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Research article

Regulatory mechanism and molecular genetic dissection of rice (*Oryza sativa* L.) grain size

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ABSTRACT

With the sharp increase of the global population, adequate food supply is a great challenge. Grain size is an essential determinant of rice yield and quality. It is a typical quantitative trait controlled by multiple genes. In this paper, we summarized the quantitative trait loci (QTL) that have been molecularly characterized and provided a comprehensive summary of the regulation mechanism and genetic pathways of rice grain size. These pathways include the ubiquitin-proteasome system, G-protein, mitogen-activated protein kinase, phytohormone, transcriptional factors, abiotic stress. In addition, we discuss the possible application of advanced molecular biology methods and reasonable breeding strategies, and prospective on the development of high-yielding and high-quality rice varieties using molecular biology techniques.

1. Introduction

Rice (*Oryza sativa* L.) is the main food crop for more than 50 % of the global population. It is estimated that by 2030, rice production needs to increase by 40 % to meet the expected requirements of the growing world population. Therefore, increasing rice production plays a critical role in ensuring world food security. There are three crucial factors of rice yield: the number of panicles per plant, the number of grains per panicle, and grain weight. The grain size of rice includes four dimensions: grain length, grain width, grain weight, and length-width ratio, which positively correlate with grain weight [1]. As a critical breeding target, the grain size of rice directly affects the yield and quality of rice [2]. Seed size is determined by the integrated signals of maternal and zygotic tissues, controls the coordinated growth of the embryo, endosperm, and seed coat. Therefore, the grain size depends on the maternal genotype [3].

In the last few decades, scientists have detected a large amount of rice grain size QTL, and cloned many rice grain size genes. These findings are valuable for illustrating the molecular regulation mechanism of rice grain and breeding new high-yield and high-quality rice varieties. In this review, we summarize recent advances in molecular identification of important QTL for rice grain size and their molecular mechanisms, conclude their signaling pathways, regulatory mechanisms, and genetic relationships in a molecular background, provide insights into the opportunities and challenges faced by breeders in the post-genomic era.

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2. QTL mapping for grain size and weight of rice

Grain size mainly composed by grain size, grain length, grain width, grain thickness, aspect ratio and grain weight, is a complex quantitative trait. Up to now, more than 400 QTL controlling grain size and weight have been identified by using various mapping populations [4,5]. Among of these QTL, there are 103 QTL related to grain length, 95 QTL related to grain width and relatively few QTL related to grain aspect ratio and grain thickness. These QTL distribute on 12 chromosomes of rice. In this review, we characterize the molecular features of several major QTL/genes for grain size and explore their role in determining the regulatory mechanisms of grain size or weight (Fig. 1). *GL2/GS2* regulating grain length and weight was mapped within the 700 kb region of RM13792 and RM8248, on chromosome two by using BC_3F_2 population derived from the cross between BobaiB (BBB) and RW11. *GL2*, an allele of *OsGRF4*, can potentially increase grain weight and grain yield. And it interacts with OsGSK2, a negative regulator of oleuropein lactones, which increased the expression of GRF4 and promoted seed development [6]. *GW2* encodes a previously RING-type protein with E3 ubiquitin ligase activity. Functional deletion of *GW2* increases the number of cells and the rate of filling, negatively regulates rice grain length and width [7].

A major QTL for grain weight and grain length, *GS3*, was mapped on rice chromosome 3 by using a backcross population crossing between Mingchuan 63 (large grain) and Chuan 7 (small grain). The *GS3* locus can explain 80–90% of grain weight and length variation in the BC_3F_2 population. These findings suggest that *GS3* may be a negative regulator that controls grain size growth [53]. *GS3* encodes a transmembrane protein containing a plant-specific organ size regulation (OSR) domain. At the cellular level, *GS3* regulates seed length by controlling cell number in the upper epidermis of the glumes [54]. FAZ1 (small grain indica variety) and WY3 (larger grain indica variety) rice varieties were selected as parents to map a new major QTL, *GL3.1*, which of grain length. *GL3.1* encodes a protein phosphatase kelch (PPKL) family-Ser/Thr phosphatase. *GL3.1-WY3* influences the phosphorylation of proteins in

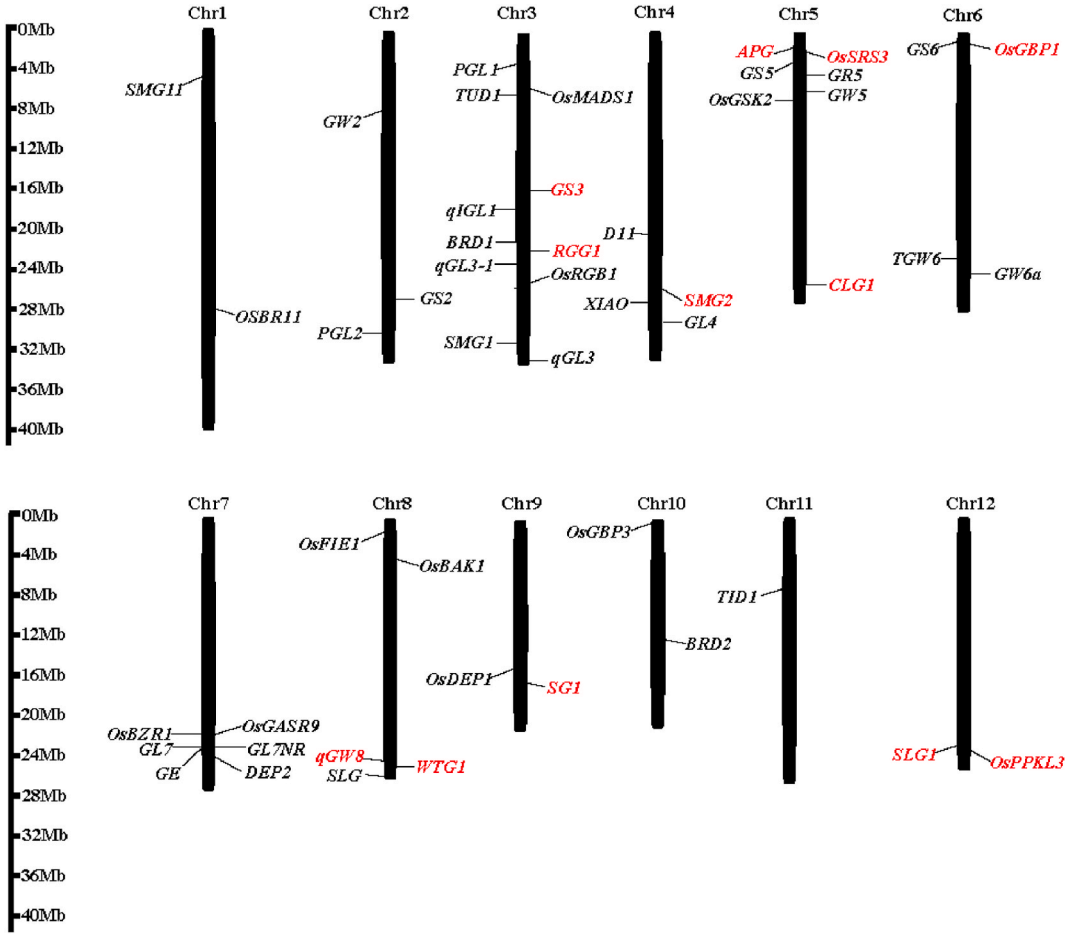

Fig. 1. Genes Associated with Rice Grain Length and Width. The genes associated with rice grain length and width are divided into two groups: positive regulators (black) and negative regulators (red). The positive regulators including: *OsBRI1* [8], *PGL2* [9], *qGL3-1* [10], *PGL1*, *GW6a* [11], *TGW6* [12], *DEP2* [13], *TID1* [14], *GW2* [15], *GS2* [16], *GSD1* [17], *GS5* [18], *GR5* [19] *GW5* [20], *APG* [21], *GS6* [22], *SMG11* [23], *SMG1* [24], *qIGL1* [25], *qGL3* [26], *BRD1* [27], *OsRGB1* [28], *OsMADS1* [29], *TUD1* [30], *XIAO* [31], *D11* [32], *OsGSK2* [33], *GL7* [34], *GL7NR* [35], *OsGASR9* [36], *GE* [37], *OsBZR1* [32], *SLG* [38], *OsBAK1* [39], *OsFIE1* [40], *OsDEP1* [41], *Brd 2* [42], *OsGBP3* [43]; The negative regulators including: *GS3* [44], *RGG1* [45], *SMG2* [46], *OsSRS3* [47], *CLG1* [2], *OsGBP1* [43], *qGW8* [48], *WTG1* [49], *SG1* [50], *SLG1* [51], *OsPPKL3* [52].

spikelets and accelerates the cell division rate, increasing grain length and crop yield [55]. GWAS detects Ninety-nine QTL for grain length. *OsLG3*, has been identified, encoding an APETALA2/ethylene-responsive element binding protein, which can regulate grain length positively and improve rice yield without influencing grain quality [56]. *qTGW3/TGW3/GL3.3*, is also located on chromosome 3 and encodes an Osk41/OsGSK5 protein kinase involved in phosphorylating the auxin inhibitor factor *OsARF4*, negatively regulating grain length. Expression analysis showed that *qTGW3* was primarily expressed in young rice spikelet and regulated rice grain length negatively by interacting with and phosphorylating *OsARF4*, a transcriptional repressor in the growth factor pathway, and inhibiting the expression of genes related to the growth factor signaling pathway during grain development [57,58].

A major grain length QTL, *GL4* controlling rice grain length was mapped on the region between RM3335 and RM5608 on chromosome 4 by using An F_2 population containing 186 lines crossing between the progressive line GIL25 with and the African cultivar IRGC102305. It was shown that single nucleotide polymorphism (SNP) mutations in the *GL4* results in the early stop termination of codons in the premature coding region, which results in small grain and seed shattering in African wild rice [59].

GW5, a major QTL controlling grain width and grain weight in rice, codes a novel nuclear protein, and plays a role in the ubiquitin-proteasome pathway, regulates cell division during seed growth [60]. *GW5* protein is localized at the plasma membrane. and inhibits the activity of the GSK2 kinase, resulting in the accumulation of unphosphorylated OsBZR1 and DLT proteins in the nucleus, which mediates the expression of brassinosteroid (BR) responsive genes and the growth responses, thereby negatively regulates the grain width of rice seeds [20]. *qGL5/OsAUX3*,a major gene negatively regulating grain length and weight were identified by using a recombinant inbred line population crossing between 9311 and Nipponbare. The growth factor *OsARF6* can directly bind to the AuxRE motif of the *qGL5/OsAUX3* promoter and positively regulate the expression of *qGL5/OsAUX3* [61].

qTGW6 for 1000-grain weight were analyzed under different environments in backcross inbred lines of Nipponbare and Kasalath. *TGW6* can enhance the accumulation of carbohydrates in plants, thereby increasing rice yield potential [62]. *TGW6* encodes a novel protein with IAA glucose hydrolase activity, affecting the transition from syncytial to cytosolic phase by controlling growth hormone activity and limiting the increase of cell number and length [63]. *GW6a/OsglHAT1*, associated with grain length and grain weight, encoding a novel GNAT-like protein with intrinsic histone acetyltransferase activity (*OsglHAT1*), was mapped on chromosome 6. *GW6a/OsglHAT1* increases grain length and weight by positively regulating cell number and grain filling rate [64].

GLW7 is a major QTL for grain length and grain width, explaining 30% of the variation in grain length and 25% in grain width in Japonica rice varieties. It was verified that *OsSPL13* is the target gene of *GLW7*. *OsSPL13*, a plant-specific transcription factor, positively regulates grain length and weight by modulating rice glume cell size [65]. *qSS7*, a major QTL determining grain length, width, and the ratio of seed length to width, was mapped on the long arm of chromosome 7. It was shown that *qSS7* can regulates grain length by modulating cell elongation in the glume lemma [66]. *GW7* encodes a TONNEAU1-recruiting motif protein. Up-regulation of *GW7*

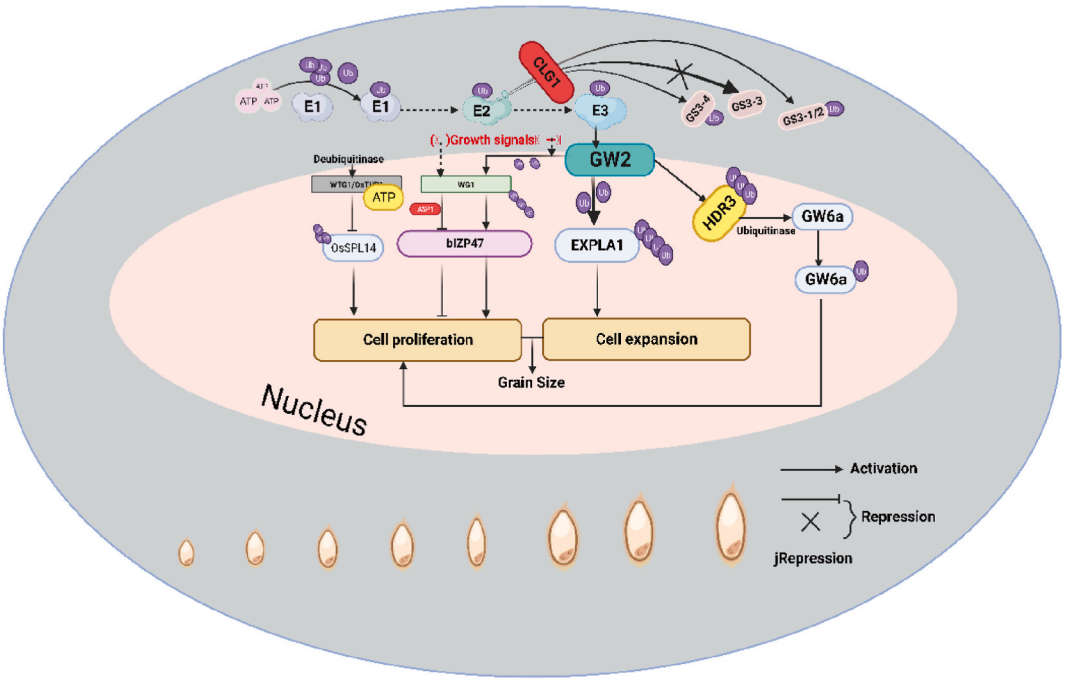

Fig. 2. E3 ubiquitin ligase *GW2* interacts with *EXPLA1*, which functions as a cell relaxation protein and promotes cell expansion. When external growth signals are enhanced, *WG1* binds to the transcriptional repressor *ASP1*, inhibiting the transcriptional activity of *OsbZIP47*, which negatively regulates rice cell proliferation. In addition, in the presence of enhanced external growth signals, *GW2* promotes the ubiquitination of *WG1*, which enhances the transcriptional activation activity of *OsbZIP47* and promotes cell growth. Furthermore, E3 ubiquitin ligase directly interacts with *EXPLA* to regulate cell expansion; *GW6a* ubiquitinates *HDR3*, a ubiquitin-containing receptor involved in seed size regulation; *CLG1* ubiquitinates *GS3-1/2/3*, but *GS3-4* is localized on the cell membrane and promotes grain length by balancing G protein signaling.

expression can promote the longitudinal cell division and reduce the transverse cell division. *OsSPL6* (*GW8*), an SBP domain transcription factor that regulating grain width, directly binds to and represses the expression of the *GW7* promoter [67]. *qLGY3* encodes MADS domain transcription factor *OsMADS1*, a key downstream effector of G protein βγ dimerization. positively regulates grain length by promoting longitudinal cell extension [29].

qGW8, a primary QTL for grain width, was detected in the long arm of chromosome 8 by using 153 single-segment substitution lines (SLS). *GW8*, a homologous gene of *OsSPL16*, positively regulates cell proliferation. High expression of *GW8* promotes cell division and grain filling, which increases grain width in rice [48]. *GS9* encoding a protein with unknown conserved functional domain, negatively regulates rice grain size by altering cell division. *GS9* interacts with the ovate family proteins *OsOFP14* and *OsOFP8*, which is regulated by the *OsGSK2* [68].

3. Regulatory mechanisms of grain size in rice

Rice grain size affects the crop's adaptability to environmental and directly determines the crop's yield. Seed size is majorly determined by Genetic factors, seed growth is coordinately controlled by both maternal tissues and zygotic tissues [3]. Research identified several signaling pathways that control grain size by regulating maternal tissue growth, including ubiquitin-proteasome pathway G protein signaling, MAPK signaling, phytohormone signaling, and transcriptional regulators; these pathways control rice grain size by affecting the development of the grain. Related studies have shown that plant abiotic stress, photosynthetic product accumulation, and grain filling rate can affect rice grain size by influencing rice grain filling. Then, we will discuss recent advances in grain size regulatory pathways in rice.

3.1. Ubiquitin-proteasome pathway

Protein ubiquitination affects many aspects of protein stability, activity, structure, and cellular metabolism. Ubiquitination usually occurs on the lysine (K) side chain of the target protein, a process that involves the coordinated action of E1-ubiquitin activating enzymes, E2-ubiquitin conjugating enzymes, and E3-ubiquitin ligases. The function of the ubiquitinated proteasome pathway in regulating the grain size of rice has received broad interest (Fig. 2).

Arabidopsis thaliana DA1, encoding a predicted ubiquitin receptor, regulating seed size by limiting cell proliferation [69]. A grain width-regulated gene with ubiquitinates activity, *OsUBP15*, was identified by map-based cloning by using the mutant material lg*1-D* and the wild type as parents. *OsUBP15* positively regulates grain width in rice [70]. *GW2* encoding a RING-type protein with E3 ubiquitin ligase activity, negatively regulates grain width and weight in rice. *GW2* can elevate rice yield without compromising seed quality, exhibiting significant potential-for increased productivity [15]. *CLG1*, which encodes an E3 ligase, positively regulates grain size and interacts with *GS3*. *CLG1* ubiquitinates *GS3*, which is subsequently degraded via the endosomal degradation pathway, leading to an increased grain size [71]. It was shown that *GW6a* interacts with *HDR3*, the ubiquitin response factor of *DA1*, regulates grain size

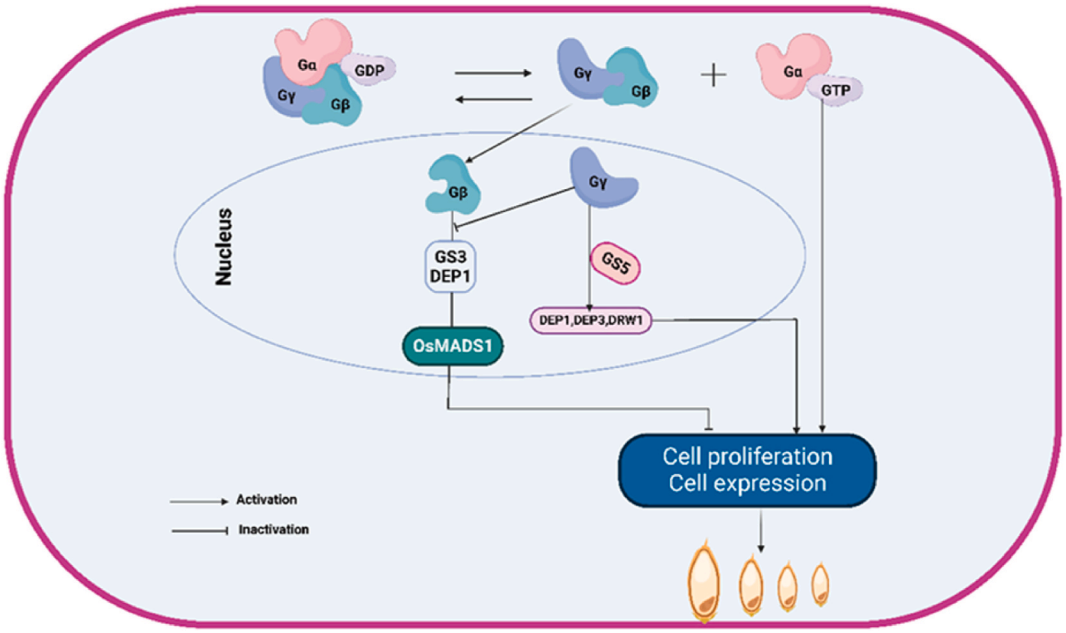

Fig. 3. G protein signaling controls grain size in rice. Gβ (*RGB1*) interacts with *GS3/DEP1* and regulates the expression of the downstream gene *OsMADS1*, which regulates the proliferation and expansion of rice cells to change the rice grain size. Meanwhile, *GS3/DEP1* antagonizes Gγ (GGC2) and competitively binds to Gβ (RGB1) to regulate seed grain size.

by altering cell proliferation in spikelet hulls [15]. Mechanistically, *HDR3* interacts with and stabilizes *GW6a* in an ubiquitin-dependent way, delaying protein degradation by the 26S proteasome, positively regulating the grain size [72]. The 26S proteasome is a multi catalytic proteasome composing of a 20S core protease and a 19S regulatory particle (RP). Studies have shown that a subunit of RP, RPT2, is involved in controlling seed size in Arabidopsis. The Arabidopsis RPT2 subunit is mainly encoded by two homologous genes, *AtRPT2a* and *AtRPT2b*. Lossing of the function of the subunit-regulated granule AAA ATPase (RPT2a) leads to a decrease in 26S proteasome activity and results in enlarged leaves, stems, flowers, fruits, seeds, and embryos [73]. *WTG1* determines grain size and shape mainly by affecting cell expansion. *WTG1* encodes an otubain-like protease with ubiquitination, and the lossing of ubiquitination activity of the mutant protein (*wtg1-1*) results in decreased rice seed size [49]. *GSE5*, encoding a plasma membrane-associated protein, interacts with the rice calmodulin *OsCaM1-1*. *GSE5* negatively regulates grain width and grain weight in rice by affecting cell proliferation in the glumes [74].

3.2. G-protein signaling

Heterotrimeric GTP-binding proteins (G proteins) are signal transduction elements that mediate membrane receptor action in eukaryotic organisms [75]. G proteins are composed of alpha (α), beta (β), and gamma (γ) subunits, usually localized at the cytoplasmic face of the plasma membrane (Fig. 3). G protein promotes GDP release and GTP binding, and plays a crucial role in plant growth and development [76]. In Arabidopsis, three genes encoding Gγ subunits (AGGs): *AGG1*, *AGG2*, and *AGG3* were identified. G protein c-subunit (*AGG3*) regulates plant morphological development in *Arabidopsis thaliana*. The homologs of *AGG3* in rice have been identified as crucial genes for grain size and yield [77].

There are one Gα (*RGA1*), one Gβ (*RGB1*) and five Gγ homologs (*RGG1*, *RGG2*, *GS3*, *qPE9-1/DEP1* and *GGC2*) in the rice. Three Gγ proteins, *DEP1*, *GGC2*, and *GS3,* antagonistically regulate grain size. *DEP1* and *GGC2* complexed with Gβ increase grain length. *GS3* having no effect on grain size by itself but reduces grain length through a competitive interaction with Gβ [78]. *qLGY3* encodes a MADS domain transcription factor, *OsMADS1*, a critical downstream effector of G-protein βγ dimer. The Gγ subunits *GS3* and *DEP1* function in direct interactions with the MADS transcription factors to enhance the transcriptional activity of *OsMADS1* and promote synergistic transactivation of common target genes to regulate grain size and shape [29].

GR5, a transcriptional activator, determines grain size by affecting cell proliferation and expansion. *GR5* physically interacts with five Gγ subunit proteins, including *RGG1*, *RGG2*, *DEP1*, *GS3*, and *GGC2*, and works in downstream of the G-protein complex to regulate the expression of downstream target genes which determine grain size [19]. GTPase-activating proteins are closely related to a family of G-protein-associated receptors regulating G-protein binding and signaling with other proteins. GRAIN SIZE AND WEIGHT 3 (*GSW3*), a GTPase-regulated protein, negatively regulates grain length and width by promoting grain glume cell division and cell growth. Mutations in a crucial SNP in the coding region of *GSW3* result in an amino acid substitution from Gln to Arg at position 161 of *GSW3*,

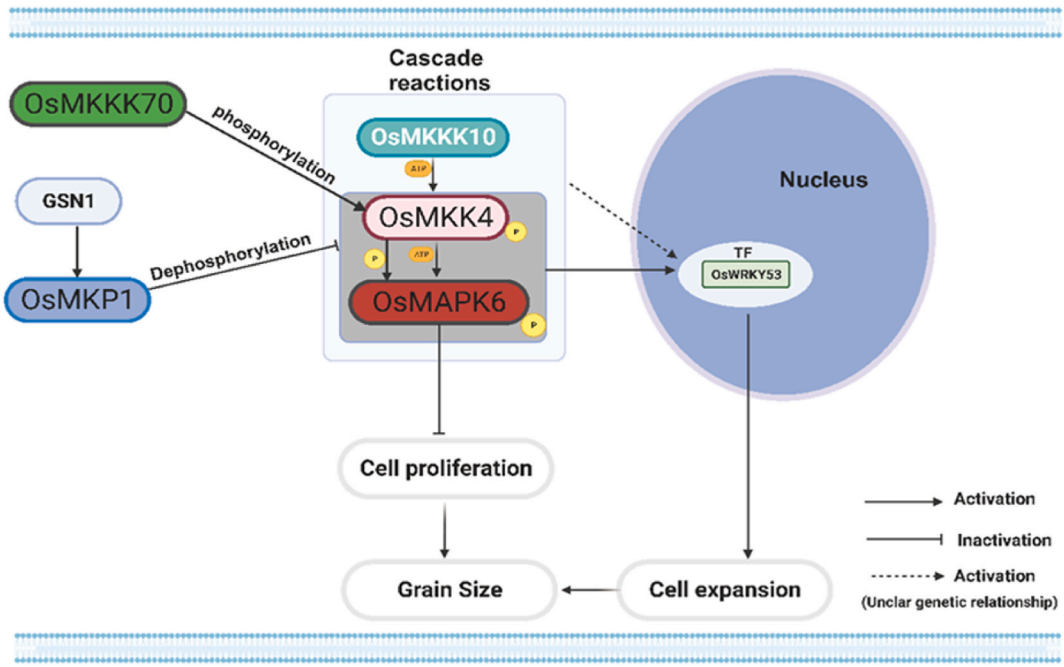

Fig. 4. Mitogen-activated protein kinase signaling pathway regulates the seed size pathway; OsMKKK10, OsMKK4, and OsMAPK6 negatively regulate rice grain length as a response. *OsWRKY53* is a target of the OsMKKK10-OsMKK4-OsMAPK6 module to regulate grain growth by promoting the expansion of cells in the husk of the spikelet. OsMKKK70, which interacts with OsMKK4, promotes the phosphorylation of OsMAPK6, which positively regulates grain size in rice.

which reduced the grain size [79].

3.3. Mitogen-activated protein kinase (MAPK) signaling

Mitogen-activated protein kinase (MAPK) cascade is a generic signal transduction module in eukaryotes [80]. Each MAPK cascade consists of a set of three sequentially acting protein kinases: MAPK, MAPK kinase (MKK), and kinase of MAPK kinase (MKKK) [81]. OsMKK10-OsMKK4-OsMPK6 participate in rice panicle morphogenesis and act in a common pathway. *GSN1* encodes OsMKP1, a mitogen-activated protein kinase phosphatase. As a negative regulator of the OsMKKK10-OsMKK4-OsMPK6, the GSN1-MAPK module coordinates the balance between grain number and grain size by integrating localized cell differentiation and proliferation [46] (Fig. 4). OsMKK4, the upstream kinase of OsMPK1, phosphorylates OsMPK1 through wounding in vivo. OsMPK1 directly interacts with the rice defense-related transcription factor OsWRKY53. The injury signaling pathway, OsMKK4-OsMPK1-OsWRKY53, may be important in regulating the interplay between abiotic and biotic stresses in plants [82].

The Oryza sativa Mitogen-Activated Protein Kinase Kinase OsMKKK70 regulates rice grain size and leaf angle by activating with OsMAPK6. OsMKKK70 is an active kinase, interacts with OsMKK4 and promotes the phosphorylation of OsMAPK6 [83].

3.4. Phytohormone signaling

Plant hormones have multiple functions in plant growth and development, responsing to stress, and metabolism. Brassinosteroids (BR), auxin, gibberellin, and cytokinin have been proven to regulate grain growth through maternal tissues. In addition, the development of endosperm is also regulated by phytohormones, consequently affecting grain size (Fig. 5).

3.4.1. Brassinosteroids (BR)

Both BR deletion and BR insensitive mutants in Arabidopsis and rice produce short-grained. SLG (BAHD acyltransferase-like protein gene) is an important regulator of BR homeostasis. *Slg-D* mutant showed mild BR-deficient phenotypes, including shorter grains [38]. *XIAO* encoding an LRR kinase co-expressed with many genes involving in the cell cycle. *XIAO* is a regulator of BR signaling and cell division. Reducing the expression of *XIAO* leads to a decrease in the rate of cell division and a reduction in the number of cells, which leads to smaller grain size [31].

In Arabidopsis, the GSK3/shaggy-like kinase brassinosteroid-insensitive 2 (BIN2) regulates the expression of downstream BR-

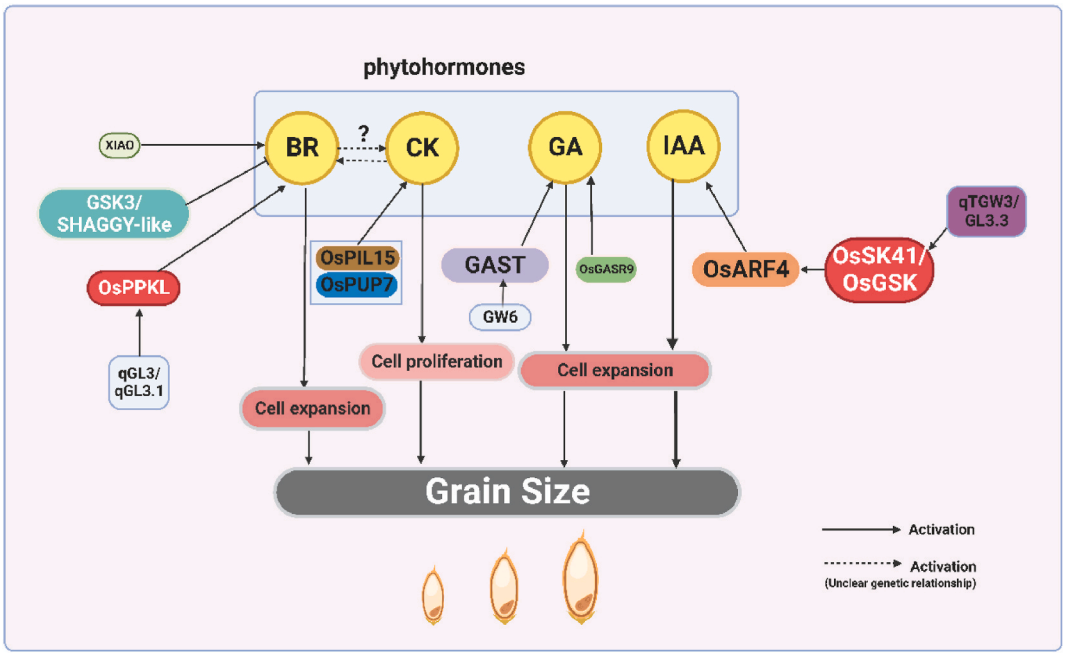

Fig. 5. Phytohormone regulation of rice seed grain size: *XIAO* is a regulator of Brassinosteroids signaling and cell division, and the reduction of BR deficiency results in a lower rate of cell division, leading to a reduction in cell number. OsPPKL, encoded by *qGL3/qGL3.1*, regulates grain size by restricting cell proliferation in the spikelet hulls; The activation decreased of *GSK2* can result in reduced grain length in rice, and there may be a cooperative relationship between BR and CK in the regulation of grain size in rice; *GW6* encodes a GA-sensitive GAST family gene, which affects GA signaling and regulates grain width and grain weight in rice. *OsGASR9* regulates rice grain size through the GA pathway. *qTGW3/GL3.3* encodes an *OsSK41/OsGSK* protein that interacts with *OsARF4* to negatively regulate rice grain size and grain weight by affecting cell expansion in the spikelet hulls. The *OsPIL15-OsPUP7* module affects grain size by increasing the number of divisions of the endosperm cells to enhance grain filling. The *OsPIL15-OsPUP7* module negatively regulated grain size and grain weight in rice.

responsive genes. It plays a critical role in the BR signaling pathway. The activation of rice GSK3/SHAGGY-like kinase (GSK2), one of the orthologs of BIN2, results in a typical BR-deficient phenotype in rice and involves a signaling pathway that regulates seed size in rice [84]. *qGL3* encodes a putative protein phosphatase (OsPPKL1) with a Kelch-like repeat structural domain. *qGL3* inhibits BR signaling by regulating the phosphorylation and stability of OsGSK3, ultimately resulting in shorter grains [85]. *GS5* positively regulates rice grain width by binding to the extracellular leucine-rich repeat (LRR) domain of OsBAK1-7, which competitively inhibits the interaction between OsBAK1-7 and OsMSBP1 and enhances BR signaling [86]. *GLW10* encoding a putative BR signaling kinase (OsBSK2), regulates grain size by affecting cell expansion. OsBSK2 directly interacts with the OsBRI1 (BR receptor kinase), regulating grain size independently of the BR signaling pathway [87].

3.4.2. Gibberellins

Gibberellin regulates seed germination, flowering, fruiting and grain size by regulating cell growth. However, present studies have mainly focused on the effects of blocking GA biosynthesis or signaling in the GA pathway on grain size, the specific signaling pathways are still unclear. *GW6* encoding a GA-regulated GAST family protein, positively regulates grain width and weight by promoting the expansion of spikelet shell cells. *GW6* is induced by GA, knockout of *GW6* downregulates the expression of GA biosynthetic genes and reduces GA content in the young panicles [88]. OsGASRs (Plant-specific GA-stimulated transcription gene family) are critical for plant growth and development. Ten OsGASR family members have been characterized in rice. *OsGASR9* is a positive regulator responding to GA, regulates plant height, grain size, and yield through the GA pathway [36].

WRKYs are transcription factors that regulate plant resistance growth and development, including height and grain size. The *sgsd3* mutant reduced sensitivity to GA and accumulates higher levels of *SLR1* (a DELLA-like inhibitor of GA signaling). *OsWRKY36* protects *SLR1* from GA-mediated degradation, suppressing GA signaling by stabilizing the expression of *SLR1* and negatively regulating grain size in rice [89].

3.4.3. Auxin

Auxin regulates the growth process of plants by determining the rate of cell division and expansion or cell development. Growth hormone signaling is mediated by a combination of growth hormone/indole-3-acetic acid transcriptional repressor and growth hormone response factor (ARF) transcription factors. Most ARF proteins contain three conserved structural domains: the N-terminal DNA-binding domain (DBD), the mid-domain (MD), and the C-terminal dimerization domain (CTD). The DBD binds specifically to AuxRE and regulates the expression of growth hormone response genes [90].

qTGW3 encodes OsSK41 (OsGSK5), a member of the GLYCOGEN SYNTHASE KINASE 3/SHAGGY-like protein family. *OsSK41* interacts with and phosphorylates AUXIN RESPONSE FACTOR 4 (*OsARF4*), loss-of-function mutations of *OsARF4* result in larger grain size in rice [26]. The dominant mutant *big grain 1* (*Bg1-D*) exhibits an oversized grain. Overexpression of *BG1* leads to a significant increase in grain size, and the mutant has an increased sensitivity to auxin and N-1-naphthylphthalamic acid (an auxin transporter inhibitor), whereas knockdown of *BG1* results in a decreased sensitivity and smaller grains [91]. *TGW6* encodes a protein with indole-3-acetic acid (IAA)-glucose hydrolase activity. During grain development, the *tgw6* allele influences grain size by controlling IAA synthesis, limiting cell number, and influencing the transition from the syncytium to the cell phase [12].

3.4.4. Cytokinins

Cytokinins influence cell proliferation and expansion by controlling the cell cycle of the apical meristem. It is important in regulating grain number and size in rice [92]. Big grain 3 (*bg3-D*) exhibits a significant increase in grain size caused by the activation of the PURINE PERMEASE gene *OsPUP4*. *OsPUP4* is localized in the plasma membrane, whereas *OsPUP7* is localized in the endoplasmic reticulum. *OsPUP4* and *OsPUP7* direct the transport of cytokinins between cells in a linear pathway that affects the long-range movement and local distribution of cytokinin [93]. The division and proliferation of endosperm cells can be promoted by the exogenous application of cytokinins, leading to an enhancement in the grain filling rate. In rice, six photoreceptor action factors have been identified. *OsPIL15*, a member of the essential helix-loop-helix transcription factor families, interacting with *OsPUP7*. Study has shown that the *OsPIL15/OsPUP7* module can regulate rice grain morphology by enhancing endosperm cell division and grain filling [94].

3.5. Transcriptional regulatory pathway

Transcriptional regulation is crucial for many plant growth and developmental processes, some transcription factors regulate seed grain type. The SPL (SQUAMOSA Promoter Binding Protein-Like) family, a plant-specific group of transcription factors, has been confirmed to participate in various developmental processes and physiological activities, exerting a significant role in rice yield-related traits. *GLW7* encoding the plant-specific transcription factor *OsSPL13*, increases the length and yield of rice grains by positively regulating the size of cells in the husk of rice [84]. *GS2* is a semi-dominant gene controlling grain size and weight in rice. *GS2* encodes the transcription factor *OsGRF4* (GROWTH REGULATING FACTOR 4), regulated by OsmiR396. A 2 bp substitution mutation in *GS2* disrupts the directional regulation of *GS2* by OsmiR396, resulting in bigger and heavier grain size [95]. One SNP (SNP1066) in the SPL12 transcriptional activation structural domain is strongly associated with grain width and increases transcriptional activity. *OsSPL12* directly binds to the 1212-bp fragment of the Indica rice. *GW5* promoter and induces the expression of *GW5*, which determines the difference in grain size between Indica and Japonica rice through enhancer-promoter structure [96]. *GW8* an allele of *OsSPL16*, is a positive regulator of cell proliferation. Overexpression of *GW8* can promote grain width and yield in rice [48]. *GW7* encodes a TONNEAU-recruiting motif protein and positively regulates grain length in rice by increasing longitudinal and decreasing transverse cell divisions. *OsSPL6* (*GW8*), an SBP domain transcription factor that regulates grain width, directly binds to and represses

the expression of the *GW7* promoter [67]. Variation in the *OsAUX3* promoter resulted in the function decreased of *OsAUX3*, and increased grain length and width. It was demonstrated that *OsARF6* is an upstream transcription factor regulating the expression of *OsAUX3*. *OsARF6* directly binds with the auxin-responsive element of the *OsAUX3* promoter and controls grain length by changing the longitudinal expansion of the glume cells and auxin distribution/content [97].

The MAD-box gene is a widely existing transcription factor in plants, which has essential functions in controlling spike development. *OsMADS1*, a MADS-box transcriptional regulator gene family member, directly regulates seed development and grain size in rice, mainly caused by abnormal lemma and palea development [98].

4. Other pathways for regulating grain size in rice

Rice grain size is also affected by abiotic stresses. *GSA1* encoding a UDP glucosyltransferase, regulates grain size by affecting cell proliferation and expansion, which are regulated by flavonoid-mediated growth auxin content and expression of related genes. Overexpression of *GSA1* results in larger rice grains and enhanced the abiotic stress tolerance of rice [99]. Small heat shock proteins (sHsps) are ATP-independent chaperone proteins, widely expressed in response to diverse environmental stresses. A single hairpin construct was designed to generate all silenced CI-sHsp rice lines [100]. Rice RNAiCI-sHsp seedlings showed reduced heat tolerance and significantly decreased seed grain length. The 14-3-3 family were widely involved in regulating plant growth and development [101]. Research demonstrates that *Os14-3-3* genes may regulate grain size in JA signaling [102,103].

The size of rice hulls directly determines the primary factor of grain size. As early as 1970, Takeda and Takahash proposed that the size of the grain husk directly determines grain size of rice [104]. *OsQUA2* is essential for maintaining a high degree of methyl esterification in the cell wall of the rice stalk-screening element (SE), which is critical for efficient Suc partitioning and grain filling [105]. *GF14f*, a member of the 14-3-3 family of proteins, showed differences in expression patterns between SS (earlier flowering superior spikelets) and IS (later flowering inferior spikelets), negatively regulate the seed size and seed filling [106]. *FGW1* encoding a protein containing the DUF630/DUF632 structural domains, directly interacts with *GF14f*, it positively regulating seed width by affecting glume cytokinesis and expansion [107].

5. Discussion and perspectives

Cultivated rice varieties that have domesticated from wild rice through both natural and artificial selection. In this process, some elite genes were selected while some were eliminated, resulting in the gradual decrease of rice genetic diversity. So, it is necessary to explore new genes and excellent alleles which regulating rice grain size in wild rice populations. At the same time, contemporary high-throughput sequencing technology with GWAS, BSA, and other bioinformatics tools can be used to discover new QTL/gene related to the regulation of rice grain size, and the molecular mechanism of grain size can be explored further by combining transcriptomic, proteomic and metabolomic tools.

In recent decades, researchers have characterized over 400 QTL related to rice grain size, and the molecular mechanisms of regulating rice grain size have been studied. The main pathways include Ubiquitination-proteasome, G proteins, mitogen-activated protein kinases, transcription factors, etc. In the previous section, we have reviewed the progress of the different pathways, and the connection between these pathways has not yet been revealed. Rice grain size is mainly determined by its genetic factors and is also affected by the external environment. *RGA1* is a positive regulator of drought tolerance in rice, and the *RGA1* allele *D1* encodes the alpha subunit of GTP-binding proteins (G proteins), regulating the size of grains through GA signaling [108,109]. Researchers can focus on the critical regulator factors of the grain size pathway, finding target genes in other pathways and connecting these pathways. Meanwhile, the researchers used forward genetics to explore the critical QTL affecting grain shape under stress, further understand the mechanism of abiotic stress on rice grain shape, and comprehensively reveal the molecular mechanisms regulating rice grain size.

Variety improvement is an important practice in the agricultural production of rice. Traditional breeding mainly uses allelic variation under different background materials to select elite strains, but the result may not be as expected. Using grain size genes for variety improvement may affect other traits of rice and result in lower yields. In our opinion, the appropriate elite genes can be selected according to the need for gene polymerization to achieve precision breeding. In polymerizing different elite genes, it is important to break the negative linkage between rice yield and quality and improve rice quality effectively. Breeders can utilize the genome editing technology CRISPR/Cas9 to accurately and efficiently polymerize superior allele genes and precisely knock out unfavorable traits in rice varieties, and rapidly obtaining new materials to improve breeding efficiency. Combine traditional and modern breeding to cultivate new high-yield and high-quality rice varieties.

Grain size is significantly different among plant species. Exploring the essential genes responsible for grain size variation among different species will help us to understand the molecular mechanism of grain size regulation on the gene origination and evolution level. The evolutionary characteristics of grain size can be explored by analyzing the genetic and genomic data of species. Grain size is a yield trait mainly linked to quality and combines the breeding objectives of achieving high yields and good quality. Therefore, exploring the molecular regulation mechanism of rice grain size and making appropriate breeding strategies to achieve high yield and quality rice is important.

Data availability statement

Data availability does not apply to this article as no new data were created or analyzed in this study.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Yuntao Yan: Writing – original draft. **Xiaoya Zhu:** Writing – original draft. **Hui Qi:** Methodology. **Haiqing Zhang:** Writing – review & editing. **Jiwai He:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] R. D, D. C, Q. Q, Molecular bases of rice grain size and quality for optimized productivity, Sci. Bull. 68 (2023), https://doi.org/10.1016/j.scib.2023.01.026.
[2] W. Yang, K. Wu, B. Wang, H. Liu, S. Guo, X. Guo, W. Luo, S. Sun, Y. Ouyang, X. Fu, K. Chong, Q. Zhang, Y. Xu, The RING E3 ligase CLG1 targets GS3 for degradation via the endosome pathway to determine grain size in rice, Mol. Plant 14 (2021) 1699–1713.
[3] N. Li, R. Xu, Y. Li, Molecular networks of seed size control in plants, Annu. Rev. Plant Biol. 70 (2019) 435–463.
[4] R. Huang, L. Jiang, J. Zheng, T. Wang, H. Wang, Y. Huang, Z. Hong, Genetic bases of rice grain shape: so many genes, so little known, Trends Plant Sci. 18 (2013) 218–226.
[5] W. Hao, H.-X. Lin, Toward understanding genetic mechanisms of complex traits in rice, J. Genet. Genom. = Yi Chuan Xue Bao 37 (2010) 653–666.
[6] R. Che, H. Tong, B. Shi, Y. Liu, S. Fang, D. Liu, Y. Xiao, B. Hu, L. Liu, H. Wang, M. Zhao, C. Chu, Control of grain size and rice yield by GL2-mediated brassinosteroid responses, Nat. Plants 2 (2015) 15195.
[7] X.-J. Song, W. Huang, M. Shi, M.-Z. Zhu, H.-X. Lin, A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase, Nat. Genet. 39 (2007) 623–630.
[8] M. Niu, H. Wang, W. Yin, W. Meng, Y. Xiao, D. Liu, X. Zhang, N. Dong, J. Liu, Y. Yang, F. Zhang, C. Chu, H. Tong, Rice DWARF AND LOW-TILLERING and the homeodomain protein OSH15 interact to regulate internode elongation via orchestrating brassinosteroid signaling and metabolism, Plant Cell 34 (2022) 3754–3772.
[9] D. Heang, H. Sassa, An atypical bHLH protein encoded by *POSITIVE REGULATOR OF GRAIN LENGTH 2* is involved in controlling grain length and weight of rice through interaction with a typical bHLH protein APG, Breed Sci. 62 (2012) 133–141.
[10] Z. Hu, H. He, S. Zhang, F. Sun, X. Xin, W. Wang, X. Qian, J. Yang, X. Luo, A kelch motif-containing serine/threonine protein phosphatase determines the large grain QTL trait in rice, J. Integr. Plant Biol. 54 (2012) 979–990.
[11] X.J. Song, T. Kuroha, M. Ayano, T. Furuta, K. Nagai, N. Komeda, S. Segami, K. Miura, D. Ogawa, T. Kamura, T. Suzuki, T. Higashiyama, M. Yamasaki, H. Mori, Y. Inukai, J. Wu, H. Kitano, H. Sakakibara, S.E. Jacobsen, M. Ashikari, Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice, Proc. Natl. Acad. Sci. USA 112 (2015) 76–81.
[12] K. Ishimaru, N. Hirotsu, Y. Madoka, N. Murakami, N. Hara, H. Onodera, T. Kashiwagi, K. Ujiie, B. Shimizu, A. Onishi, H. Miyagawa, E. Katoh, Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield, Nat. Genet. 45 (2013) 707–711.
[13] F. Li, W. Liu, J. Tang, J. Chen, H. Tong, B. Hu, C. Li, J. Fang, M. Chen, C. Chu, Rice DENSE AND ERECT PANICLE 2 is essential for determining panicle outgrowth and elongation, Cell Res. 20 (2010) 838–849.
[14] S. Segami, I. Kono, T. Ando, M. Yano, H. Kitano, K. Miura, Y. Iwasaki, Small and round seed 5 gene encodes alpha-tubulin regulating seed cell elongation in rice, Rice 5 (2012) 4.
[15] X.-J. Song, W. Huang, M. Shi, M.-Z. Zhu, H.-X. Lin, A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase, Nat. Genet. 39 (2007) 623–630.
[16] J. Hu, Y. Wang, Y. Fang, L. Zeng, J. Xu, H. Yu, Z. Shi, J. Pan, D. Zhang, S. Kang, L. Zhu, G. Dong, L. Guo, D. Zeng, G. Zhang, L. Xie, G. Xiong, J. Li, Q. Qian, A rare allele of GS2 enhances grain size and grain yield in rice, Mol. Plant 8 (2015) 1455–1465.
[17] W. Wu, X. Liu, M. Wang, R.S. Meyer, X. Luo, M.-N. Ndjiondjop, L. Tan, J. Zhang, J. Wu, H. Cai, C. Sun, X. Wang, R.A. Wing, Z. Zhu, A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domestication, Nat. Plants 3 (2017) 17064.
[18] Y. Li, C. Fan, Y. Xing, Y. Jiang, L. Luo, L. Sun, D. Shao, C. Xu, X. Li, J. Xiao, Y. He, Q. Zhang, Natural variation in GS5 plays an important role in regulating grain size and yield in rice, Nat. Genet. 43 (2011) 1266–1269.
[19] Y. Wang, Y. Lv, H. Yu, P. Hu, Y. Wen, J. Wang, Y. Tan, H. Wu, L. Zhu, K. Wu, B. Chai, J. Liu, D. Zeng, G. Zhang, L. Zhu, Z. Gao, G. Dong, D. Ren, L. Shen, Q. Zhang, Q. Li, L. Guo, G. Xiong, Q. Qian, J. Hu, GR5 acts in the G protein pathway to regulate grain size in rice, Plant Commun 5 (2024) 100673.
[20] J. Liu, J. Chen, X. Zheng, F. Wu, Q. Lin, Y. Heng, P. Tian, Z. Cheng, X. Yu, K. Zhou, X. Zhang, X. Guo, J. Wang, H. Wang, J. Wan, GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice, Nat. Plants 3 (2017) 17043.
[21] D. Heang, H. Sassa, Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice, PLoS One 7 (2012) e31325.
[22] L. Sun, X. Li, Y. Fu, Z. Zhu, L. Tan, F. Liu, X. Sun, X. Sun, C. Sun, GS6, a member of the GRAS gene family, negatively regulates grain size in rice, J. Integr. Plant Biol. 55 (2013) 938–949.
[23] N. Fang, R. Xu, L. Huang, B. Zhang, P. Duan, N. Li, Y. Luo, Y. Li, SMALL GRAIN 11 controls grain size, grain number and grain yield in rice, Rice 9 (2016) 64.
[24] P. Duan, Y. Rao, D. Zeng, Y. Yang, R. Xu, B. Zhang, G. Dong, Q. Qian, Y. Li, SMALL GRAIN 1, which encodes a mitogen-activated protein kinase kinase 4, influences grain size in rice, Plant J. 77 (2014) 547–557.
[25] J. Niu, F. Wang, C. Yang, Q. Ye, J. Huang, Y. La, Q. Wang, J. Dai, T. Hu, L. Sang, P. Zhang, Y. Zou, Z. Zhai, J. Jin, D. Abdulmajid, J. Guo, H. Chen, H. La, Identification of Increased Grain Length 1 (IGL1), a novel gene encoded by a major QTL for modulating grain length in rice, Theor. Appl. Genet. 137 (2024) 24, https://doi.org/10.1007/s00122-023-04531-7.
[26] Z. Hu, S.-J. Lu, M.-J. Wang, H. He, L. Sun, H. Wang, X.-H. Liu, L. Jiang, J.-L. Sun, X. Xin, W. Kong, C. Chu, H.-W. Xue, J. Yang, X. Luo, J.-X. Liu, 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 (2018) 736–749.
[27] M. Mori, T. Nomura, H. Ooka, M. Ishizaka, T. Yokota, K. Sugimoto, K. Okabe, H. Kajiwara, K. Satoh, K. Yamamoto, H. Hirochika, S. Kikuchi, Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis, Plant Physiol. 130 (2002) 1152–1161.

[28] D. Zhang, M. Zhang, J. Liang, RGB1 regulates grain development and starch accumulation through its effect on OsYUC11-mediated auxin biosynthesis in rice endosperm cells, Front. Plant Sci. 12 (2021) 585174.
[29] Q. Liu, R. Han, K. Wu, J. Zhang, Y. Ye, S. Wang, J. Chen, Y. Pan, Q. Li, X. Xu, J. Zhou, D. Tao, Y. Wu, X. Fu, G-protein βγ subunits determine grain size through interaction with MADS-domain transcription factors in rice, Nat. Commun. 9 (2018) 852.
[30] X. Hu, Q. Qian, T. Xu, Y. Zhang, G. Dong, T. Gao, Q. Xie, Y. Xue, The U-box E3 ubiquitin ligase TUD1 functions with a heterotrimeric G α subunit to regulate brassinosteroid-mediated growth in rice, PLoS Genet. 9 (2013) e1003391.
[31] Y. Jiang, L. Bao, S.-Y. Jeong, S.-K. Kim, C. Xu, X. Li, Q. Zhang, XIAO is involved in the control of organ size by contributing to the regulation of signaling and homeostasis of brassinosteroids and cell cycling in rice: a gene regulating brassinosteroid response in rice, Plant J. 70 (2012) 398–408.
[32] X. Zhu, W. Liang, X. Cui, M. Chen, C. Yin, Z. Luo, J. Zhu, W.J. Lucas, Z. Wang, D. Zhang, Brassinosteroids promote development of rice pollen grains and seeds by triggering expression of Carbon Starved Anther, a MYB domain protein, Plant J. 82 (2015) 570–581.
[33] J. Lyu, D. Wang, P. Duan, Y. Liu, K. Huang, D. Zeng, L. Zhang, G. Dong, Y. Li, R. Xu, B. Zhang, X. Huang, N. Li, Y. Wang, Q. Qian, Y. Li, Control of grain size and weight by the GSK2-LARGE1/OML4 pathway in rice, Plant Cell 32 (2020) 1905–1918.
[34] Y. Zhou, J. Miao, H. Gu, X. Peng, M. Leburu, F. Yuan, H. Gu, Y. Gao, Y. Tao, J. Zhu, Z. Gong, C. Yi, M. Gu, Z. Yang, G. Liang, Natural variations in *SLG7* regulate grain shape in rice, Genetics 201 (2015) 1591–1599.
[35] Y. Wang, G. Xiong, J. Hu, L. Jiang, H. Yu, J. Xu, Y. Fang, L. Zeng, E. Xu, J. Xu, W. Ye, X. Meng, R. Liu, H. Chen, Y. Jing, Y. Wang, X. Zhu, J. Li, Q. Qian, Copy number variation at the GL7 locus contributes to grain size diversity in rice, Nat. Genet. 47 (2015) 944–948.
[36] X. Li, S. Shi, Q. Tao, Y. Tao, J. Miao, X. Peng, C. Li, Z. Yang, Y. Zhou, G. Liang, OsGASR9 positively regulates grain size and yield in rice (Oryza sativa), Plant Sci. 286 (2019) 17–27.
[37] W. Yang, M. Gao, X. Yin, J. Liu, Y. Xu, L. Zeng, Q. Li, S. Zhang, J. Wang, X. Zhang, Z. He, Control of rice embryo development, shoot apical meristem maintenance, and grain yield by a novel cytochrome P450, Mol. Plant 6 (2013) 1945–1960.
[38] Z. Feng, C. Wu, C. Wang, J. Roh, L. Zhang, J. Chen, S. Zhang, H. Zhang, C. Yang, J. Hu, X. You, X. Liu, X. Yang, X. Guo, X. Zhang, F. Wu, W. Terzaghi, S.-K. Kim, L. Jiang, J. Wan, *SLG* controls grain size and leaf angle by modulating brassinosteroid homeostasis in rice, J. Exp. Bot. 67 (2016) 4241–4253.
[39] D. Li, L. Wang, M. Wang, Y.-Y. Xu, W. Luo, Y.-J. Liu, Z.-H. Xu, J. Li, K. Chong, Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield, Plant Biotechnol. J. 7 (2009) 791–806.
[40] X. Cheng, M. Pan, Z. E, Y. Zhou, B. Niu, C. Chen, Functional divergence of two duplicated *Fertilization Independent Endosperm* genes in rice with respect to seed development, Plant J. 104 (2020) 124–137.
[41] X. Huang, Q. Qian, Z. Liu, H. Sun, S. He, D. Luo, G. Xia, C. Chu, J. Li, X. Fu, Natural variation at the DEP1 locus enhances grain yield in rice, Nat. Genet. 41 (2009) 494–497.
[42] Z. Hong, M. Ueguchi-Tanaka, S. Fujioka, S. Takatsuto, S. Yoshida, Y. Hasegawa, M. Ashikari, H. Kitano, M. Matsuoka, The rice *brassinosteroid-deficient dwarf 2* mutant, defective in the rice homolog of Arabidopsis DIMINUTO/DWARF1, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone, Plant Cell 17 (2005) 2243–2254.
[43] R. Gong, H. Cao, J. Zhang, K. Xie, D. Wang, S. Yu, Divergent functions of the GAGA -binding transcription factor family in rice, Plant J. 94 (2018) 32–47, https://doi.org/10.1111/tpj.13837.
[44] C. Fan, Y. Xing, H. Mao, T. Lu, B. Han, C. Xu, X. Li, Q. Zhang, 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 (2006) 1164–1171.
[45] Y. Tao, J. Miao, J. Wang, W. Li, Y. Xu, F. Wang, Y. Jiang, Z. Chen, F. Fan, M. Xu, Y. Zhou, G. Liang, J. Yang, RGG1, involved in the cytokinin regulatory pathway, controls grain size in rice, Rice 13 (2020) 76.
[46] T. Guo, K. Chen, N.-Q. Dong, C.-L. Shi, W.-W. Ye, J.-P. Gao, J.-X. Shan, H.-X. Lin, 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 (2018) 871–888.
[47] K. Kitagawa, S. Kurinami, K. Oki, Y. Abe, T. Ando, I. Kono, M. Yano, H. Kitano, Y. Iwasaki, A novel kinesin 13 protein regulating rice seed length, Plant Cell Physiol. 51 (2010) 1315–1329.
[48] S. Wang, K. Wu, Q. Yuan, X. Liu, Z. Liu, X. Lin, R. Zeng, H. Zhu, G. Dong, Q. Qian, G. Zhang, X. Fu, Control of grain size, shape and quality by OsSPL16 in rice, Nat. Genet. 44 (2012) 950–954.
[49] K. Huang, D. Wang, P. Duan, B. Zhang, R. Xu, N. Li, Y. Li, WIDE AND THICK GRAIN 1, which encodes an otubain-like protease with deubiquitination activity, influences grain size and shape in rice, Plant J. 91 (2017) 849–860.
[50] H. Nakagawa, A. Tanaka, T. Tanabata, M. Ohtake, S. Fujioka, H. Nakamura, H. Ichikawa, M. Mori, *SHORT GRAIN1* decreases organ elongation and brassinosteroid response in rice, Plant Physiol. 158 (2012) 1208–1219.
[51] Y. Xu, L. Zhang, S. Ou, R. Wang, Y. Wang, C. Chu, S. Yao, Natural variations of SLG1 confer high-temperature tolerance in indica rice, Nat. Commun. 11 (2020) 5441.
[52] X. Zhang, J. Wang, J. Huang, H. Lan, C. Wang, C. Yin, Y. Wu, H. Tang, Q. Qian, J. Li, H. Zhang, 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 (2012) 21534–21539.
[53] C. Fan, Y. Xing, H. Mao, T. Lu, B. Han, C. Xu, X. Li, Q. Zhang, 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 (2006) 1164–1171.
[54] N. Takano-Kai, H. Jiang, A. Powell, S. McCouch, I. Takamure, N. Furuya, K. Doi, A. Yoshimura, Multiple and independent origins of short seeded alleles of GS3 in rice, Breed Sci. 63 (2013) 77–85.
[55] P. Qi, Y.-S. Lin, X.-J. Song, J.-B. Shen, W. Huang, J.-X. Shan, M.-Z. Zhu, L. Jiang, J.-P. Gao, H.-X. Lin, The novel quantitative trait locus GL3.1 controls rice grain size and yield by regulating Cyclin-T1;3, Cell Res. 22 (2012) 1666–1680.
[56] J. Yu, H. Xiong, X. Zhu, H. Zhang, H. Li, J. Miao, W. Wang, Z. Tang, Z. Zhang, G. Yao, Q. Zhang, Y. Pan, X. Wang, M.A.R. Rashid, J. Li, Y. Gao, Z. Li, W. Yang, X. Fu, Z. Li, OsLG3 contributing to rice grain length and yield was mined by Ho-LAMap, BMC Biol. 15 (2017) 28.
[57] Z. Hu, S.-J. Lu, M.-J. Wang, H. He, L. Sun, H. Wang, X.-H. Liu, L. Jiang, J.-L. Sun, X. Xin, W. Kong, C. Chu, H.-W. Xue, J. Yang, X. Luo, J.-X. Liu, 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 (2018) 736–749.
[58] D. Xia, H. Zhou, R. Liu, W. Dan, P. Li, B. Wu, J. Chen, L. Wang, G. Gao, Q. Zhang, Y. He, 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 (2018) 754–756.
[59] W. Wu, X. Liu, M. Wang, R.S. Meyer, X. Luo, M.-N. Ndjiondjop, L. Tan, J. Zhang, J. Wu, H. Cai, C. Sun, X. Wang, R.A. Wing, Z. Zhu, A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domestication, Nat. Plants 3 (2017) 17064.
[60] J. Weng, S. Gu, X. Wan, H. Gao, T. Guo, N. Su, C. Lei, X. Zhang, Z. Cheng, X. Guo, J. Wang, L. Jiang, H. Zhai, J. Wan, Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight, Cell Res. 18 (2008) 1199–1209.
[61] J. Qiao, H. Jiang, Y. Lin, L. Shang, M. Wang, D. Li, X. Fu, M. Geisler, Y. Qi, Z. Gao, Q. Qian, A novel miR167a-OsARF6-OsAUX3 module regulates grain length and weight in rice, Mol. Plant 14 (2021) 1683–1698.
[62] K. Ishimaru, Identification of a locus increasing rice yield and physiological analysis of its function, Plant Physiol. 133 (2003) 1083–1090.
[63] K. Ishimaru, N. Hirotsu, Y. Madoka, N. Murakami, N. Hara, H. Onodera, T. Kashiwagi, K. Ujiie, B.-I. Shimizu, A. Onishi, H. Miyagawa, E. Katoh, Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield, Nat. Genet. 45 (2013) 707–711.
[64] X.J. Song, T. Kuroha, M. Ayano, T. Furuta, K. Nagai, N. Komeda, S. Segami, K. Miura, D. Ogawa, T. Kamura, T. Suzuki, T. Higashiyama, M. Yamasaki, H. Mori, Y. Inukai, J. Wu, H. Kitano, H. Sakakibara, S.E. Jacobsen, M. Ashikari, Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice, Proc. Natl. Acad. Sci. USA 112 (2015) 76–81.
[65] L. Si, J. Chen, X. Huang, H. Gong, J. Luo, Q. Hou, T. Zhou, T. Lu, J. Zhu, Y. Shangguan, E. Chen, C. Gong, Q. Zhao, Y. Jing, Y. Zhao, Y. Li, L. Cui, D. Fan, Y. Lu, Q. Weng, Y. Wang, Q. Zhan, K. Liu, X. Wei, K. An, G. An, B. Han, OsSPL13 controls grain size in cultivated rice, Nat. Genet. 48 (2016) 447–456.

[66] X. Qiu, R. Gong, Y. Tan, S. Yu, Mapping and characterization of the major quantitative trait locus qSS7 associated with increased length and decreased width of rice seeds, Theor. Appl. Genet. 125 (2012) 1717–1726.
[67] S. Wang, S. Li, Q. Liu, K. Wu, J. Zhang, S. Wang, Y. Wang, X. Chen, Y. Zhang, C. Gao, F. Wang, H. Huang, X. Fu, The OsSPL16-GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality, Nat. Genet. 47 (2015) 949–954.
[68] D.-S. Zhao, Q.-F. Li, C.-Q. Zhang, C. Zhang, Q.-Q. Yang, L.-X. Pan, X.-Y. Ren, J. Lu, M.-H. Gu, Q.-Q. Liu, GS9 acts as a transcriptional activator to regulate rice grain shape and appearance quality, Nat. Commun. 9 (2018) 1240.
[69] Y. Li, L. Zheng, F. Corke, C. Smith, M.W. Bevan, Control of final seed and organ size by the *DA1* gene family in *Arabidopsis thaliana*, Genes Dev. 22 (2008) 1331–1336.
[70] C. Shi, Y. Ren, L. Liu, F. Wang, H. Zhang, P. Tian, T. Pan, Y. Wang, R. Jing, T. Liu, F. Wu, Q. Lin, C. Lei, X. Zhang, S. Zhu, X. Guo, J. Wang, Z. Zhao, J. Wang, H. Zhai, Z. Cheng, J. Wan, Ubiquitin specific protease 15 has an important role in regulating grain width and size in rice, Plant Physiol. 180 (2019) 381–391.
[71] W. Yang, K. Wu, B. Wang, H. Liu, S. Guo, X. Guo, W. Luo, S. Sun, Y. Ouyang, X. Fu, K. Chong, Q. Zhang, Y. Xu, The RING E3 ligase CLG1 targets GS3 for degradation via the endosome pathway to determine grain size in rice, Mol. Plant 14 (2021) 1699–1713.
[72] Q. Gao, N. Zhang, W.-Q. Wang, S.-Y. Shen, C. Bai, X.-J. Song, 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 (2021) 3331–3347.
[73] J. Kurepa, S. Wang, Y. Li, D. Zaitlin, A.J. Pierce, J.A. Smalle, Loss of 26S proteasome function leads to increased cell size and decreased cell number in Arabidopsis shoot organs, Plant Physiol. 150 (2009) 178–189.
[74] P. Duan, J. Xu, D. Zeng, B. Zhang, M. Geng, G. Zhang, K. Huang, L. Huang, R. Xu, S. Ge, Q. Qian, Y. Li, Natural variation in the promoter of GSE5 contributes to grain size diversity in rice, Mol. Plant 10 (2017) 685–694.
[75] D.C. New, J.T. Wong, The evidence for G-protein-coupled receptors and heterotrimeric G proteins in protozoa and ancestral metazoa, Biol. Signals Recept. 7 (1998) 98–108.
[76] C.R. McCudden, M.D. Hains, R.J. Kimple, D.P. Siderovski, F.S. Willard, G-protein signaling: back to the future, Cell. Mol. Life Sci. 62 (2005) 551–577.
[77] D. Chakravorty, Y. Trusov, W. Zhang, B.R. Acharya, M.B. Sheahan, D.W. McCurdy, S.M. Assmann, J.R. Botella, An atypical heterotrimeric G-protein γ-subunit is involved in guard cell K+-channel regulation and morphological development in Arabidopsis thaliana: identification of a novel class of G-protein γ-subunits, Plant J. 67 (2011) 840–851.
[78] S. Sun, L. Wang, H. Mao, L. Shao, X. Li, J. Xiao, Y. Ouyang, Q. Zhang, A G-protein pathway determines grain size in rice, Nat. Commun. 9 (2018) 851.
[79] F. Bai, H. Ma, Y. Cai, M.Q. Shahid, Y. Zheng, C. Lang, Z. Chen, J. Wu, X. Liu, L. Wang, Natural allelic variation in *GRAIN SIZE AND WEIGHT 3* of wild rice regulates the grain size and weight, Plant Physiol. (2023), https://doi.org/10.1093/plphys/kiad320 kiad320.
[80] MAPK Group, Mitogen-activated protein kinase cascades in plants: a new nomenclature, Trends Plant Sci. 7 (2002) 301–308.
[81] J. Xu, S. Zhang, Mitogen-activated protein kinase cascades in signaling plant growth and development, Trends Plant Sci. 20 (2015) 56–64, https://doi.org/10.1016/j.tplants.2014.10.001.
[82] S.J. Yoo, S.-H. Kim, M.-J. Kim, C.-M. Ryu, Y.C. Kim, B.H. Cho, K.-Y. Yang, Involvement of the OsMKK4-OsMPK1 cascade and its downstream transcription factor OsWRKY53 in the wounding response in rice, Plant Pathol. J. 30 (2014) 168–177.
[83] Z. Liu, E. Mei, X. Tian, M. He, J. Tang, M. Xu, J. Liu, L. Song, X. Li, Z. Wang, Q. Guan, Q. Xu, Q. Bu, OsMKKK70 regulates grain size and leaf angle in rice through the OsMKK4-OsMAPK6-OsWRKY53 signaling pathway, J. Integr. Plant Biol. 63 (2021) 2043–2057.
[84] H. Tong, L. Liu, Y. Jin, L. Du, Y. Yin, Q. Qian, L. Zhu, C. Chu, DWARF and LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-like kinase to mediate brassinosteroid responses in rice, Plant Cell 24 (2012) 2562–2577.
[85] X. Gao, J.-Q. Zhang, X. Zhang, J. Zhou, Z. Jiang, P. Huang, Z. Tang, Y. Bao, J. Cheng, H. Tang, W. Zhang, H. Zhang, J. Huang, Rice qGL3/OsPPKL1 functions with the GSK3/SHAGGY-like kinase OsGSK3 to modulate brassinosteroid signaling, Plant Cell 31 (2019) 1077–1093.
[86] C. Xu, Y. Liu, Y. Li, X. Xu, C. Xu, X. Li, J. Xiao, Q. Zhang, Differential expression of GS5 regulates grain size in rice, J. Exp. Bot. 66 (2015) 2611–2623.
[87] H. Yuan, Z. Xu, W. Chen, C. Deng, Y. Liu, M. Yuan, P. Gao, H. Shi, B. Tu, T. Li, L. Kang, B. Ma, Y. Wang, J. Wang, X. Chen, S. Li, P. Qin, OsBSK2, a putative brassinosteroid-signalling kinase, positively controls grain size in rice, J. Exp. Bot. 73 (2022) 5529–5542.
[88] C. Shi, N. Dong, T. Guo, W. Ye, J. Shan, H. Lin, A quantitative trait locus *GW6* controls rice grain size and yield through the gibberellin pathway, Plant J. 103 (2020) 1174–1188.
[89] J. Lan, Q. Lin, C. Zhou, Y. Ren, X. Liu, R. Miao, R. Jing, C. Mou, T. Nguyen, X. Zhu, Q. Wang, X. Zhang, X. Guo, S. Liu, L. Jiang, J. Wan, Small grain and semi-dwarf 3, a WRKY transcription factor, negatively regulates plant height and grain size by stabilizing SLR1 expression in rice, Plant Mol. Biol. 104 (2020) 429–450.
[90] D. Weijers, J. Friml, SnapShot: auxin signaling and transport, Cell 136 (2009) 1172–1172.e1.
[91] L. Liu, H. Tong, Y. Xiao, R. Che, F. Xu, B. Hu, C. Liang, J. Chu, J. Li, C. Chu, Activation of *Big Grain1* significantly improves grain size by regulating auxin transport in rice, Proc. Natl. Acad. Sci. USA 112 (2015) 11102–11107.
[92] J. Jin, L. Hua, Z. Zhu, L. Tan, X. Zhao, W. Zhang, F. Liu, Y. Fu, H. Cai, X. Sun, P. Gu, D. Xie, C. Sun, GAD1 encodes a secreted peptide that regulates grain number, grain length, and Awn development in rice domestication, Plant Cell 28 (2016) 2453–2463.
[93] Y. Xiao, D. Liu, G. Zhang, S. Gao, L. Liu, F. Xu, R. Che, Y. Wang, H. Tong, C. Chu, Big Grain 3, encoding a purine permease, regulates grain size via modulating cytokinin transport in rice, J. Integr. Plant Biol. 61 (2019) 581–597.
[94] X. Ji, Y. Du, F. Li, H. Sun, J. Zhang, J. Li, T. Peng, Z. Xin, Q. Zhao, The basic helix-loop-helix transcription factor, OsPIL15, regulates grain size via directly targeting a purine permease gene OsPUP7 in rice, Plant Biotechnol. J. 17 (2019) 1527–1537.
[95] P. Duan, S. Ni, J. Wang, B. Zhang, R. Xu, Y. Wang, H. Chen, X. Zhu, Y. Li, Regulation of OsGRF4 by OsmiR396 controls grain size and yield in rice, Nat. Plants 2 (2015) 15203.
[96] X.-F. Zhang, C.-Y. Yang, H.-X. Lin, J.-W. Wang, H.-W. Xue, Rice SPL12 coevolved with GW5 to determine grain shape, Sci. Bull. 66 (2021) 2353–2357.
[97] J. Qiao, H. Jiang, Y. Lin, L. Shang, M. Wang, D. Li, X. Fu, M. Geisler, Y. Qi, Z. Gao, Q. Qian, A novel miR167a-OsARF6-OsAUX3 module regulates grain length and weight in rice, Mol. Plant 14 (2021) 1683–1698.
[98] P. Li, H. Li, Z. Liu, Y. Zhuang, M. Wei, Y. Gu, Y. Liu, X. Sun, Y. Tang, L. Yue, L. Lu, D. Luo, W. Huang, S. Tu, S. Wang, Characterization of the 'oat-like rice' caused by a novel allele OsMADS1Olr reveals vital importance of OsMADS1 in regulating grain shape in Oryza sativa L, Rice 13 (2020) 73.
[99] N.-Q. Dong, Y. Sun, T. Guo, C.-L. Shi, Y.-M. Zhang, Y. Kan, Y.-H. Xiang, H. Zhang, Y.-B. Yang, Y.-C. Li, H.-Y. Zhao, H.-X. Yu, Z.-Q. Lu, Y. Wang, W.-W. Ye, J.-X. Shan, H.-X. Lin, UDP-glucosyltransferase regulates grain size and abiotic stress tolerance associated with metabolic flux redirection in rice, Nat. Commun. 11 (2020) 2629.
[100] N.K. Sarkar, S. Kotak, M. Agarwal, Y.-K. Kim, A. Grover, Silencing of class I small heat shock proteins affects seed-related attributes and thermotolerance in rice seedlings, Planta 251 (2020) 26, https://doi.org/10.1007/s00425-019-03318-9.
[101] L. Lu, Z. Diao, D. Yang, X. Wang, X. Zheng, X. Xiang, Y. Xiao, Z. Chen, W. Wang, Y. Wu, D. Tang, S. Li, The 14-3-3 protein GF14c positively regulates immunity by modulating the protein homoeostasis of the GRAS protein OsSCL7 in rice, Plant Cell Environ. 45 (2022) 1065–1081.
[102] J. Shin, K. Heidrich, A. Sanchez-Villarreal, J.E. Parker, S.J. Davis, TIME FOR COFFEE represses accumulation of the MYC2 transcription factor to provide time-of-day regulation of jasmonate signaling in Arabidopsis, Plant Cell 24 (2012) 2470–2482, https://doi.org/10.1105/tpc.111.095430.
[103] Y. Song, H. Yang, W. Zhu, H. Wang, J. Zhang, Y. Li, The Os14-3-3 family genes regulate grain size in rice, S1673-S8527(23)00216-3, J. Genet. Genomics (2023), https://doi.org/10.1016/j.jgg.2023.10.005.
[104] K. Takeda, M.-E. Takahashi, Unbalanced growth in floral glumes and caryopsis in rice. : I.varietal difference in the degree of unbalance and the occurrence of mulformed grains. (Genetical studies on rice plant, XXXXV), Jpn. J. Breed. 20 (1970) 337–343, https://doi.org/10.1270/jsbbs1951.20.337.
[105] Y. Xu, J. Sechet, Y. Wu, Y. Fu, L. Zhu, J. Li, Y. Zhang, E. Gineau, C. Gaertner, J. Zhou, X. Fan, Y. Liu, L. Zhou, G. Mouille, X. Lin, Rice sucrose partitioning mediated by a putative pectin methyltransferase and homogalacturonan methylesterification, Plant Physiol. 174 (2017) 1595–1608.
[106] Z. Zhang, H. Zhao, F. Huang, J. Long, G. Song, W. Lin, The 14-3-3 protein GF14f negatively affects grain filling of inferior spikelets of rice (Oryza sativa L.), Plant J. 99 (2019) 344–358.

[107] Y. Li, P. He, X. Wang, H. Chen, J. Ni, W. Tian, X. Zhang, Z. Cui, G. He, X. Sang, FGW1, a protein containing DUF630 and DUF632 domains, regulates grain size and filling in Oryza sativa L, Crop J. (2023), https://doi.org/10.1016/j.cj.2023.03.015. S2214514123000466.
[108] S. Sun, L. Wang, H. Mao, L. Shao, X. Li, J. Xiao, Y. Ouyang, Q. Zhang, A G-protein pathway determines grain size in rice, Nat. Commun. 9 (2018) 851, https://doi.org/10.1038/s41467-018-03141-y.
[109] M. Ueguchi-Tanaka, Y. Fujisawa, M. Kobayashi, M. Ashikari, Y. Iwasaki, H. Kitano, M. Matsuoka, Rice dwarf mutant d1, which is defective in the alpha subunit of the heterotrimeric G protein, affects gibberellin signal transduction, Proc. Natl. Acad. Sci. USA 97 (2000) 11638–11643.