOT* (*OXmACOT*) promoted rice grain filling rate and grain weight significantly. Moreover, the contents of palmitic acid and stearic acid

(18:0) in *OXmACOT* transgenic rice decreased, while those of the oleic acid and linoleic acid increased (Zhao et al. 2019).

Suppressing the expression of *OsLTP36*, encoding a lipid transporter, resulted in decreased contents of fatty acids and proteins, smaller and loose starch grains, and some other growth defects, including the seed setting rate, 1000-grain weight, chalkiness, and seed germination rate (Wang et al. 2015b). Moreover, lipoxygenase (LOX) catalyzes lipid oxidation, which leads to aging and a decrease of nutrient level in rice (Cho and Lim 2016). *LOX-2* and *LOX-3* regulate the degradation of fatty acids negatively, and suppression of their expression or loss-of-function mutation effectively prolonged the storage time and maintained a high nutritional value of rice (Long et al. 2013). Meanwhile, reducing the expression of *LOX-3* could effectively reduce the degradation of β-carotene in golden rice (Huang et al. 2014; Zhou et al. 2014). A recent study showed that mutation of *OsPLDα1*, encoding a phospholipase, changed lipid metabolites and reduced the phytic acid content strikingly (Khan et al. 2019). Further analysis indicated that the mutant brown rice shows some changes in ECQ properties, including a decreased AC, setback viscosity, and gelatinization temperature (GT), as well as an increased disintegration rate, corresponding to improved ECQ and NQ (Khan et al. 2020).

Lysine

The types of amino acids and the proportion of essential amino acids also determine the nutritional quality of rice. Lysine (Lys) is considered as the first limiting essential amino acid in humans; however, its content in milled rice is quite low. Several strategies have been used to enhance the lysine content in rice, including overexpression of lysine-rich proteins and modulation of lysine metabolism pathways (Yang et al. 2021a). For example, overexpression of lysine-rich histone proteins, RLRH1 and RLRH2, increased the lysine content of rice by 35% (Wong et al. 2015). With regards to modulation of lysine metabolism pathway, one method is to enhance the expression of lysine biosynthesis genes, another is to block the catabolism of lysine, thus promoting the lysine content of rice. AK and DHPS, two rate-limiting enzymes in the lysine biosynthesis pathway, are under strict feedback inhibition by lysine. By overexpressing modified Lys-insensitive AK or DHPS, the free lysine content increase by 6.6- to 21.7-fold. When simultaneously expressing these two enzymes, the level of free lysine increased by 58.5-fold (Yang et al. 2020, 2021a). Suppressing the expression of *LKR/SDH* gene, encoding lysine ketoglutaric acid reductase/saccharopine dehydropine dehydrogenase (LKR/SDH), attenuated lysine catabolism and remarkably

promoted the free lysine content in rice (Wu et al. 2016a; Yang et al. 2016b; Yang et al. 2018b; Zheng and Wang 2014). The rice varieties with high free lysine content had no significant differences in yield and other main agronomic traits except for plant height and grain color (Yang et al. 2016b). The changed endosperm color of high-lysine rice is mainly caused by activation of the jasmonic acid pathway by the high abundance of free lysine and subsequent enhanced serotonin biosynthesis (Yang et al. 2018b).

Carotenoids

Regardless of the fact that more than 700 kinds of carotene have been found in nature, only α-carotene, β-carotene, lutein, lycopene, zeaxanthin, and astaxanthin have been shown to be beneficial for health (Federico and Schmidt 2016). Carotenoids are important phytonutrients with antioxidant properties, and are used widely in foods and feedstuffs as supplements. In addition, carotenoids can also be used as antioxidants to prevent seed aging and promote seed vigor, leading to successful germination (Federico and Schmidt 2016). The synthesis of carotenoids in seeds is closely related to the ABA biosynthesis pathway, the dominant pathway for seed dormancy.

Rice carotenoids biosynthesis is blocked in the first enzymatic step. Biofortification is an effective way to produce and accumulate carotenoids in rice grains. Therefore, the major objective of golden rice (GR) development is to improve its carotenoids content. Driven by the *Gt1* promoter, the daffodils-originating *PSY* gene and *Erwinia uredovora*-originating *CRTI* gene were transformed into rice, thus generating rice grains with β-carotene accumulation (Ye et al. 2000). Golden rice 2 (GR2) was developed by transferring the maize phytene synthase gene *Zmpac1* and the carotene desaturase gene *CrtI* from soil bacteria *Pantoea ananatis* into japonica Kaybonnet rice. The content of β-carotene in the rice grain was 23 times higher than that in the first generation of GR (Paine et al. 2005). Recently, astaxanthin biosynthesis was bioengineered in the rice endosperm by introducing *sZmPSY1*, *sPaCrtI*, *sCrBKT*, and *sHpBHY*, four genes encoding the enzymes phytoene synthase, phytoene desaturase, β-carotene ketolase, and β-carotene hydroxylase, respectively, thus generating multiple healthy rice germplasms, including β-carotene-enriched Golden Rice, Canthaxanthin Rice, and Astaxanthin Rice (Zhu et al. 2018). Another study indicated that co-expression of *tHMG1*, *ZmPSY1*, and *PaCRTI* could boost the carotenoids flux through the MVA pathway, thus increasing the accumulation of carotenoids in the rice endosperm markedly (Tian et al. 2019b). The promotion of carotenoids in rice relies on insertions of target genes at random sites via

conventional transgenic methods; therefore, the marker gene still exists in the genome of transgenic rice. Alternatively, an expression cassette including two carotenoid biosynthesis genes were introduced using targeted insertion at a safe site in the rice genome via CRISPR/Cas9, thus generating marker-free carotenoid-enriched rice (Dong et al. 2020).

Minerals, Taking Fe and Zn as Examples

At present, more than 90 Fe-related QTLs have been identified in the rice genome, among which 17 are stable and 25 harbor Fe-related genes nearby or within the QTL (Swamy et al. 2021). A common mechanism of transporters and chelators mediates iron and zinc absorption and transport; therefore, most instances of increased iron content in rice are accompanied by a parallel increase in zinc (Kawakami and Bhullar 2018).

There are two sources of iron in seeds, absorption by the roots from soil solution, followed by direct transfer to seeds and reactivation from different tissues and organs during seed development (Ishimaru et al. 2006, 2007a). Two Ferritin (FER) genes, *OsFER1* and *OsFER2*, have been identified in rice (Stein et al. 2009). *OsFER2* is more sensitive to external iron supply than *OsFER1,* implying its major role in rice FE chelation. Seed-specific overexpression of *OsFER2* promotes the accumulation of iron and zinc in milled rice seeds by 2.1 and 1.37-fold, respectively (Paul et al. 2012).

Nicotinamide (NA) is a ubiquitous metal-chelated non-protein amino acid in terrestrial plants with important role in metal transport. Increasing the expression of the NA synthase gene (*NAS*) is a useful biofortification method to promote the Fe and Zn contents of rice. There are three *NAS* genes in rice, *OsNAS1*, *OsNAS2*, and *OsNAS3* (Nozoye et al. 2019). Knockout of *OsNAS3* reduced the iron content in rice flag leaves and seeds, while *OsNAS3* overexpression had the opposite phenotype (Lee et al. 2009). Yellow Stripe 1-Like 9 (encoded by *OsYSL9*) transports the iron-sodium/DMA complex from the endosperm to the embryo during seed development. An *OsYSL9* null mutant showed a decreased iron content in embryos, but an increased iron content in the endosperm (Senoura et al. 2017).

OsVIT1 and OsVIT2 are another two transporters that regulate the iron content in rice seeds. Knockout of the two genes promotes the amount of Fe/Zn in rice seeds, but decreases contents of these metals in rice leaves, suggesting that OsVIT1 and OsVIT2 play important roles in controlling Fe/Zn translocation between source and sink organs (Zhang et al. 2012b).

ZIP transporters include zinc regulated transporters (ZRT) and iron regulated transporters (IRT). The rice *ZIP* gene family contains 16 members, including 14 *ZRT* genes and two *IRT* genes (Sasaki et al. 2015). Overexpression of *OsIRT1* reduces plant height, tiller, and yield of rice, but increases the content of Fe and Zn in rice grains (Lee and An 2009). In addition, overexpression of *OsZIPs*, such as *OsZIP4*, *OsZIP5*, and *OsZIP8*, promotes the Zn content in roots, but reduces the content in shoots and grains (Ishimaru et al. 2007b; Lee et al. 2010a, b).

In general, three major strategies can be used to enhance the iron content in rice, including overexpression of *NAS* genes, endosperm-specific expression of *FERs*, and promoting source-to-endosperm Fe remobilization (Kawakami and Bhullar 2021).

2-Acetyl-1-Pyrroline (2-AP)

2-AP is the main aroma substance in scented rice (Kovach et al. 2009; Bradbury et al. 2010). The *Badh2* gene, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-AP by exhausting γ-aminobutyraldehyde (AB-ald), a presumed 2AP precursor. The significant increase of 2-AP levels in fragrant rice varieties greatly improves the aroma of milled rice. The null *badh2* alleles, with a protein frameshift mutation, enhance 2-AP biosynthesis and hence the aroma of rice (Chen et al. 2008).

Genes Regulating Other Seed Structures and Their Roles in Grain Qualities
The Embryo

The rice embryo, containing most of the genetic information of rice, has the highest concentration of nutrients, including proteins, fatty acids, vitamins, and minerals. Giant embryo rice is a rice mutant whose embryo is about two to three times larger than the normal embryo. The phenotype of giant embryo rice is determined by the *GIANT EMBRYO* (*GE*) gene, encoding cytochrome P450 protein CYP78A13. *GE* mutation leads to large embryo and small endosperm, while overexpression of *GE* had the opposite phenotype (Nagasawa et al. 2013).

In plants, GABA is mainly catalyzed by glutamate decarboxylase (GAD), producing decarboxylate glutamate (Akama et al. 2009; Shelp et al. 2012). However, the higher GABA content in the grains of developing giant embryo rice mainly comes from the upregulation of the polyamine (PA) derivative pathway and the downregulation of GABA catabolism activity (Zhao et al. 2017). The C-terminal extension of OsGAD2 acts as a powerful self-inhibitory domain, and truncation of this domain caused the enzyme to function constitutively and actively. Rice endosperm specific expression of *OsGAD2ΔC* enhanced the GABA content by about tenfold (Shelp et al. 2012). In addition, the combination of seed-specific overexpression of truncated GAD with suppression of GABA-T, enhanced the GABA content strikingly (75–350 mg/100 g) in milled rice

(Shimajiri et al. 2013). Recently, the coding region of CaMBD from the *OsGAD3* gene was knocked out using CRISPR/Cas9 technology, thus improving rice GABA content by 7 times, accompanied by increased grain weight and protein content (Akama et al. 2020).

The Aleurone Layer

Brown rice is unpolished rice after shelling, which is composed of the endosperm, embryo, and rice bran. Compared with milled rice, brown rice contains more macronutrients, vitamins, minerals, and other functional substances, such as GABA, is thus more beneficial to human health. The proportion of nutrients among various rice components are different, thus optimization of rice structure is expected to change the nutritional quality of rice (Zheng and Wang 2014). Most nutrients of the seeds are stored in the endosperm. The triploid endosperm of rice develops from the fertilized polar nucleus. The endosperm at the filling stage consists of the aleurone layer, subaleurone layer, and starch endosperm, from outside to inside, respectively (Wu et al. 2016a). The cells of the aleurone layer of the mature endosperm are living, whereas the cells in the starch endosperm are dead. As a transitional cell type, the subaleurone layer cells accumulate both starch and protein in the early stage of development and differentiate into the starch endosperm in the late stage of endosperm development. The aleurone layer contains a large number of nutrient elements, such as proteins, vitamins, and minerals (Becraft and Yi 2011).

TA1, encoding a single-stranded DNA binding protein, OsmtSSB1 (located in mitochondria), is highly expressed in the aleurone layer, subaleurone layer and the caryopsis embryo, but not in the starch endosperm. The thickness of the aleurone layer of the mutant *ta1* was about twice as thick as that of the wild-type, and the nutritional quality was greatly improved. In the *ta1* mutant, the content of all nutrient elements increased (Li et al. 2021). In the mutant *ta2-1*, its grain transparency, 1000-grain weight, seed setting rate, total starch and AC were decreased; however, its nutritional composition of shelled grains increased, including the total protein, lipid, iron, zinc, calcium, dietary fiber, antioxidants, phenols, and vitamins (Liu et al. 2018a).

Although brown rice is rich in nutrients, it is accepted by few consumers in the market because of its poor taste. How to balance taste and nutrition is an urgent problem that should be solved in the future. Currently, using germinated brown rice seems to be a reasonable alternative, which maintains the nutritional quality of rice but improves its edible quality (Cho and Lim 2016).

The Seed Coat

Brown rice or dehulled rice refers to rice with the hull removed, and further polishing generates milled white rice, which is generally sold in the market and consumed by people. However, brown rice contains most of the nutritional substances, including dietary fiber, vitamins, and phenolics, most of which are lost during the polishing process. Therefore, brown rice, as the whole grain, contains more nutritional components. Moreover, brown rice with a red, purple, or black pericarp is more beneficial to human health than traditional white pericarp rice because of the accumulation of more antioxidant compounds. Red rice pigmentation is controlled by the joint action of two genes *Rc* and *Rd* (Sweeney et al. 2006; Furukawa et al. 2007). *Rc*, encoding a basic helix ring helix (bHLH) transcription factor, is responsible for the accumulation of the pigment, and *Rd/OsDFR*, encoding a dihydroflavonol 4-reductase, enhances the accumulation of procyanidin in the brown grain pericarp (Sweeney et al. 2006; Furukawa et al. 2007). *Rc* and *Rd* together cause the peel to be red and most cultivated rice varieties that produce white grains have a 14 bp frameshift deletion of the seventh exon of *Rc* (Furukawa et al. 2007). Recently, the recessive *Rc* allele with 14 bp deletion was functionally restored to an in-frame mutation via the CRISPR/Cas9 method, which successfully transformed three excellent white grain varieties into red grains, thus remarkably promoting the content of procyanidin and anthocyanin (Zhu et al. 2019).

The black rice pigment is also regulated by the joint action of two genes, *Pb* and *Pp* (Hu et al. 1996; Wang and Shu 2007). The *Pb* locus is composed of two genes, encoding a MYC transcription factor and a bHLH16 transcription factor, which are involved in the synthesis of anthocyanin and procyanidins, respectively. The expression of *Pb* seems to be the cause of pigment accumulation in the pericarp of brown grains, whereas the expression of *Pp* increases the content of pigment, resulting in purple grains. The copy number of the *Pp* gene correlates with the intensity of purple pigmentation. In the absence of *Pp*, *Pb* plants produce brown grains, while *Pp* plants without *Pb* have white grains (Tanaka et al. 2008; Rahman et al. 2013; Mbanjo et al. 2020).

The gene corresponding to black rice is *Kala4/OsB2*, encoding a bHLH transcription factor. The structural rearrangement of its promoter region leads to its ectopic expression, resulting in a black pericarp. *OsB2* regulates a number of genes encoding anthocyanin synthesis related enzymes, including *F3H*, *DFR*, and *ANS* (Oikawa et al. 2015).

Recently, a *C-S-A* gene model of rice husk pigmentation was proposed. *C1* and *A1* jointly determine the color change, while *S1* diversifies the pigment tissue. *C1*

encodes an R2R3-MYB transcription factor and acts as a color producing gene. *S1* encodes a bHLH protein that functions in a tissue-specific manner. C1 interacts with S1 and activates the expression of *A1*, which encodes dihydroflavonol reductase, thus generating purple rice pigment. The involvement of functional A1 leads to high accumulation of cyano 3-o-glucoside, while A1 mutation results in a brown pigment. Instead of C1, rice pigment color is produced by the synergistic regulation of S1 and other MYB transcription factors (Sun et al. 2018b).

More recently, a study showed that *OsTTG1*, the *WD40* gene in rice, is an important regulator of anthocyanin biosynthesis. The OsTTG1 protein is located in the nucleus and directly interacts with Kala4, OsC1, OsDFR, and Rc, which are determinants of pigments or anthocyanin biosynthesis. Knockout of *OsTTG1* reduced the anthocyanin content of the mutant grain to only 0.15% of that of wild-type plants, suggesting that OsTTG1 is a vital regulator of rice anthocyanin biosynthesis (Yang et al. 2021c).

Conclusion and Perspectives

Rice grain quality, a typical quantitative trait, is influenced by complex genetic regulation and environmental factors. Rice quality generally includes milling quality, appearance quality, nutritional quality, and eating and cooking quality. The structure and composition of the rice seed are correlate closely with different aspects of rice quality. For example, grain size not only affects rice AQ, but also influences rice MQ and ECQ. Moreover, the rice endosperm, as the edible part of rice, is composed of starch, protein, lipids, and other micronutrients. The quantity and quality of these component are major contributors to rice ECQ, AQ, and NQ. Therefore, modifying and optimizing the structure and composition of rice seed are crucial to promoting its quality. Considering that rice growth and development are controlled by numerous genes and complicated regulation networks, the cloning of key genes involved in regulating specific grain trait and the dissection their molecular mechanisms will provide valuable gene resources and essential information for breeding high quality rice. In the present review, we summarized the cloned genes and their molecular functions that determine each part of rice seeds and their corresponding roles in regulating rice quality. In particular, the genes involved in controlling seed size and endosperm components are highlighted, which are also the two major determinants of rice quality.

Although impressive progress has been made in rice quality research, few cloned genes are suitable for use in high quality rice breeding programs. In addition, a huge gap still exists in our understanding of the regulatory network of rice grain quality. Therefore, a number

of urgent problems remain to be solved. First, there is a contradiction between grain yield and rice quality; therefore, how to improve rice quality without sacrificing rice yield is an important issue. Second, it is a great challenge to explore the upstream and downstream components of known rice quality regulators, as well as the crosstalk between various regulation pathways. Third, the evaluation of the effects of most cloned rice quality genes or their elite alleles are limited and fragmented, being based on the analysis in a only a few rice varieties, especially some so-called model varieties. The performance of quality traits are genetic and environment-dependent, which often causes the silencing of some reported high quality genes in breeding practice. Fourth, some rice quality determinants often play contradictory roles in the context of different quality traits. For example, the protein content correlates positively with rice NQ. However, the higher the protein content, the more protein bodies accumulate among starch grains, resulting in increased chalkiness and the deterioration of ECQ. For lipids, the oxidation of unsaturated fatty acids helps to improve the flavor of rice. However, it is not conducive to long-term storage because the hydrolysis and oxidation of lipids will worsen the appearance and taste of rice. Finally, we lack universal and unified rice quality evaluation standards. Rice quality is relative and context-specific; thus the evaluation of rice quality depends on their application mode and consumer background, including region, age, or even religion.

Although rice quality research remains challenging, certain strategies should be employed in future studies. Most importantly, more genes encoding proteins with specific roles in the control of rice quality should be cloned, sequenced, and functionally analyzed, thus enlarging the high quality gene pool. In addition, powerful and accurate technologies can be used in basic research and in breeding practices of high quality rice, such as genome-wide selection, enhanced selection of major genes for grain quality, and precise gene knock-in or knock-out. For example, based on re-sequencing data of 200 japonica rice varieties in central China, Xiao et al. (2021) revealed a number of superior alleles for rice quality and rice blast resistance by using genome-wide association mapping and selection approach. Next, the alleles related to blast-resistance and excellent rice ECQ, such as Wx^{mp} allele, corresponding to low amylose content, were successfully introduced into two high-yield rice cultivars. Hence, two elite rice lines, XY99 and JXY1, with both ECQ and blast resistance improved were efficiently developed (Xiao et al. 2021). In addition, the corresponding breeding character targets, as well as universal and objective rice quality

evaluation standards according to regional or population preferences of consumers, should be established as soon as possible, which will help to accelerate high quality rice research.

Abbreviations

AAP10: Amino acid permease 10; ABA: Abscisic acid; AC: Amylose content; ACOT: Acyl-CoA thioesterase; AGPases: ADP-glucose pyrophosphorylases; AK2: Aspartate kinase 2; ALK: Alkali digestion; AQ: Appearance quality; AP2: APETALA2; Badh2: Betaine aldehyde dehydrogenase 2; BAK1: BRI1-ASSOCI-ATED RECEPTOR KINASE; BG3: Big grain 3; BR: Brassinosteroid; BRD1: BR-defi-cient dwarf1; BRI1: BRASSINOSTEROID-INSENSITIVE 1; BZR1: BRASSINAZOLE-RESISTANT 1; CaMBD: Calmodulin-binding domain; CLG1-1: Chang Li Geng1-1; CORVET: The class C core vacuole/endosome tethering; D1: Dwarf 1; DBE: Debranching enzyme; DEP1: Dense and erect panicle 1; DHPS: Dihydrodipico-linate synthase; DLT: Dwarf and low-tillering; ECQ: Eating and cooking quality; FAD: Fatty acid desaturase; FER1: Ferritin gene 1; FGR1: Floury and growth retardation 1; FLO6: FLOURY ENDOSPERM 6; FLR: FERONIA-like receptor; FSE1: Floury Shrunken Endosperm1; GA: Gibberellin; GABA: γ-Aminobutyric acid; GAD: Glutamate decarboxylase; GAD1: Grain length and awn development 1; GBSS: Granule-bound starch synthase; GC: Gel consistency; GE: GIANT EMBRYO; GGC: G protein gamma subunit; GL3.1: Grain length 3.1; GluA1: Glutelin type-A1; GPA1: GLUTELIN PRECURSOR ACCUMULATION1; GR: Golden rice; GRF4: Growth-regulating factor 4; GS3: QTL for grain size on chromosome 3; GS5: Grain size on chromosome 5; GS9: Grain shape gene on chromosome 9; GSK2: GSK3/SHAGGY-like kinase 2; GSN1: Grain size and number1; GT: Gelati-nization temperature; GW2: Grain weight and width on chromosome 2; GW5: QTL for grain width and weight on chromosome 5; GW6: Grain width 6; GW6a: Grain weight on chromosome 6-a; GZF1: GluB-1-binding Zinc Finger 1; HDR3: Homolog of DA1 on rice chromosome 3; IRT: Iron regulated transporters; ISA: Isoamylase; LAC: Laccase; LG1: Large grain 1; LHT1: Lysine-histidine-type transporter 1; LKR: Lysine ketoglutaric acid reductase; LOX-2: Lipoxygenase 2; Lys: Lysine; MADS6: MADS-box transcription factor 6; MAPK: Mitogen-activated protein kinase; MKKs: MAPK kinases; MKKKs: MKK kinases; MQ: Milling quality; NAC20: NAC domain-containing protein 20; NAS: Nicotinamide synthase; NF-YB1: Nuclear transcription factor Y subunit B1; NQ: Nutrition quality; OFP: OVATE family protein; PA: Polyamine; PGL1: Positive regulator of grain length 1; PHI-1: Phosphorylation inducible protein 1; PHO: Starch phosphorylase; pk2: Plastidic pyruvate kinase 2; PLDα1: Phospholipase Dα 1; POW1: Put on weight 1; PPDKB: Pyruvate phosphate dikinase; PPKL1: Protein phosphatase with Kelch-like repeat domain 1; QTL: Quantitative trait locus; Rab5a: Small GTPase RAT BRAIN 5a; RBP: RNA-binding protein; RGA: G protein alpha subunit; RGB: G protein beta subunit; RGG: Heterotrimeric G protein γ subunit; RISC: RNA-induced silencing complex; RLRH1: Rice lysine rich histone proteins 1; RVA: Rapid visco analyzer; SBE: Starch branching enzyme; SDH: Saccharo-pine dehydropine dehydrogenase; SMOS1: Small organ size 1; SNARE: Soluble N-ethylmaleimide sensitive factor attachment protein receptor; SNB: SUPERNUMERARY BRACT; SPL: SQUAMOSA promoter binding protein-like; SRS5: Small and round seed 5; SSH1: Suppression of shattering 1; SSRGs: Starch synthesis related genes; SSS: Soluble starch synthase; TA1: Thick aleurone 1; TF: Transcription factor; TGW6: Thousand-grain weight 6; TTG1: TRANSPARENT TESTA GLABRA1; TUD1: Taihu Dwarf 1; UBP15: Ubiquitin-specific protease 15; UPP: Ubiquitin proteasome pathway; VIT1: Vacuolar iron transporter 1; WTG1: Wide and thick grain 1; Wx: Waxy; YSL9: Yellow stripe1-like 9; YUC11: YUCCA (YUC) flavin-containing monooxygenase 11; ZRT: Zinc regulated transporters.

Availability of Data and Materials
Not applicable.

Declarations

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
The manuscript has been approved by all authors.

Competing interests
The authors declare no competing interest.

Author details
[1] Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding/Jiangsu Key Laboratory of Crop Genetics and Physiology/State Key Laboratory of Hybrid Rice, College of Agriculture, Yangzhou University, Yangzhou 225009, Jiangsu, China. [2] Co-Innovation Center for Modern Production Technology of Grain Crops of Jiangsu Province/Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, Jiangsu, China.

References
Akabane T, Suzuki N, Tsuchiya W et al (2021) Expression, purification and crystallization of *TGW6*, which limits grain weight in rice. Protein Expr Purif 188:105975

Akama K, Takaiwa F (2007) C-terminal extension of rice glutamate decarboxy-lase (*OsGAD2*) functions as an autoinhibitory domain and overexpres-sion of a truncated mutant results in the accumulation of extremely high levels of GABA in plant cells. J Exp Bot 58:2699–2707

Akama K, Kanetou J, Shimosaki S et al (2009) Seed-specific expression of truncated *OsGAD2* produces GABA-enriched rice grains that influence a decrease in blood pressure in spontaneously hypertensive rats. Transgenic Res 18:865–876

Akama K, Akter N, Endo H et al (2020) An in vivo targeted deletion of the calmodulin-binding domain from rice glutamate decarboxylase 3 (*OsGAD3*) increases γ-aminobutyric acid content in grains. Rice 13:20

Ashikari M, Wu J, Yano M et al (1999) Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the alpha-subunit of GTP-binding protein. Proc Natl Acad Sci USA 96:10284–10289

Aya K, Hobo T, Sato-Izawa K et al (2014) A novel AP2-type transcription factor, SMALL ORGAN SIZE1, controls organ size downstream of an auxin signaling pathway. Plant Cell Physiol 55:897–912

Azizi P, Osman M, Hanafi M et al (2019) Molecular insights into the regulation of rice kernel elongation. Crit Rev Biotechnol 39:904–923

Bao J (2014) Genes and QTLs for rice grain quality improvement. Rice Germ-plasm Genet Improv 9:239–278

Bao JS, Corke H, Sun M (2006) Nucleotide diversity in starch synthase IIa and validation of single nucleotide polymorphisms in relation to starch gelatinization temperature and other physicochemical properties in rice (*Oryza sativa* L.). Theor Appl Genet 113:1171–1183

Baysal C, He W, Drapal M et al (2020) Inactivation of rice starch branching enzyme IIb triggers broad and unexpected changes in metabo-lism by transcriptional reprogramming. Proc Natl Acad Sci U S A 117:26503–26512

Becraft PW, Yi G (2011) Regulation of aleurone development in cereal grains. J Exp Bot 62:1669–1675

Bello BK, Hou Y, Zhao J et al (2019) NF-YB1-YC12-bHLH144 complex directly activates *Wx* to regulate grain quality in rice (*Oryza sativa* L.). Plant Biotechnol J 17:1222–1235

Bradbury L, Fitzgerald TL, Henry RJ et al (2010) The gene for fragrance in rice. Plant Biotechnol J 3:363–370

Buttery R, Ling L, Juliano B et al (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agric Food Chem 31:823–826

Cai Y, Li S, Jiao G et al (2018) *OsPK2* encodes a plastidic pyruvate kinase involved in rice endosperm starch synthesis, compound granule formation and grain filling. Plant Biotechnol J 16:1878–1891

Che R, Tong H, Shi B et al (2015) Control of grain size and rice yield by GL2-mediated brassinosteroid responses. Nat Plants 2:15195

Chen S, Wu J, Yang Y et al (2006) The fgr gene responsible for rice fragrance was restricted within 69 kb. Plant Sci 171:505–514

Chen S, Yang Y, Shi W et al (2008) *Badh2*, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. Plant Cell 20:1850–1861

Chen Y, Sun A, Wang M et al (2014) Functions of the CCCH type zinc finger protein *OsGZF1* in regulation of the seed storage protein GluB-1 from rice. Plant Mol Biol 84:621–634

Chen E, Huang X, Tian Z et al (2019) The genomics of oryza species provides insights into rice domestication and heterosis. Annu Rev Plant Biol 70:639–665

Chen Z, Lu Y, Feng L et al (2020) Genetic dissection and functional differentiation of *ALKᵃ* and *ALKᵇ*, two natural alleles of the *ALK/SSIIa* gene, responding to low gelatinization temperature in rice. Rice 13:39

Chen H, Yu H, Jiang W et al (2021) Overexpression of ovate family protein 22 confers multiple morphological changes and represses gibberellin and brassinosteroid signalings in transgenic rice. Plant Sci 304:110734

Cheng F, Zhong LJ, Wang F et al (2005) Differences in cooking and eating properties between chalky and translucent parts in rice grains. Food Chem 90:39–46

Cho DH, Lim ST (2016) Germinated brown rice and its bio-functional compounds. Food Chem 196:259–271

Chou HL, Tian L, Kumamaru T et al (2017) Multifunctional RNA binding protein OsTudor-SN in storage protein mRNA transport and localization. Plant Physiol 175:1608–1623

Chou HL, Tian L, Fukuda M et al (2019) The role of RNA-binding protein OsTudor-SN in post-transcriptional regulation of seed storage proteins and endosperm development. Plant Cell Physiol 60:2193–2205

Ding W, Lin L, Zhang B et al (2015) OsKASI, a β-ketoacyl-[acyl carrier protein] synthase I, is involved in root development in rice (*Oryza sativa* L.). Planta 242:203–213

Dong OX, Yu S, Jain R et al (2020) Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9. Nat Commun 11:1178

Doroshenk KA, Tian L, Crofts AJ et al (2014) Characterization of RNA binding protein RBP-P reveals a possible role in rice glutelin gene expression and RNA localization. Plant Mol Biol 85:381–394

Duan M, Sun S (2005) Profiling the expression of genes controlling rice grain quality. Plant Mol Biol 59:165–178

Duan P, Rao Y, Zeng D et al (2014) *SMALL GRAIN 1*, which encodes a mitogen-activated protein kinase kinase 4, influences grain size in rice. Plant J 77:547–557

Duan P, Ni S, Wang J et al (2015) Regulation of *OsGRF4* by OsmiR396 controls grain size and yield in rice. Nat Plants 2:15203

Duan P, Xu J, Zeng D et al (2017) Natural variation in the promoter of *GSE5* contributes to grain size diversity in rice. Mol Plant 10:685–694

Evenson RE, Gollin D (2003) Assessing the impact of the green revolution, 1960 to 2000. Science 300:758–762

Fan C, Xing Y, Mao H et al (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Federico ML, Schmidt MA (2016) Modern breeding and biotechnological approaches to enhance carotenoid accumulation in seeds. Subcell Biochem 79:345–358

Fujisawa Y, Kato T, Ohki S et al (1999) Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice. Proc Natl Acad Sci USA 96:7575–7580

Fujita N, Yoshida M, Kondo T et al (2007) Characterization of SSIIIa-deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm. Plant Physiol 144:2009–2023

Furukawa T, Maekawa M, Oki T et al (2007) The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. Plant J 49:91–102

Gao Z, Zeng D, Cui X et al (2003) Map-based cloning of the *ALK* gene, which controls the gelatinization temperature of rice. Sci China C Life Sci 46:661–668

Gao X, Zhang J, Zhang X et al (2019) Rice *qGL3/OsPPKL1* functions with the GSK3/SHAGGY-like kinase *OsGSK3* to modulate brassinosteroid signaling. Plant Cell 31:1077–1093

Gao Q, Zhang N, Wang W et al (2021) The ubiquitin-interacting motif-type ubiquitin receptor HDR3 interacts with and stabilizes the histone acetyltransferase *GW6a* to control the grain size in rice. Plant Cell 33:3331–3347

Guo T, Chen K, Dong N et al (2018) *GRAIN SIZE AND NUMBER1* negatively regulates the OsMKKK10-OsMKK4-OsMPK6 cascade to coordinate the trade-off between grain number per panicle and grain size in rice. Plant Cell 30:871–888

Guo N, Gu M, Hu J et al (2020a) Rice *OsLHT1* Functions in leaf-to-panicle nitrogen allocation for grain yield and quality. Front Plant Sci 11:1150

Guo N, Hu J, Yan M et al (2020b) *Oryza sativa* Lysine-Histidine-type Transporter 1 functions in root uptake and root-to-shoot allocation of amino acids in rice. Plant J 103:395–411

Hao Y, Wang Y, Wu M et al (2019) The nuclear-localized PPR protein OsNPPR1 is important for mitochondrial function and endosperm development in rice. J Exp Bot 70:4705–4720

Hao J, Wang D, Wu Y et al (2021) The GW2-WG1-OsbZIP47 pathway controls grain size and weight in rice. Mol Plant 14:1266–1280

He W, Wang L, Lin Q et al (2021) Rice seed storage proteins: Biosynthetic pathways and the effects of environmental factors. J Integr Plant Biol 63:1999–2019

Heang D, Sassa H (2012) Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice. PLoS ONE 7:e31325

Hirano K, Yoshida H, Aya K et al (2017) *SMALL ORGAN SIZE 1* and *SMALL ORGAN SIZE 2/DWARF AND LOW-TILLERING* form a complex to integrate Auxin and Brassinosteroid signaling in rice. Mol Plant 10:590–604

Hiroyuki S, Yasuhiro S, Makoto S et al (2002) Molecular characterization of Wx-mq, a novel Mutant gene for low-amylose content in endosperm of rice (*Oryza sativa* L.). Breed Sci 2:131–135

Hong Z, Ueguchi-Tanaka M, Shimizu-Sato S et al (2002) Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase, prevents the organized arrangement and polar elongation of cells in the leaves and stem. Plant J 32:495–508

Hong Z, Ueguchi-Tanaka M, Fujioka S et al (2005) The Rice brassinosteroid-deficient *dwarf2* mutant, defective in the rice homolog of Arabidopsis *DIMINUTO/DWARF1*, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone. Plant Cell 17:2243–2254

Hori K, Suzuki K, Iijima K et al (2016) Variation in cooking and eating quality traits in Japanese rice germplasm accessions. Breed Sci 66:309–318

Hu J, Anderson B, Wessler SR (1996) Isolation and characterization of rice R genes: evidence for distinct evolutionary paths in rice and maize. Genetics 142:1021–1031

Hu Z, He H, Zhang S et al (2012) A Kelch motif-containing serine/threonine protein phosphatase determines the large grain QTL trait in rice. J Integr Plant Biol 54:979–990

Hu X, Qian Q, Xu T et al (2013) The U-box E3 ubiquitin ligase *TUD1* functions with a heterotrimeric G α subunit to regulate Brassinosteroid-mediated growth in rice. PLoS Genet 9:e1003391

Hu J, Wang Y, Fang Y et al (2015) A rare allele of *GS2* enhances grain size and grain yield in rice. Mol Plant 8:1455–1465

Hu Z, Lu S, Wang M et al (2018) A novel QTL qTGW3 encodes the GSK3/SHAGGY-Like Kinase OsGSK5/OsSK41 that interacts with OsARF4 to negatively regulate grain size and weight in rice. Mol Plant 11:736–749

Hu J, Huang L, Chen G et al (2021) The elite alleles of *OsSPL4* regulate grain size and increase grain yield in rice. Rice 14:90

Huang X, Qian Q, Liu Z et al (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. Nat Genet 41:494–497

Huang R, Jiang L, Zheng J et al (2013) Genetic bases of rice grain shape: so many genes, so little known. Trends Plant Sci 18:218–226

Huang J, Cai M, Long Q et al (2014) *OsLOX2*, a rice type I lipoxygenase, confers opposite effects on seed germination and longevity. Transgenic Res 23:643–655

Huang K, Wang D, Duan P et al (2017) *WIDE AND THICK GRAIN 1*, which encodes an otubain-like protease with deubiquitination activity, influences grain size and shape in rice. Plant J 91:849–860

Huang L, Li Q, Zhang C et al (2020) Creating novel Wx alleles with fine-tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system. Plant Biotechnol J 18:2164–2166

Huang L, Tan H, Zhang C et al (2021a) Starch biosynthesis in cereal endosperms: an updated review over the last decade. Plant Commun 2:100237

Huang L, Gu Z, Chen Z et al (2021b) Improving rice eating and cooking quality by coordinated expression of the major starch synthesis-related genes, SSII and Wx, in endosperm. Plant Mol Biol 106:419–432

Hui S, Li H, Mawia AM et al (2021) Production of aromatic three-line hybrid rice using novel alleles of BADH2. Plant Biotechnol J 20:59–74

Inoue H, Higuchi K, Takahashi M et al (2003) Three rice nicotianamine synthase genes, OsNAS1, OsNAS2, and OsNAS3 are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. Plant J 36:366–381

Ishimaru Y, Suzuki M, Tsukamoto T et al (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J 45:335–346

Ishimaru Y, Kim S, Tsukamoto T et al (2007a) Mutational reconstructed ferric chelate reductase confers enhanced tolerance in rice to iron deficiency in calcareous soil. Proc Natl Acad Sci USA 104:7373–7378

Ishimaru Y, Masuda H, Suzuki M et al (2007b) Overexpression of the *OsZIP4* zinc transporter confers disarrangement of zinc distribution in rice plants. J Exp Bot 58:2909–2915

Ishimaru K, Hirotsu N, Madoka Y et al (2013) Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield. Nat Genet 45:707–711

Jeon JS, Ryoo N, Hahn TR et al (2010) Starch biosynthesis in cereal endosperm. Plant Physiol Biochem 48:383–392

Jiang L, Ma X, Zhao S et al (2019) The APETALA2-Like Transcription Factor SUPERNUMERARY BRACT Controls Rice Seed Shattering and Seed Size. Plant Cell 31:17–36

Jin J, Hua L, Zhu Z et al (2016) *GAD1* Encodes a secreted peptide that regulates grain number, grain length, and awn development in rice domestication. Plant Cell 28:2453–2463

Kabir M, Nonhebel H (2021) Reinvestigation of *THOUSAND-GRAIN WEIGHT 6* grain weight genes in wheat and rice indicates a role in pollen development rather than regulation of auxin content in grains. Theor Appl Genet 134:2051–2062

Kato C, Mizutani T, Tamaki H et al (2004) Characterization of heterotrimeric G protein complexes in rice plasma membrane. Plant J 38:320–331

Kawakami Y, Bhullar NK (2018) Molecular processes in iron and zinc homeostasis and their modulation for biofortification in rice. J Integr Plant Biol 60:1181–1198

Kawakami Y, Bhullar NK (2021) Delineating the future of iron biofortification studies in rice: challenges and future perspectives. J Exp Bot 72:2099–2113

Kawakatsu T, Takaiwa F (2010) Cereal seed storage protein synthesis: fundamental processes for recombinant protein production in cereal grains. Plant Biotechnol J 8:939–953

Kawakatsu T, Yamamoto MP, Touno SM et al (2009) Compensation and interaction between RISBZ1 and RPBF during grain filling in rice. Plant J 59:908–920

Khan MSS, Basnet R, Islam SA et al (2019) Mutational analysis of OsPLDalpha1 reveals its involvement in phytic acid biosynthesis in rice grains. J Agric Food Chem 67:11436–11443

Khan MSS, Basnet R, Ahmed S et al (2020) Mutations of *OsPLDa1* increase lysophospholipid content and enhance cooking and eating quality in rice. Plants 9:390–400

Kovach M, Calingacion M, Fitzgerald M et al (2009) The origin and evolution of fragrance in rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 106:14444–14449

le Qu Q, Takaiwa F (2004) Evaluation of tissue specificity and expression strength of rice seed component gene promoters in transgenic rice. Plant Biotechnol J 2:113–125

le Qu Q, Xing YP, Liu WX et al (2008) Expression pattern and activity of six glutelin gene promoters in transgenic rice. J Exp Bot 59:2417–2424

Lee S, An G (2009) Over-expression of *OsIRT1* leads to increased iron and zinc accumulations in rice. Plant Cell Environ 32:408–416

Lee S, Chiecko JC, Kim SA et al (2009) Disruption of *OsYSL15* leads to iron inefficiency in rice plants. Plant Physiol 150:786–800

Lee S, Jeong HJ, Kim SA et al (2010a) *OsZIP5* is a plasma membrane zinc transporter in rice. Plant Mol Biol 73:507–517

Lee S, Kim SA, Lee J et al (2010b) Zinc deficiency-inducible *OsZIP8* encodes a plasma membrane-localized zinc transporter in rice. Mol Cells 29:551–558

Leng Y, Xue D, Yang Y et al (2014) Mapping of QTLs for eating and cooking quality-related traits in rice (*Oryza sativa* L.). Euphytica 197:99–108

Li N, Li Y (2016) Signaling pathways of seed size control in plants. Curr Opin Plant Biol 33:23–32

Li Y, Fan C, Xing Y et al (2011) Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nat Genet 43:1266–1269

Li D, Wang L, Wang M et al (2009) Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield. Plant Biotechnol J 7:791–806

Li S, Gao F, Xie K et al (2016) The OsmiR396c-OsGRF4-OsGIF1 regulatory module determines grain size and yield in rice. Plant Biotechnol J 14:2134–2146

Li Q, Huang L, Chu R et al (2018a) Down-regulation of *SSSII-2* gene expression results in novel low-amylose rice with soft, transparent grains. J Agric Food Chem 66:9750–9760

Li Q, Lu J, Yu J et al (2018b) The brassinosteroid-regulated transcription factors BZR1/BES1 function as a coordinator in multisignal-regulated plant growth. Biochim Biophys Acta Gene Regul Mech 1861:561–571

Li N, Xu R, Li Y (2019) Molecular networks of seed size control in plants. Annu Rev Plant Biol 70:435–463

Li DQ, Wu XB, Wang HF et al (2021) Defective mitochondrial function by mutation in THICK ALEURONE 1 encoding a mitochondrion-targeted single-stranded DNA-binding protein leads to increased aleurone cell layers and improved nutrition in rice. Mol Plant 14:1343–1361

Liu L, Ma X, Liu S et al (2009) Identification and characterization of a novel *Waxy* allele from a Yunnan rice landrace. Plant Mol Biol 71:609–626

Liu HL, Yin ZJ, Xiao L et al (2012) Identification and evaluation of ω-3 fatty acid desaturase genes for hyperfortifying α-linolenic acid in transgenic rice seed. J Exp Bot 63:3279–3287

Liu L, Tong H, Xiao Y et al (2015a) Activation of *Big Grain1* significantly improves grain size by regulating auxin transport in rice. Proc Natl Acad Sci USA 112:11102–11107

Liu S, Hua L, Dong S et al (2015b) *OsMAPK6*, a mitogen-activated protein kinase, influences rice grain size and biomass production. Plant J 84:672–681

Liu J, Chen J, Zheng X et al (2017) *GW5* acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nat Plants 3:17043

Liu J, Wu X, Yao X et al (2018a) Mutations in the DNA demethylase OsROS1 result in a thickened aleurone and improved nutritional value in rice grains. Proc Natl Acad Sci USA 115:11327–11332

Liu Q, Han R, Wu K et al (2018b) G-protein βγ subunits determine grain size through interaction with MADS-domain transcription factors in rice. Nat Commun 9:852

Liu J, Li M, Zhang Q et al (2020) Exploring the molecular basis of heterosis for plant breeding. J Integr Plant Biol 62:287–298

Liu Z, Mei E, Tian X et al (2021) OsMKKK70 regulates grain size and leaf angle in rice through the OsMKK4-OsMAPK6-OsWRKY53 signaling pathway. J Integr Plant Biol 63:2043–2057

Long Q, Zhang W, Wang P et al (2013) Molecular genetic characterization of rice seed lipoxygenase 3 and assessment of its effects on seed longevity. J Plant Biol 56:232–242

Long W, Wang Y, Zhu S et al (2018) FLOURY SHRUNKEN ENDOSPERM1 connects phospholipid metabolism and amyloplast development in rice. Plant Physiol 177:698–712

Luo J, Liu H, Zhou T et al (2013) *An-1* encodes a basic helix-loop-helix protein that regulates awn development, grain size, and grain number in rice. Plant Cell 25:3360–3376

Lyu J, Wang D, Duan P et al (2020) Control of grain size and weight by the GSK2-LARGE1/OML4 pathway in rice. Plant Cell 32:1905–1918

Mao H, Sun S, Yao J et al (2010) Linking differential domain functions of the *GS3* protein to natural variation of grain size in rice. Proc Natl Acad Sci U S A 107:19579–19584

Mbanjo EGN, Kretzschmar T, Jones H et al (2020) The genetic basis and nutritional benefits of pigmented rice grain. Front Genet 11:229

Miao J, Yang Z, Zhang D et al (2019) Mutation of *RGG2*, which encodes a type B heterotrimeric G protein γ subunit, increases grain size and yield production in rice. Plant Biotechnol J 17:650–664

Miao C, Wang D, He R et al (2020) Mutations in MIR396e and MIR396f increase grain size and modulate shoot architecture in rice. Plant Biotechnol J 18:491–501

Mikami I, Uwatoko N, Ikeda Y et al (2008) Allelic diversification at the *wx* locus in landraces of Asian rice. Theor Appl Genet 116:979–989

Miura S, Koyama N, Crofts N et al (2021) Generation and starch characterization of non-transgenic BEI and BEIIb double mutant rice (Oryza sativa) with ultra-high level of resistant starch. Rice 14:3

Mori M, Nomura T, Ooka H et al (2002) Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis. Plant Physiol 130:1152–1161

Morrison W (1988) Lipids in cereal starches: a review. J Cereal Sci 8:1–15

Nagasawa N, Hibara K, Heppard EP et al (2013) GIANT EMBRYO encodes CYP78A13, required for proper size balance between embryo and endosperm in rice. Plant J 75:592–605

Nakanishi H, Ogawa I, Ishimaru Y et al (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe2+ transporters OsIRT1 and OsIRT2 in rice. Soil Sci Plant Nutr 52:464–469

Nayar S, Sharma R, Tyagi AK et al (2013) Functional delineation of rice *MADS29* reveals its role in embryo and endosperm development by affecting hormone homeostasis. J Exp Bot 64:4239–4253

Nozoye T, von Wirén N, Sato Y et al (2019) Characterization of the nicotianamine exporter ENA1 in rice. Front Plant Sci 10:502

Oikawa T, Maeda H, Oguchi T et al (2015) The birth of a black rice gene and its local spread by introgression. Plant Cell 27:2401–2414

Paine JA, Shipton CA, Chaggar S et al (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Paul S, Ali N, Gayen D et al (2012) Molecular breeding of *Osfer 2* gene to increase iron nutrition in rice grain. GM Crops Food 3:310–316

Peng C, Wang Y, Liu F et al (2014) FLOURY ENDOSPERM6 encodes a CBM48 domain-containing protein involved in compound granule formation and starch synthesis in rice endosperm. Plant J 77:917–930

Pereira C, Lourenço VM, Menezes R et al (2021) Rice compounds with impact on diabetes control. Foods 10:1992

Pfister B, Zeeman SC (2016) Formation of starch in plant cells. Cell Mol Life Sci 73:2781–2807

Pingali PL (2012) Green revolution: impacts, limits, and the path ahead. Proc Natl Acad Sci USA 109:12302–12308

Qi P, Lin Y, Song X et al (2012) The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1;3. Cell Res 22:1666–1680

Qiao J, Jiang H, Lin Y et al (2021) A novel *miR167a-OsARF6-OsAUX3* module regulates grain length and weight in rice. Mol Plant 14:1683–1698

Rahman MM, Lee KE, Lee ES (2013) The genetic constitutions of complementary genes Pp and Pb determine the purple color variation in pericarps with cyanidin-3-O-glucoside depositions in black rice. J Plant Biol 56:24–31

Ren Y, Wang Y, Liu F et al (2014) GLUTELIN PRECURSOR ACCUMULATION3 encodes a regulator of post-Golgi vesicular traffic essential for vacuolar protein sorting in rice endosperm. Plant Cell 26:410–425

Ren Y, Wang Y, Pan T et al (2020) *GPA5* encodes a Rab5a effector required for post-golgi trafficking of rice storage proteins. Plant Cell 32:758–777

Ren Y, Huang Z, Jiang H et al (2021) A heat stress responsive NAC transcription factor heterodimer plays key roles in rice grain filling. J Exp Bot 72:2947–2964

Sasaki A, Yamaji N, Mitani-Ueno N et al (2015) A node-localized transporter *OsZIP3* is responsible for the preferential distribution of Zn to developing tissues in rice. Plant J 84:374–384

Segami S, Kono I, Ando T et al (2012) Small and round seed 5 gene encodes alpha-tubulin regulating seed cell elongation in rice. Rice 5:4

Segami S, Takehara K, Yamamoto T et al (2017) Overexpression of *SRS5* improves grain size of brassinosteroid-related dwarf mutants in rice (*Oryza sativa* L.). Breed Sci 67:393–397

Senoura T, Sakashita E, Kobayashi T et al (2017) The iron-chelate transporter *OsYSL9* plays a role in iron distribution in developing rice grains. Plant Mol Biol 95:375–387

Seung D, Smith AM (2019) Starch granule initiation and morphogenesis-progress in Arabidopsis and cereals. J Exp Bot 70:771–784

Shelp BJ, Bozzo GG, Trobacher CP et al (2012) Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. Plant Sci 193–194:130–135

Shi J, Cao Y, Fan X et al (2012) A rice microsomal delta-12 fatty acid desaturase can enhance resistance to cold stress in yeast and *Oryza sativa*. Mol Breed 29:743–757

Shi C, Ren Y, Liu L et al (2019) Ubiquitin Specific Protease 15 has an important role in regulating grain width and size in rice. Plant Physiol 180:381–391

Shi C, Dong N, Guo T et al (2020) A quantitative trait locus *GW6* controls rice grain size and yield through the gibberellin pathway. Plant J 103:1174–1188

Shimajiri Y, Oonishi T, Ozaki K et al (2013) Genetic manipulation of the γ-aminobutyric acid (GABA) shunt in rice: overexpression of truncated glutamate decarboxylase (GAD2) and knockdown of γ-aminobutyric acid transaminase (GABA-T) lead to sustained and high levels of GABA accumulation in rice kernels. Plant Biotechnol J 11:594–604

Shomura A, Izawa T, Ebana K et al (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40:1023–1028

Si L, Chen J, Huang X et al (2016) *OsSPL13* controls grain size in cultivated rice. Nat Genet 48:447–456

Song X, Huang W, Shi M et al (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Song XJ, Kuroha T, Ayano M et al (2015) Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. Proc Natl Acad Sci USA 112:76–81

Stein RJ, Ricachenevsky FK, Fett JP (2009) Differential regulation of the two rice ferritin genes (OsFER1 and OsFER2). Plant Sci 177:563–569

Sun L, Li X, Fu Y et al (2013) *GS6*, a member of the GRAS gene family, negatively regulates grain size in rice. J Integr Plant Biol 55:938–934

Sun X, Ling S, Lu Z et al (2014) *OsNF-YB1*, a rice endosperm-specific gene, is essential for cell proliferation in endosperm development. Gene 551:214–221

Sun P, Zhang W, Wang Y et al (2016) *OsGRF4* controls grain shape, panicle length and seed shattering in rice. J Integr Plant Biol 58:836–847

Sun S, Wang L, Mao H et al (2018a) A G-protein pathway determines grain size in rice. Nat Commun 9:851

Sun X, Zhang Z, Chen C et al (2018b) The C-S-A gene system regulates hull pigmentation and reveals evolution of anthocyanin biosynthesis pathway in rice. J Exp Bot 69:1485–1498

Sunohara H, Kawai T, Shimizu-Sato S et al (2009) A dominant mutation of TWISTED DWARF 1 encoding an alpha-tubulin protein causes severe dwarfism and right helical growth in rice. Genes Genet Syst 84:209–218

Swamy BPM, Marathi B, Ribeiro-Barros AIF et al (2021) Iron biofortification in rice: an update on quantitative trait loci and candidate genes. Front Plant Sci 12:647341

Sweeney MT, Thomson MJ, Pfeil BE et al (2006) Caught red-handed: *Rc* encodes a basic helix-loop-helix protein conditioning red pericarp in rice. Plant Cell 18:283–294

Tanabe S, Ashikari M, Fujioka S et al (2005) A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, dwarf11, with reduced seed length. Plant Cell 17:776–790

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733–749

Tao Y, Miao J, Wang J et al (2020) RGG1, involved in the cytokinin regulatory pathway, controls grain size in rice. Rice 13:76

Tian X, Li X, Zhou W et al (2017) Transcription factor *OsWRKY53* positively regulates Brassinosteroid signaling and plant architecture. Plant Physiol 175:1337–1349

Tian L, Chou HL, Zhang L et al (2018) RNA-Binding Protein RBP-P is required for glutelin and prolamine mRNA localization in rice endosperm cells. Plant Cell 30:2529–2552

Tian L, Chou HL, Zhang L et al (2019a) Targeted endoplasmic reticulum localization of storage protein mRNAs requires the RNA-Binding Protein RBP-L. Plant Physiol 179:1111–1131

Tian YS, Wang B, Peng RH et al (2019b) Enhancing carotenoid biosynthesis in rice endosperm by metabolic engineering. Plant Biotechnol J 17:849–851

Tian L, Doroshenk KA, Zhang L et al (2020) Zipcode RNA-Binding Proteins and membrane trafficking proteins cooperate to transport glutelin mRNAs in rice endosperm. Plant Cell 32:2566–2581

Tian X, He M, Mei E et al (2021) *WRKY53* integrates classic brassinosteroid sign-aling and the mitogen-activated protein kinase pathway to regulate rice architecture and seed size. Plant Cell 33:2753–2775

Tong H, Jin Y, Liu W et al (2009) DWARF AND LOW-TILLERING, a new member of the GRAS family, plays positive roles in brassinosteroid signaling in rice. Plant J 58:803–816

Tong H, Liu L, Jin Y et al (2012) DWARF AND LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-like kinase to mediate brassi-nosteroid responses in rice. Plant Cell 24:2562–2577

Umemoto T, Yano M, Satoh H et al (2002) Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor Appl Genet 104:1–8

Utsunomiya Y, Samejima C, Takayanagi Y et al (2011) Suppression of the rice heterotrimeric G protein β-subunit gene, *RGB1*, causes dwarfism and browning of internodes and lamina joint regions. Plant J 67:907–916

Wanchana S, Toojinda T, Tragoonrung S et al (2003) Duplicated coding sequence in the *waxy* allele of tropical glutinous rice (*Oryza sativa* L.). Plant Sci 165:1193–1199

Wang C, Shu Q (2007) Fine mapping and candidate gene analysis of purple pericarp gene *Pb* in rice (*Oryza sativa* L.). Chin Sci Bull 52:3097–3104

Wang C, Washida H, Crofts AJ et al (2008) The cytoplasmic-localized, cytoskeletal-associated RNA binding protein OsTudor-SN: evidence for an essential role in storage protein RNA transport and localization. Plant J 55:443–454

Wang ZY, Zheng FQ, Shen GZ et al (2010) The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. Plant J 7:613–622

Wang S, Wu K, Yuan Q et al (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet 44:950–954

Wang JC, Xu H, Zhu Y et al (2013) *OsbZIP58*, a basic leucine zipper transcrip-tion factor, regulates starch biosynthesis in rice endosperm. J Exp Bot 64:3453–3466

Wang S, Li S, Liu Q et al (2015a) The OsSPL16-GW7 regulatory module deter-mines grain shape and simultaneously improves rice yield and grain quality. Nat Genet 47:949–954

Wang X, Zhou W, Lu Z et al (2015b) A lipid transfer protein, *OsLTPL36*, is essential for seed development and seed quality in rice. Plant Sci 239:200–208

Wang Y, Xiong G, Hu J et al (2015c) Copy number variation at the GL7 locus contributes to grain size diversity in rice. Nat Genet 47:944–948

Wang A, Hou Q, Si L et al (2019) The PLATZ transcription factor GL6 affects grain length and number in rice. Plant Physiol 180:2077–2090

Wang J, Chen Z, Zhang Q et al (2020a) The NAC transcription factors OsNAC20 and OsNAC26 regulate starch and storage protein synthesis. Plant Physiol 184:1775–1791

Wang S, Yang Y, Guo M et al (2020b) Targeted mutagenesis of amino acid transporter genes for rice quality improvement using the CRISPR/Cas9 system. Crop J 3:457–464

Wang L, Wang D, Yang Z et al (2021) Roles of FERONIA-like receptor genes in regulating grain size and quality in rice. Sci China Life Sci 64:294–310

Weng J, Gu S, Wan X et al (2008) Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. Cell Res 18:1199–1209

Wong HW, Liu Q, Sun SS (2015) Biofortification of rice with lysine using endog-enous histones. Plant Mol Biol 87:235–248

Wu CY, Adach T, Hatano T et al (1998) Promoters of rice seed storage protein genes direct endosperm-specific gene expression in transgenic rice. Plant Cell Physiol 39:885–889

Wu X, Liu J, Li D et al (2016a) Rice caryopsis development II: Dynamic changes in the endosperm. J Integr Plant Biol 58:786–798

Wu Y, Fu Y, Zhao S et al (2016b) CLUSTERED PRIMARY BRANCH 1, a new allele of *DWARF11*, controls panicle architecture and seed size in rice. Plant Biotechnol J 14:377–386

Wu W, Liu X, Wang M et al (2017) A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domes-tication. Nat Plants 3:17064

Wu M, Ren Y, Cai M et al (2019) Rice FLOURY ENDOSPERM10 encodes a pen-tatricopeptide repeat protein that is essential for the trans-splicing of mitochondrial nad1 intron 1 and endosperm development. New Phytol 223:736–750

Wu K, Wang S, Song W et al (2020) Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. Science 367:eaaz2046

Xia D, Zhou H, Liu R et al (2018) *GL3.3*, a novel QTL encoding a GSK3/SHAGGY-like Kinase, epistatically interacts with *GS3* to produce extra-long grains in rice. Mol Plant 11:754–756

Xiao Y, Liu D, Zhang G et al (2017) Brassinosteroids Regulate OFP*1*, a DLT inter-acting protein, to modulate plant architecture and grain morphology in rice. Front Plant Sci 8:1698

Xiao Y, Liu D, Zhang G et al (2019) *Big Grain3*, encoding a purine permease, regulates grain size via modulating cytokinin transport in rice. J Integr Plant Biol 61:581–597

Xiao Y, Zhang G, Liu D et al (2020) GSK2 stabilizes OFP3 to suppress brassinos-teroid responses in rice. Plant J 102:1187–1201

Xiao N, Pan C, Li Y et al (2021) Genomic insight into balancing high yield, good quality, and blast resistance of japonica rice. Genome Biol 22:283

Xie K, Wu C, Xiong L (2006) Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. Plant Physiol 142:280–293

Xiong Y, Ren Y, Li W et al (2019) *NF-YC12* is a key multi-functional regula-tor of accumulation of seed storage substances in rice. J Exp Bot 70:3765–3780

Xu C, Liu Y, Li Y et al (2015) Differential expression of *GS5* regulates grain size in rice. J Exp Bot 66:2611–2623

Xu R, Duan P, Yu H et al (2018) Control of grain size and weight by the OsMKKK10-OsMKK4-OsMAPK6 signaling pathway in rice. Mol Plant 11:860–873

Xu R, Li N, Li Y (2019) Control of grain size by G protein signaling in rice. J Integr Plant Biol 61:533–540

Xu Z, Yu M, Yin Y et al (2020) Generation of selectable marker-free soft trans-genic rice with transparent kernels by downregulation of SSSII-2. Crop J 8:53–61

Xu X, E Z, Zhang D et al (2021) OsYUC11-mediated auxin biosynthesis is essen-tial for endosperm development of rice. Plant Physiol 185:934–950

Xue M, Liu L, Yu Y et al (2019) Lose-of-function of a rice nucleolus-localized pentatricopeptide repeat protein is responsible for the floury endosperm14 mutant phenotypes. Rice 12:100

Yamakawa H, Hirose T, Kuroda M et al (2007) Comprehensive expression profil-ing of rice grain filling-related genes under high temperature using DNA microarray. Plant Physiol 144:258–277

Yamamoto MP, Onodera Y, Touno SM et al (2006) Synergism between RPBF Dof and RISBZ1 bZIP activators in the regulation of rice seed expression genes. Plant Physiol 141:1694–1707

Yamamuro C, Ihara Y, Wu X et al (2000) Loss of function of a rice brassinoster-oid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell 12:1591–1606

Yan Y, Wei M, Li Y et al (2021) MiR529a controls plant height, tiller number, panicle architecture and grain size by regulating SPL target genes in rice (*Oryza sativa* L.). Plant Sci 302:110728

Yang C, Shen W, He Y et al (2016a) OVATE family protein 8 positively mediates brassinosteroid signaling through interacting with the GSK3-like kinase in rice. PLoS Genet 12:e1006118

Yang QQ, Zhang CQ, Chan ML et al (2016b) Biofortification of rice with the essential amino acid lysine: molecular characterization, nutritional evaluation, and field performance. J Exp Bot 67:4285–4296

Yang C, Ma Y, He Y et al (2018a) *OsOFP19* modulates plant architecture by integrating the cell division pattern and brassinosteroid signaling. Plant J 93:489–501

Yang QQ, Zhao DS, Zhang CQ et al (2018b) A connection between lysine and serotonin metabolism in rice endosperm. Plant Physiol 176:1965–1980

Yang C, Guo M, Sun S et al (2019) Natural variation of *OsGluA2* is involved in grain protein content regulation in rice. Nat Commun 10:1949

Yang Q, Zhao D, Liu Q (2020) Connections between amino acid metabolisms in plants: lysine as an example. Front Plant Sci 11:928

Yang QQ, Yu WH, Wu HY et al (2021a) Lysine biofortification in rice by modulating feedback inhibition of aspartate kinase and dihydrodipicolinate synthase. Plant Biotechnol J 19:490–501

Yang W, Wu K, Wang B et al (2021b) The RING E3 ligase CLG1 targets GS3 for degradation via the endosome pathway to determine grain size in rice. Mol Plant 14:1699–1713

Yang X, Wang J, Xia X et al (2021c) *OsTTG1*, a WD40 repeat gene, regulates anthocyanin biosynthesis in rice. Plant J 107:198–214

Ye X, Al-Babili S, Klöti A et al (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Yin LL, Xue HW (2012) The *MADS29* transcription factor regulates the degradation of the nucellus and the nucellar projection during rice seed development. Plant Cell 24:1049–1065

Yin W, Xiao Y, Niu M et al (2020) ARGONAUTE2 enhances grain length and salt tolerance by activating BIG GRAIN3 to modulate cytokinin distribution in rice. Plant Cell 32:2292–2306

Ying J, Ma M, Bai C et al (2018) *TGW3*, a major QTL that negatively modulates grain length and weight in rice. Mol Plant 11:750–753

Yu S, Xu C, Tan Y et al (2000) Analyzing quantitative trait loci for yield using a vegetatively replicated F 2 population from a cross between the parents of an elite rice hybrid. TAG Theor Appl Genet 101:248–254

Yu J, Miao J, Zhang Z et al (2018) Alternative splicing of *OsLG3b* controls grain length and yield in japonica rice. Plant Biotechnol J 16:1667–1678

Yu M, Wu M, Ren Y et al (2021) Rice FLOURY ENDOSPERM 18 encodes a pentatricopeptide repeat protein required for 5′ processing of mitochondrial nad5 messenger RNA and endosperm development. J Integr Plant Biol 63:834–847

Zemach A, Kim MY, Silva P et al (2010) Local DNA hypomethylation activates genes in rice endosperm. Proc Natl Acad Sci U S A 107:18729–18734

Zeng D, Liu T, Ma X et al (2020) Quantitative regulation of *Waxy* expression by CRISPR/Cas9-based promoter and 5′UTR-intron editing improves grain quality in rice. Plant Biotechnol J 18:2385–2387

Zhan P, Wei X, Xiao Z et al (2021) GW10, a member of P450 subfamily regulates grain size and grain number in rice. Theor Appl Genet 134:3941–3950

Zhang J, Nallamilli BR, Mujahid H et al (2010) *OsMADS6* plays an essential role in endosperm nutrient accumulation and is subject to epigenetic regulation in rice (*Oryza sativa*). Plant J 64:604–617

Zhang X, Wang J, Huang J et al (2012a) Rare allele of *OsPPKL1* associated with grain length causes extra-large grain and a significant yield increase in rice. Proc Natl Acad Sci USA 109:21534–21539

Zhang Y, Xu YH, Yi HY et al (2012b) Vacuolar membrane transporters *OsVIT1* and *OsVIT2* modulate iron translocation between flag leaves and seeds in rice. Plant J 72:400–410

Zhang YC, Yu Y, Wang CY et al (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. Nat Biotechnol 31:848–852

Zhang C, Zhou L, Zhu Z et al (2016) Characterization of grain quality and starch fine structure of two japonica rice (*Oryza Sativa*) cultivars with good sensory properties at different temperatures during the filling stage. J Agric Food Chem 64:4048–4057

Zhang C, Chen S, Ren X et al (2017) Molecular structure and physicochemical properties of starches from rice with different amylose contents resulting from modification of OsGBSSI activity. J Agric Food Chem 65:2222–2232

Zhang H, Xu H, Feng M et al (2018a) Suppression of OsMADS7 in rice endosperm stabilizes amylose content under high temperature stress. Plant Biotechnol J 16:18–26

Zhang M, Su J, Zhang Y et al (2018b) Conveying endogenous and exogenous signals: MAPK cascades in plant growth and defense. Curr Opin Plant Biol 45:1–10

Zhang C, Zhu J, Chen S et al (2019a) *Wx^{lv}*, the ancestral allele of rice *Waxy* Gene. Mol Plant 12:1157–1166

Zhang D, Zhang M, Zhou Y et al (2019b) The rice G protein γ subunit *DEP1/qPE9-1* positively regulates grain-filling process by increasing auxin and cytokinin content in rice grains. Rice 12:91

Zhang C, Yang Y, Chen Z et al (2020) Characteristics of grain physicochemical properties and the starch structure in rice carrying a mutated ALK/SSIIa gene. J Agric Food Chem 68:13950–13959

Zhang C, Yang Y, Chen S et al (2021a) A rare *Waxy* allele coordinately improves rice eating and cooking quality and grain transparency. J Integr Plant Biol 63:889–901

Zhang D, Zhang M, Liang J (2021b) RGB1 regulates grain development and starch accumulation through its effect on OsYUC11-mediated auxin biosynthesis in rice endosperm cells. Front Plant Sci 12:585174

Zhang L, Wang R, Xing Y et al (2021c) Separable regulation of *POW1* in grain size and leaf angle development in rice. Plant Biotechnol J 19:2517–2531

Zhao GC, Xie MX, Wang YC et al (2017) Molecular mechanisms underlying γ-aminobutyric acid (GABA) accumulation in giant embryo rice seeds. J Agric Food Chem 65:4883–4889

Zhao D, Li Q, Zhang C et al (2018) *GS9* acts as a transcriptional activator to regulate rice grain shape and appearance quality. Nat Commun 9:1240

Zhao YF, Peng T, Sun HZ et al (2019) miR1432-OsACOT (Acyl-CoA thioesterase) module determines grain yield via enhancing grain filling rate in rice. Plant Biotechnol J 17:712–723

Zheng Y, Wang Z (2014) Protein accumulation in aleurone cells, sub-aleurone cells and the center starch endosperm of cereals. Plant Cell Rep 33:1607–1615

Zhong J, He W, Peng Z et al (2020) A putative AGO protein, *OsAGO17*, positively regulates grain size and grain weight through OsmiR397b in rice. Plant Biotechnol J 18:916–928

Zhou S, Xue H (2020) The rice PLATZ protein *SHORT GRAIN6* determines grain size by regulating spikelet hull cell division. J Integr Plant Biol 62:847–864

Zhou Y, Zhu J, Li Z et al (2009) Deletion in a quantitative trait gene *qPE9-1* associated with panicle erectness improves plant architecture during rice domestication. Genetics 183:315–324

Zhou G, Ren N, Qi J et al (2014) The 9-lipoxygenase Osr9-LOX1 interacts with the 13-lipoxygenase-mediated pathway to regulate resistance to chewing and piercing-sucking herbivores in rice. Physiol Plant 152:59–69

Zhou H, Wang L, Liu G et al (2016) Critical roles of soluble starch synthase SSIIIa and granule-bound starch synthase Waxy in synthesizing resistant starch in rice. Proc Natl Acad Sci USA 113:12844–12849

Zhou H, Li P, Xie W et al (2017a) Genome-wide association analyses reveal the genetic basis of stigma exsertion in rice. Mol Plant 10:634–644

Zhou W, Wang X, Zhou D et al (2017b) Overexpression of the 16-kDa α-amylase/trypsin inhibitor RAG2 improves grain yield and quality of rice. Plant Biotechnol J 15:568–580

Zhou Y, Tao Y, Zhu J et al (2017c) GNS4, a novel allele of DWARF11, regulates grain number and grain size in a high-yield rice variety. Rice 10:34

Zhou H, Xia D, Zhao D et al (2021a) The origin of *Wx^{la}* provides new insights into the improvement of grain quality in rice. J Integr Plant Biol 63:878–888

Zhou S, Li Q, Liu Q et al (2021b) DNA demethylases remodel DNA methylation in rice gametes and zygote and are required for reproduction. Mol Plant 14:1569–1583

Zhu L, Gu M, Meng X et al (2012) High-amylose rice improves indices of animal health in normal and diabetic rats. Plant Biotechnol J 10:353–362

Zhu X, Liang W, Cui X et al (2015) Brassinosteroids promote development of rice pollen grains and seeds by triggering expression of Carbon Starved Anther, a MYB domain protein. Plant J 82:570–581

Zhu Q, Zeng D, Yu S et al (2018) From golden rice to aSTARice: Bioengineering astaxanthin biosynthesis in rice endosperm. Mol Plant 11:1440–1448

Zhu Y, Lin Y, Chen S et al (2019) CRISPR/Cas9-mediated functional recovery of the recessive rc allele to develop red rice. Plant Biotechnol J 17:2096–2105

Zuo J, Li J (2014) Molecular genetic dissection of quantitative trait loci regulating rice grain size. Annu Rev Genet 48:99–118

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