REVIEW

Toward An Optimum Return From Crop Plants

Xian-Jun Song · Motoyuki Ashikari

Abstract The increasing world population and global climate change have made optimizing crop plant return both crucial and urgent. Furthermore, improved understanding of crop yield potential as well as the implementation of related crop management strategies might be important components of a new green revolution. Notably, grain size (weight) and grain number are both essential components of crop yield potential; great progress has recently been made in understanding the genetic and molecular regulation of these traits. This knowledge would be helpful for improving crop yields using traditional and marker-assisted selective breeding strategies combined with genetic modification of local elite varieties.

Keywords Grain size and grain number · Grain productivity · Genetic and molecular regulator · Crop plants

Introduction

Food security issues have moved to the top of the global agricultural agenda in response to recent soaring global food prices and are a long-term concern for humans worldwide. It is of central importance to enhance crop production in order to feed the increasing world population. Our understanding of global climate issues is incomplete, and climate change will continue in the next decade, which may have profound and direct impacts on global food security [8]. Furthermore, the recent increase in planting of crops for bioenergy at the expense of food production will further complicate future food security [71, 75]. Thus,

breeders throughout the world are currently faced with the challenge of improving crop yields to avoid a potentially severe global food shortage.

Many important agronomic traits, such as crop yield, are controlled simultaneously by multiple genes (namely, quantitative trait loci, QTLs) and are strongly influenced by the environment [38]. Therefore, to dissect such complex traits into several indispensable components would contribute to our understanding of this intractable issue. For example, in rice, the yield components usually contain three major elements: grain weight, number of grains per panicle, and panicle number per plant [20, 67, 81]. Similarly, wheat yield potential is determined by three major components: number of tillers per plant, average number of kernels per spike, and average kernel weight [6]. Undoubtedly, the disaggregation of crop yield into several essential components will provide achievable targets not only for basic research on yield formation mechanisms but also for use in crop yield breeding programs.

In recent years, substantial progress has been made in exploring the molecular basis of plant yield potential. A number of key genetic and molecular regulators of yield component traits, especially seed size and number, have been identified. Examples of these regulators are *Gn1a* [3] and *Ghd7* [81], which control rice grain number, and *GW2* [67] and *qSW5* [65], which primarily affect grain size. These factors have all shown the potential for increasing overall yield. This review aims to summarize the genetic and molecular regulators related to seed size development and to the different number of seeds per panicle.

Control of seed size–weight

In flowering plants, successful seed set depends on the success of double fertilization, which results in the

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formation of a healthy seed and hence the coordinated development of the resultant diploid embryo, triploid endosperm, and maternal ovule (the integuments ultimately constitute the seed coat). Each part of a seed represents a different genetic origin [39, 58]. This unique aspect of the seed makes its developmental pattern different from that of other plant organs, such as the shoot, root, or leaf. In monocots, such as cereals, and some dicots, the endosperm cells contribute the major volume of the mature seed and supply nutrients to the embryo upon germination, while, in *Arabidopsis* and many other dicots, the endosperm cells eventually degenerate and are replaced by the embryo. However, in all cases, the initial growth of the endosperm is the major determinant of seed growth [69]. Moreover, the final sizes of plant organs, including seeds, are primarily defined by internal developmental signals [45, 53, 54]. A summary of our knowledge of genetic and molecular regulation of seed size follows. For convenience, we provide a classification outline of the regulating factors, such as genomic imprinting, plant hormone signals, metabolic signals and developmental pathways, and the RING-type E3 ubiquitin ligase.

Genomic imprinting effects on the control of seed size

As far as the control of seed development and seed size are concerned, genomic imprinting effects are indispensable. Evidence comes from *Arabidopsis* interploidy crosses, in which viable seeds are larger than normal when the paternal genome is in excess, whereas extra maternal genome copies produce smaller viable seeds [48, 58]. Similar results were obtained in reciprocal crosses between wild-type and transgenic DNA methyltransferase gene *MET1* antisense plants [46, 79].

Genomic imprinting is a form of epigenetic modification, in which the alleles of the imprinting genes are expressed differently in the filial generations, depending on whether they are inherited from the male or female parent [27]. In flowering plants, the endosperm is an important site of imprinting [26], and the established function of genomic imprinting is to govern endosperm development before and after fertilization by repressing target gene expression, and thereby influencing seed development; thus, mutations in the imprinting genes usually lead to seed abortion [13, 29, 42, 43]. A key feature of genomic imprinting is that the imprinted alleles carry some form of differential epigenetic mark, such as DNA methylation and/or histone modification [39]. To date, two hypotheses have been proposed to explain endosperm genomic imprinting; one is the parental conflict theory, key points of which involve different patterns in allocation of resources from maternal tissue to offspring

depending on the origin of paternal or maternal alleles [44, 79]; the other hypothesis is a dosage effect, in which seed development primarily depends on a normal ratio of maternal and paternal genomes in the endosperm [64, 79], where any disturbance of the normal ratio will lead to seed abortion or viable seeds of different sizes.

In the developing endosperm, maternal alleles of *Arabidopsis FIS* class genes *MEDEA* (*MEA*), *FERTILIZATION INDEPENDENT SEED2* (*FIS2*), *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*), and *MSI1* are expressed, whereas the paternal alleles are silenced due to imprinting repression [10, 29, 40, 42, 43, 56]. *MEA* and *FIE* encode a SET domain and WD motif protein, respectively, both of which are similar to relevant members of the *Drosophila* polycomb group (PcG) proteins [29, 57]. PcG proteins function in large complexes to methylate histones and modify chromatin, thus maintaining allele-specific suppression of their target genes [27, 60]. A direct interaction was established between MEA and FIE [46]; however, there is no interaction between either MEA or FIE and FIS2, a zinc finger motif-containing protein [47]. Subsequently, the WD-40 domain protein MIS1 was shown to be a component of the MEA–FIE complex that plays an essential role in seed development [42, 43]. However, the maternal expression of *MEA* depends on the activity of *DEMETER* (*DEM*), a helix–hairpin–helix DNA glycosylase, which is exclusively expressed in the central cell and is responsible for the removal of DNA methylation of the maternal *MEA* allele, where the expressed maternal allele of *MEA* mediates its silencing of the paternal allele [13, 27]. Usually, in a cross using the *fis* mutant (*mea, fis2, fie,* and *mis1*) as the female parent, almost all seeds undergo endosperm over-proliferation, embryo arrest, and seed abortion [34]. In animals, PcG complexes regulate genes responsible for cell proliferation. One function of *FIS* is to prevent the onset of central cell proliferation and endosperm development prior to fertilization and to repress endosperm growth and development after fertilization [34].

The only known paternally expressed and maternally repressed gene, *PHERES1* (*PHE1*), which encodes an ancient type I class of MADS-box protein, is under the direct epigenetic control of PcG proteins [42, 43], and the expression of *MEA* is causal to the parent-of-origin-dependent expression of *PHE1* [44]. Hence, it was proposed that the upregulated expression of maternal *PHE1* in the *mea* mutant causes the endosperm and embryo of *mea* seeds to overproliferate after fertilization and eventually abort [42, 43]. Consistent with this, *PHE1* is expressed transiently after fertilization in both the embryo and the endosperm [42, 43]. Furthermore, reduced expression of *PHE1* in a *mea* mutant background can rescue the *mea* seed abortion phenotype [42, 43]. It is noteworthy that reducing *PHE1* expression levels in the *mea* background

using an antisense transgenic strategy cannot only rescue *mea* seed abortion but also results in its transgenic lines bearing heritable and enlarged seeds [42, 43].

Besides the well-studied regulation of *FIS* genes, various other known genes act maternally to control seed size. One example of maternal sporophytic control of seed size development is associated with *transparent testa glabra 2* (*ttg2*) in *Arabidopsis*. The mutant *ttg2* was originally found to be defective for proanthocyanidin synthesis and mucilage deposition in the seed coat [37]. Interestingly, seed size is much reduced in *ttg2/ttg2* seeds, with a 15% reduction in seed length. The reduction of *ttg2* seed size is coupled to a reduction in cell elongation in the integument and also to precocious endosperm cellularization. However, the *ttg2* mutation is proposed to primarily affect cell elongation in the integument [24]. Consistently, *TTG2* encodes a transcription factor of the WRKY family that is expressed strongly in the seed integument and at low levels in the endosperm [24, 37]. As well as *TTG2*, *APETALA2* (*AP2*) is also involved in maternal sporophytic control of seed size in *Arabidopsis*. Apart from its well-defined role in flower development in *Arabidopsis*, however, loss-of-function *ap2* mutations cause the production of seeds that can have twice the mass of wild-type seeds, indicating that *AP2* also plays an important role in determining seed size and weight [36]. The *ap2* mutant embryos are larger and have increased cell numbers and cell size. Reciprocal cross experiments revealed the maternal effect of *AP2* on seed size [58]. *AP2* encodes an AP2/EREBP transcription factor, and the mechanisms by which it carries out its role in regulating seed size are still not fully understood, except that the levels of soluble sugars during seed development point to the possibility of an alteration in sugar metabolism (accumulation patterns of hexose and sucrose; [58]).

Another maternally controlled seed size mutant, *low-beta-amylase1* (*lba1*) was identified in *Arabidopsis* as a missense mutation of the *AtUPF1* gene, yielding seeds that are on average 22% longer and 35% heavier than wild-type seeds [82]. Transgenic expression of wild-type *AtUPF1* in the mutant background restored its seed-size phenotype [82]. AtUPF1 is an RNA helicase, playing a central role in nonsense-mediated mRNA decay (NMD), which specifically recognizes aberrant mRNAs and targets them for degradation ([5], reviewed by Baker [82]). The *lba1* mutant was originally isolated as a mutant exhibiting reduced sugar-induced expression of *Atβ-Amy* [52]. Because the NMD pathway is an mRNA surveillance mechanism, seed-size alteration in the *lba1* mutant is thought to be due to altered posttranscriptional regulation of NMD-targeted gene (s) [82]. Thus, identification of the targeted gene(s) promises to uncover a novel maternal control mechanism underlying seed-size development.

Plant hormone signals influence final seed size

In higher plants, hormones influence numerous aspects of growth and development [15]. The effects that plant hormones exert on seed-size development can be seen in mutants that are defective in plant hormone biosynthesis or perception [53].

Two rice dwarf mutants, *dwarf1* (*d1*; [4]) and *dwarf11* (*d11*; [72]), were identified and found to have very similar seed phenotypes, exhibiting shorter grains with dramatically reduced seed length. At the same time, Fujisawa et al. [23] used transgenic rice with antisense suppression of the *D1* gene that produced dwarf rice plants with a similar seed phenotype to the *d1* mutant. The *d1* mutant has phenotypes characteristic of GA deficiency [59], and *D1* is the only gene in rice encoding a prototypical heterotrimeric G protein α-subunit, which presumably functions in the GA signal transduction pathway [4]. The G protein is thought to transmit the gibberellin signal(s) responsible for the normal development of rice internodes and seeds [23], whereas *d11* is a novel cytochrome P450 gene mutant implicated in brassinosteroid (BR) biosynthesis [72]. However, mechanisms underlying seed-size regulation are still unclear for both mutants. Other BR-biosynthesis-defective mutants in rice, such as *ebisu dwarf* (*dwarf2/d2*; [33]), *BR-deficient dwarf1* (*brd1*; [32]), and *BR-deficient dwarf2* (*brd2*; [31]), also bear smaller seeds than the wild type; therefore, GA signal transduction and the BR biosynthesis pathway affect crop grain size.

Another mutant in *Arabidopsis*, *megaintegumenta* (*mnt*), which increases seed size and weight, revealed a link between auxin signaling and seed-size control [63]. Extra cell division in the integuments surrounding *mnt* mutant ovules, resulting in the formation of enlarged seed coats, was proposed to be a major driving force for increased seed size and weight (i.e., integument-led seed growth; [63]). This may in part share similar mechanisms with the rice *GW2* gene in controlling seed size and weight [67]. Both cases provide an indication of the importance of growth of the ovule before fertilization in determining final seed size [63]. The *mnt* mutant phenotype is caused by a complete loss of function of the *AUXIN RESPONSE FACTOR 2* (*ARF2*), which encodes a transcription factor mediating gene expression in response to auxin. Future identification of the direct target(s) of *ARF2* will help to clarify the entire scenario.

Cytokinin signals may also exert an influence on seed-size development in higher plants. Evidence comes from experiments in which enhanced cytokinin breakdown occurred in genetically engineered transgenic plants. The mature viable seeds of engineered transgenic *Arabidopsis* plants that individually overexpress two cytokinin oxidase–dehydrogenase genes, *AtCKX1* and *AtCKX3*, were both

greatly enlarged and approximately twice the weight of wild-type seeds [77]. The enlarged transgenic seeds show a similar increase in embryo size attributable to increases in both cell number and cell size [77]. Similarly, plants carrying the combined mutations of three cytokinin receptor genes, AHK2, AHK3, and CRE1/AHK4, bear seeds that are more than twice as large as those of wild-type plants, primarily due to an increase in the size of their corresponding embryos. This is proposed to be a direct effect of loss of receptor functions and their role in growth control [61]. However, genetic data indicate that the embryo size alteration of the triple-cytokinin-receptor mutant is largely under maternal and/or endospermal control [61].

New studies on an Arabidopsis da1 mutant have established a link between abscisic acid (ABA) signals and seed-size regulation. Compared to the wild type, the average seed weight of da1 increased up to 32%, and total seed yield per plant increased more than 10% [45]. Moreover, molecular cloning of the DA1 gene indicates that it encodes a predicted ubiquitin receptor, and overexpression of the mutant da1 allele in the wild-type background leads to significantly larger seeds than in the wild type [45]. Expression of DA1 can be induced by ABA, and sensitivity to ABA is greatly reduced in the mutant da1, indicating that ABA signaling may be involved in plant seed-size control. In addition, the DA1 gene may control seed size by restricting the duration of cell division [45], as it has been previously shown that seed ABA content influences cell cycle arrest [21], suggesting the importance of future investigations of DA1 regulation of seed size and development.

RING-type E3 ubiquitin ligase is involved in control of grain size and crop yield

The "really interesting new gene" (RING) finger proteins are a newly defined family containing a characteristic conserved RING finger motif and play important roles in many cellular processes, including cell cycle progression, oncogenesis, signal transduction, and development [68]. Since first being recognized in 1991, the family has increased to >200 proteins across a diverse group of eukaryotes [68]. A number of RING-type proteins function as E3 ubiquitin ligases, recruiting the target proteins for ubiquitin-dependent degradation by the 26S proteasome [19, 35, 62, 80].

A newly identified RING-type protein, GW2, was reported to contribute to the molecular regulation of grain width and weight in rice [67]. In a near isogenic line (NIL; GW2) harboring a large grain allele, grain width increased 26.2% compared to the small grain parental line. Accordingly, the 1,000-grain weight in NIL (GW2) showed

a significant increase of up to 49.8%. Most strikingly, the grain yield per plant of NIL (GW2) increased by 19.7%, although a tradeoff between grain size and grain number occurred, where the number of grains per main panicle was reduced by 29.9% in NIL (GW2). Nevertheless, GW2 is a useful locus for high-yield crop breeding [67].

Map-based cloning delimited GW2 to an 8.2-kb region on chromosome 2 in which only one predicted open reading frame was found, and subsequent sequence analysis identified a 1-bp deletion resulting in a premature stop codon in exon 4 of the large grain allele, which was proposed to explain the natural grain size variation between parental lines [67]. GW2 encodes a RING domain protein with in vitro E3 ubiquitin ligase activity, and transgenic plants expressing different levels of GW2 can significantly alter the grain size of recipient lines [67]. A reasonable explanation for natural allelic variation is that the truncated protein (encoded by the large grain allele) has lost its substrate-binding domain [67], and future identification and characterization of its target substrate(s) might reveal more information about this type of regulation. Furthermore, data from histological analyses of spikelet hulls before heading suggest that loss of GW2 function increased cell number, and thus the wild-type GW2 negatively regulated cell division by targeting its substrate(s) to proteasomes for proteolysis [67].

To rule out the possibility that increased grain size is associated with lower grain quality, several rice grain quality indices were tested, and the results revealed no reduction in cooking or eating quality in NIL (GW2) grains [67]. Moreover, GW2 homologs have been found in maize (Zea mays) and wheat (Triticum aestivum); therefore, a crop variety with a suitable grain size could be obtained by genetically modifying GW2 [50].

Metabolic signals and developmental pathways: crucial regulators of final seed size

As described previously, ap2 mutations result in increased embryo cell number and cell size, probably via metabolic alteration of hexose to sucrose ratios, and thus play a role in controlling seed mass in Arabidopsis [58]. Consistent with this, studies with Vicia faba (fava bean) have suggested that large and small variations in seed size and weight are mainly due to a discrepancy in the carbohydrate status of developing seeds, which is closely correlated with accumulation patterns of cell-wall-bound invertase (VfCWINV1) activity and its mRNA levels, in agreement with mitotic activity of the developing cotyledons [76]. A longer cell division phase in the "large" embryo is associated with a prolonged phase with a higher ratio of hexose to sucrose [76]. In V. faba, VfCWINV1 is mainly expressed in the

unloading area of the seed coat and is critical to supplying hexoses to fast-dividing embryo and endosperm tissues [7]. Hence, the coordinated regulation of embryo development with that of the maternal seed coat using metabolic signals controls embryo cell division and, consequently, seed size [76].

The first plant invertase-deficient mutant, *miniature1* (*mn1*), was identified in maize (*Z. mays*; [51]). Compared to the wild type, the *mn1* endosperm has only 55% of the cell number and 25% of the endosperm volume, indicating that the small size of the *mn1* endosperm is due to a combined effect of impaired cell proliferation and reduced cell size [74]. It has been established that the number of cells formed in the cotyledons or the endosperm is equal to the capacity of the storage organ to accumulate dry matter [76]. Thus, the *mn1* mutant is marked by a drastic reduction in endosperm weight relative to that of the wild type [12]. The *Mn1* gene encodes an endosperm-specific isoform of cell-wall-bound invertase, INCW2, which functions in providing hexoses for mitoses, creating appropriate sugar signals [11]. Conversely, INCW2 deficiency may cause a prematurely high ratio of sucrose to hexose in the endosperm, which may favor cell differentiation and starch accumulation over cell division [74].

Comparisons of the activities of the carbon-metabolizing enzymes, sucrose synthase (SuSy; EC2.4.1.13), UDP-glucose pyrophosphorylase (UGPase; EC2.7.7.9), and ADP-glucose pyrophosphorylase (AGPase; EC2.7.7.27), during seed development between two genotypes of *Vigna radiata* (mung bean) differing in seed weight were carried out, and the results showed that these enzymes follow a similar pattern; that is, compared to those in small seeds, enzyme activities in large seeds were maintained for a longer period of time [14]. In maize, mutation of the endosperm-specific starch synthetic enzyme gene *Shrunken2* (*Sh2*) increased mature seed weight by 11–18% [28]. *Sh2* encodes the large subunit of the heterotetrameric starch synthesis enzyme, adenosine diphosphoglucose pyrophosphorylase (AGP; EC2.7.7.27), and its mutant had reduced sensitivity to phosphate, an inhibitor of AGP, giving rise to increased seed weight [28]. Interestingly, the increase in seed weight is not associated with a reduced number of seeds per ear [28]. In addition, transgenic wheat ectopically expressing a modified form of the maize *Sh2* gene (*Sh2r6hs*) exhibits increased AGP activity in developing seeds and produces an average of 38% more seed weight per plant [66].

Mutations of cytosolic glutamine synthetase (GS1) cause reduced seed size, highlighting the importance of nitrogen (N) metabolism in the control of grain production. In maize, mutation of the GS1 isoenzyme gene *Gln1-4* leads to a series of reductions in the *Gln1-4* transcript, the amount of expressed GS1 protein, and GS1 activity and, consequently, causes a specific decrease in grain size and production [49].

The Gln1-4 protein is localized to the bundle sheath cells in leaf tissue and is proposed to have a catabolic function in the reassimilation of ammonium released during protein breakdown to provide Gln for kernel growth during grain filling [49]. Consistent with this, the rice knockout mutant of the GS1 homologous gene *OsGS1;1* results in a severe reduction in grain filling and thus grain size and production [70]. However, the *OsGS1;1* gene has pleiotropic effects on plant development, including plant height, leaf blade length, panicle size, and grain filling [70], whereas the maize *Gln1-4* gene is specifically involved in kernel development [49].

The isolation of two small *Arabidopsis* seed mutants enabled the identification of two sporophytically acting genes, *HAIKU2* (*IKU2*) and *MINISEED3* (*MINI3*), located on chromosomes 3 and 1, respectively [48]. Cloning of *IKU2* and *MINI3* enabled the characterization of a leucine-rich repeat (LRR) kinase gene and a WRKY class transcription factor, *WRKY10*; interestingly, expression pattern analyses indicated that both genes, and another mutant locus, *iku1* [25], may function in the same regulatory pathway governing seed-size development [48]. Compared to the wild type, *iku2* and *mini3* mutants exhibit a series of similar phenotypes, such as precocious cellularization in endosperm development, retardation in embryo development, and reduction of embryo size [48]. More crucially, both mutant homozygotes can bear viable seeds and are comparable to the wild type in silique size and number of seeds per silique [48]. Coincidently, mutations in another *Arabidopsis* putative serine–threonine LRR receptor kinase gene, *EXS*, caused strikingly reduced seed size [9]. Consistent with the smaller seeds of *exs*, these seeds develop slowly, and development of the embryos of smaller seeds is significantly delayed by specifically reducing the cell size [9]. Also, endosperm development in *exs* mutants is significantly slower than in the wild type [9]. Thus, the *EXS* gene may act to enhance growth in the embryo and endosperm so as to influence final seed-size determination.

Regulators of cell division and gene transcription influence seed–fruit size

The control of cell division during organogenesis plays a crucial role in determining the intrinsic final size of organs in higher plants [54]. The role of regulators of cell division can be seen in the case of the *Arabidopsis* regulatory gene, *AINTEGUMENTA* (*ANT*), which is proposed to act as an organ size checkpoint, regulating intrinsic organ (including seed) size by maintaining meristematic competence [54]. Ectopic expression of the 35S::*ANT* transgene displayed multiple organ (including seed) hyperplasia, resulting mainly from an increase in cell number in both *Arabidopsis* and tobacco plants. This increase results from an extended

period of cell proliferation and organ growth. Conversely, loss-of-function *ant* mutants exhibit defective integument and female gametophyte development [41]. *ANT* encodes a plant-specific AP2 transcription factor, and while the mechanism by which it affects seed size is still undetermined, it presumably acts in part through regulating cell division in integuments [41].

The first QTL isolated from plants and known to control tomato fruit size, *fw2.2*, is an established regulator of cell division [16]. *fw2.2* could explain approximately 30% of the fruit weight transition from modern large-fruited cultivars to wild small-fruited relatives, by encoding a RAS-like G-protein that presumably modulates fruit size by controlling carpel cell number before anthesis [22]. Subsequent studies using the small- and larger-fruited NILs indicated that heterochronic regulatory mutations are sufficient for explaining allelic differences in *fw2.2* [18]. Also, beyond a primary and direct effect imparted by *fw2.2* on fruit size, it also modifies sink–source relationships at the whole-plant level, bringing changes to fruit number, inflorescence number, and flower–fruit abortion rates [55]. Therefore, allelic differences at *fw2.2* were found to have no obvious effects on overall yield changes in field tests [55, 73].

Apart from *fw2.2* regulating cell division, which contributes to drastic fruit size alterations in tomato plants, changes in organ (locule) number of tomato fruits represent another big step in the evolution of extreme fruit size [17, 73]. Recently, a major locus, *fascinated*, was isolated, and it was found that allelic variation underlying phenotype change points to a regulatory change in a YABBY-like transcription factor [17]. Because the increase in locule number can cause up to a 50% increase in fruit size, the identification and characterization of the *fascinated* gene holds great promise for fine-tuning tomato fruit size in breeding programs [17, 73].

Genes with no currently assigned biochemical functions are associated with seed size increases and yield improvement

With more and more genes associated with agriculturally important traits being identified in recent years, the number of genes with no assigned biochemical function has also increased. One major QTL for rice grain width, *qSW5* (*QTL for seed width on chromosome 5*), was recently isolated and characterized [65]. A field test showed that NIL(*qSW5*) can cause a >10% grain yield reduction because of reduced grain size [65]. Although no definable biochemical function has currently been assigned to *qSW5*, experimental data showed that a deletion has caused a marked increase in seed width and mass by increasing the number of cells in the outer glume of the rice flower [65].

Molecular regulators of grain number traits

Grain number is also a trait of great interest because of its direct contribution to grain productivity. Although compensation effects, i.e., negative correlations, are often observed between grain size and grain number [1, 30, 55, 58, 67], one example of a primary grain-number-regulating QTL, *Gn1a*, was found to be a regulator of grain yield per plant [3], indicative of a useful locus for increasing grain productivity.

In rice, a QTL for grain number per panicle, *Gn1*, was mapped on chromosome 1 and later found to consist of two loci, QTL*Gn1a* and QTL*Gn1b*, with a single QTL*Gn1a* locus increasing grain number by 21% [3]. *Gn1a* encodes a gene for cytokinin oxidase–dehydrogenase (*OsCKX2*), which can effectively degrade cytokinin; hence, reduced expression of *OsCKX2* is causal to higher accumulated cytokinin in inflorescence meristems and to increased grain number, leading to a higher yield potential [3]. Moreover, two agriculturally useful traits, grain number (*Gn1* locus) and plant height (*sd1*, the rice Green Revolution gene), have been successfully combined into a common genetic background (QTL pyramiding), thereby generating a new superior variety having both beneficial traits [3].

Also in rice, a single locus (QTL*Ghd7*) was recently identified on chromosome 7 and confirmed to simultaneously exert major effects on three classes of agriculturally important traits including grain number, plant height, and heading date [81]. Of note, NIL plants carrying the large grain-number allele increased grain weight per plant by 50.9%, mainly due to increased grain number per panicle [81]. Cloning, transgenic, and expressional analysis of *Ghd7* showed that this locus corresponds to a CCT (CO, CO-like, and TIME of CAB1) domain protein and functions in the photoperiodic flowering pathway upstream of *Ehd1* and *Hd3a* [81], implying an alteration of panicle size and grain number as a consequence of *Ghd7* response to photoperiod. In addition, under long-day conditions, elevated *Ghd7* expression increases plant height and panicle size and thus grain number, with a concomitant significant delay in flowering [81]. Nevertheless, the use of *Ghd7* alone in breeding practices could not simultaneously improve both heading date and crop-yield traits.

In addition, metabolic regulators also play important roles in determining grain number in plants. Transgenic plants with the pSh2::Sh2rhs construct in wheat enhanced seed AGP activity, resulting in an increase in yield (see above). This increased yield is primarily due to the increase in seed number per plant [66]. Since AGP functions to catalyze the rate-limiting reaction in starch biosynthesis in plants, the results imply that genetic modification of the corresponding regulating enzymes along this pathway helps to increase yield in crop plants.

Like *Gln1-4*, another maize GS1 isoenzyme gene, *Gln1-3*, also functions in the control of grain production. In the knockout mutant *gln1-3*, kernel number is the yield component that is preferentially affected [49]. Conversely, when *Gln1-3* was constitutively overexpressed in leaves, an increase in the amount of GS1 protein and a ~30% increase in grain yield were observed; moreover, this increase in grain yield was proportional to the increase in GS1 enzyme activity [49]. Additionally, Gln1-3 was localized to mesophyll cells and is proposed to provide sufficient N assimilates to the developing ear to avoid kernel abortion [49]. In the double mutant *gln1-3gln1-4*, a cumulative effect exists; that is, both grain size and grain number were reduced [49]. Thus, increasing N-use efficiency is a potential tool for improving yield in crop plants.

Conclusions and perspectives

An increasing number of key regulators of grain size and grain number have recently been cloned and functionally characterized. Numerous lines of evidence point to diversified pathways contributing to the control of both traits, and this control may involve some overlap of several pathways. The overlap can also be seen in a recent finding in which transgenic rice plants with elevated BR levels exclusively in the stems, leaves, and roots bear more and heavier seeds than wild-type plants, resulting in 15% to 44% increases in grain production per plant [78]. Interestingly, these phenotypic changes were associated with alterations of CO_2 assimilation and sugar metabolic signals in the flag leaves [78], suggestive of multiple-aspect regulation of grain weight and grain number. Additionally, cross talk between maternal and zygotic control of seed size has been reported [24].

Tremendous efforts have been made to enhance our knowledge of the molecular regulation of both grain size and grain number, and several examples that exhibit successful yield-improving potential have been provided; however, these findings provide only an initial step toward a thorough understanding of crop yield formation. Therefore, in future investigations, apart from improving our understanding of existing regulating factors and fully exploiting these for practical breeding programs, it is necessary to identify and characterize many more novel factors involved in the control of seed size and seed number. To this end, several aspects have been highlighted here. First, it will be helpful to create several suitable exotic genetic libraries as starting materials [83]. Second, emerging genetic approaches should be adopted in order to promote and facilitate crop improvement [71]. In particular, QTL pyramiding provides a useful strategy for effective crop breeding [2, 71].

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