REVIEW PAPER

Designing climate-resilient rice with ideal grain quality suited for high-temperature stress

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Abstract

To ensure rice food security, the target outputs of future rice breeding programmes should focus on developing climate-resilient rice varieties with emphasis on increased head rice yield coupled with superior grain quality. This challenge is made greater by a world that is increasingly becoming warmer. Such environmental changes dramatically impact head rice and milling yield as well as increasing chalkiness because of impairment in starch accumulation and other storage biosynthetic pathways in the grain. This review highlights the knowledge gained through gene discovery via quantitative trait locus (QTL) cloning and structural–functional genomic strategies to reduce chalk, increase head rice yield, and develop stable lines with optimum grain quality in challenging environments. The newly discovered genes and the knowledge gained on the influence of specific alleles related to stability of grain quality attributes provide a robust platform for marker-assisted selection in breeding to design heat-tolerant rice varieties with superior grain quality. Using the chalkiness trait in rice as a case study, we demonstrate here that the emerging field of systems genetics can help fast-track the identification of novel alleles and gene targets that can be pyramided for the development of environmentally robust rice varieties that possess improved grain quality.

Key words: Chalk, functional genomics, genetics, grain quality, milling and head rice yield, stress tolerance, systems biology, systems genetics.

Introduction

The world's rice (*Oryza sativa* L.) production is predicted to be severely affected by the global rise in temperature associated with climate change (Neelin *et al.*, 2006). Furthermore, the global population is expected to grow to 9 billion by 2050 (Godfray *et al.*, 2010). To feed the rapidly growing Asian population (projected to increase from 4.3 billion to 5.2 billion by 2050) that depends on rice as a staple food, paddy yield should not just be enhanced, but grain quality requirements need to be met to ensure consumer acceptance. Consumers primarily assess rice grain quality based on physical (milled

yield, translucency, size, shape, and colour) and sensory properties, with a strong emphasis on cooking quality and aroma (Ebron, 2013). Environmental stresses not only affect milling yield, but they also lead to a significantly elevated proportion of chalky grains which in turn alters starch and cooking quality (Cooper *et al.*, 2003; Cheng *et al.*, 2005*b*; Fabre *et al.*, 2005; Lanning *et al.*, 2011, 2012; Li *et al.*, 2011; Lanning and Siebenmorgen, 2013). Thus abiotic stresses, in particular high temperatures, have a negative effect on various grain quality traits (Fig. 1) and they also reduce sensory attributes (Chun

Abbreviations: Amy, amylase; AC, amylose content; DAF, days after flowering; FLO, floury-endosperm; GC, gel consistency; GIF, grain incomplete filling; GBSS, granule-bound starch synthase; GS, grain size; GT, gelatinization temperature; GW, grain weight; GWAS, genome-wide association studies; HRY, head rice yield; MRY, milled rice yield; PGWC, percentage of grain with chalkiness; QTL, quantitative trait locus; SBE, starch branching enzyme; SNP, single nucleotide polymorphism; SS, starch synthase.
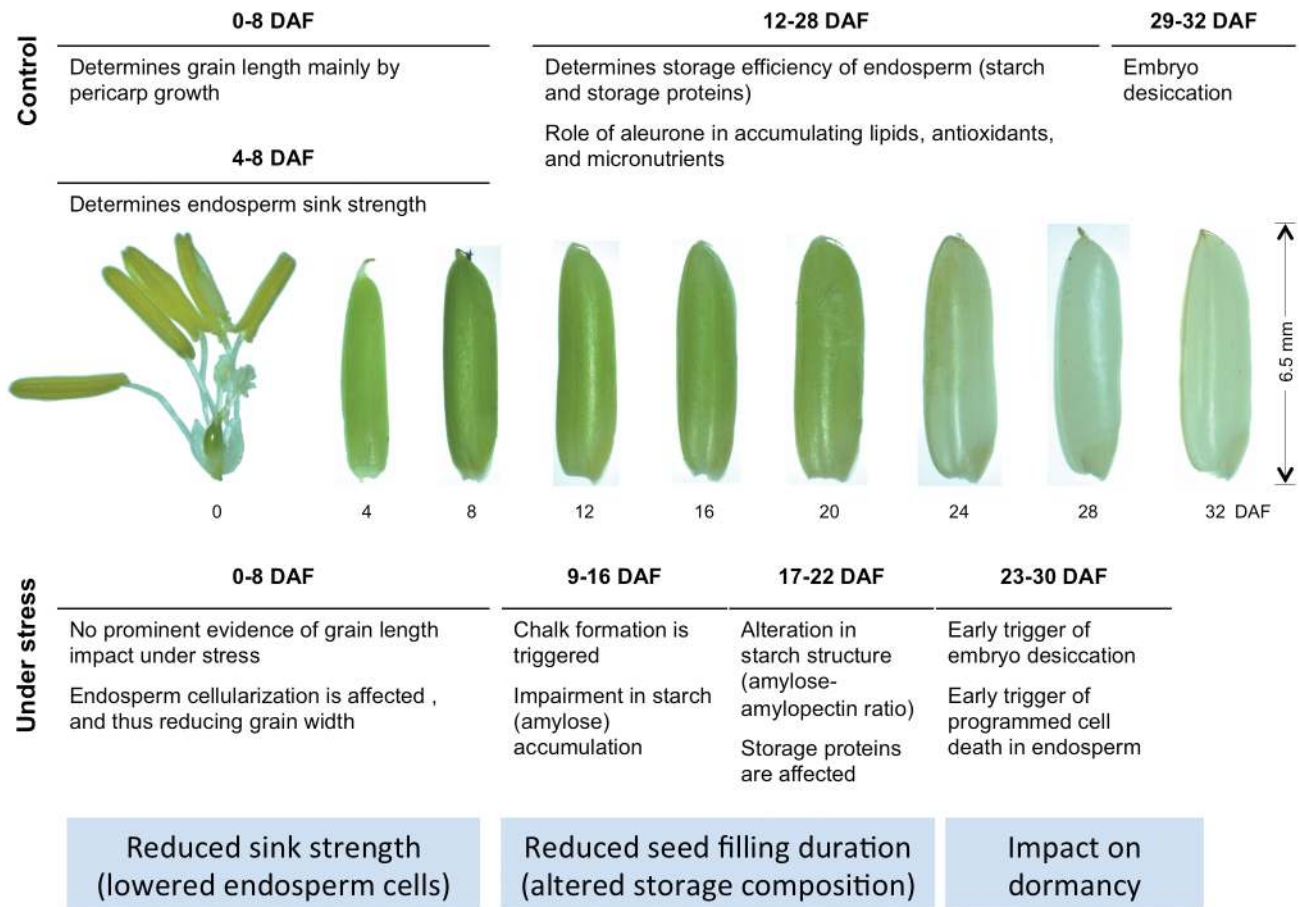
Fig. 1. The ontology of seed development covering import phase transitions. High temperature stress-induced perturbations occurring during seed development affect the grain quality, cooking quality, and eating quality of rice.

et al., 2009), which leads to a reduction in overall consumer acceptance. Excellent reviews are available that describe rice grain quality from a traditional biochemistry perspective (Champagne, 2008; Fitzgerald *et al.*, 2008, 2009; Champagne *et al.*, 2010; Cuevas *et al.*, 2010; Huang *et al.*, 2013; Liu *et al.*, 2013; Siebenmorgen *et al.*, 2013; Zhao and Fitzgerald, 2013). However, the implications of abiotic stresses on grain quality and its associated molecular and physiological mechanisms are not yet fully understood. This review will focus on the impact of heat stress perturbations on grain quality with special emphasis on enhancing head rice yield (HRY; intact grain after milling) and reducing chalk (the opaque area in the rice grain).

Compared with that of other crops, the productivity of rice is more accurately measured after milling (Lyman *et al.*, 2013). Although annual world rice production reaches 750 Mt of paddy (http://faostat.fao.org/site/339/default.aspx), the final milled rice yield (MRY; including head rice and broken grains) corresponds to 490 Mt. This implies that as many as 260 Mt year^{-1} are lost after milling (including hull and bran). Furthermore, the cost of broken rice is significantly undervalued in the world market. This can be avoided with the availability of rice varieties whose grains are not prone to breakage during post-harvest storage and processing. To improve food security and rice quality, rice breeders need to prioritize improving HRY (mass percentage of intact whole rice after milling) over MRY (mass percentage of milled rice

including both whole grains and broken grains) at the global production level (Juliano, 1998, 2003). However, improving this parameter in rice varieties is complicated because it is negatively affected by environmental stresses such as exposure to high night temperature, extreme day temperatures, and severe drought (Lyman *et al.*, 2013; Siebenmorgen *et al.*, 2013; Zhao and Fitzgerald, 2013; Usui *et al.*, 2014). The significant environmental effect on HRY is mostly due to high-temperature stress during the sensitive phase of gametogenesis (Cooper *et al.*, 2008; Jagadish *et al.*, 2010; Muthurajan *et al.*, 2011; Lyman *et al.*, 2013). Milling quality attributes are also influenced by unsynchronized flowering and seed filling, chemical properties of the rice grain, as well as abrupt changes in moisture content during harvest and storage (Juliano, 2007).

Chalk is one among many other variable parameters that influence MRY and HRY (Fitzgerald and Resurreccion, 2009; Lanning *et al.*, 2011). It is defined as the opaque portion found in an otherwise translucent white endosperm that is associated with loose packing of storage starch and protein (Lanning *et al.*, 2011; Lyman *et al.*, 2013; Usui *et al.*, 2014). The occurrence of temperature stress during the early to middle stage of seed development triggers non-uniform filling and impairment in storage biosynthesis, leading to chalk formation. The gaps formed due to aborted starch granule formation are thought to be responsible for making chalky grains more brittle and for forming fissures along the grain.

As a result, chalky grains crack easily during grain processing, which reduces HRY as a consequence of the elevated amount of broken grains (Lisle *et al.*, 2000). Despite these generalizations, however, a clear association between chalk and breakage susceptibility during milling still needs to be empirically established.

The recent progress made in the area of genetics and genomics, and the body of knowledge surrounding the molecular and physiological mechanisms associated with perturbations to temperature stress during rice seed development in the light of how grain quality in rice is affected have not been systematically reviewed. In this review, we highlight the strategies to develop rice grains with improved grain quality particularly in improving HRY and minimizing chalkiness as key quality parameters to improve rice food security under a warmer climate by integrating the knowledge on genetics and genomics gained thus far using a systems genetics approach.

Unravelling the genetic basis of head rice yield and chalk

Rice HRY is a complex, multigenic trait genetically controlled primarily by the triploid endosperm and the diploid maternal tissues (Pooni *et al.*, 1992; Zhu and Weir, 1994). Several quantitative trait loci (QTLs) have been identified for milling quality in different rice mapping populations using seeds with variable grain shapes (Dong *et al.*, 2004; Jiang *et al.*, 2005; Kepiro *et al.*, 2008; Yuan *et al.*, 2010; Nelson *et al.*, 2011). In addition, 14 QTLs were recorded for grain fissuring (Septiningsih *et al.*, 2003; Pinson *et al.*, 2013). These previous studies revealed that grain size and shape are highly correlated with HRY and quality (Zheng *et al.*, 2007). Although increased grain length is negatively associated with HRY, targeted improvement in grain width and thickness was shown to improve MRY and HRY (Siebenmorgen and Meullenet, 2004). Thus, HRY in slender grains needs to be improved for consumers who prefer long grains.

The genome view of mapped grain quality QTLs suggests that the genetic regions responsible for HRY and chalk overlapped with grain size and shape QTLs on chromosomes 3, 5, and 6 (Supplementary Fig. S1, Table S1 available at *JXB* online). Among the cloned genes related to grain size and shape, *GS3*, *GW2*, and *GW5/qSW5* act as negative regulators of grain size, while *GS5* and *GW8* act as positive regulators of cell proliferation in rice. Genes associated with grain size, shape, and weight belong to selective proteolysis, and key regulators of cellularization and brassinosteroid signalling (Fan *et al.*, 2006; Song *et al.*, 2007; Shomura *et al.*, 2008; Huang *et al.*, 2013; Sreenivasulu and Wobus, 2013). Until now, the haplotypic variations that contribute to the stability of grain size under abiotic stress exposure in domesticated rice have not been explored. Furthermore, the underlying QTL regions for milling quality have yet to be fine-mapped and cloned to unravel the interconnections between grain dimension genes. Factors such as genotype×environment (G×E) interactions are known to affect rice milling quality (Gravois *et al.*, 1991), and therefore a comprehensive understanding of MRY and

HRY requires the identification of QTL alleles and/or G×E interactions. If heterogeneity occurs in grain size and shape because of G×E interactions, mill settings need to be optimized for every variety and production environment. Thus, achieving stability of grain dimension traits continues to remain important for the milling industry.

The genetic components underlying the inter-relationships between various grain quality parameters and milling quality remain unclear. Interestingly, many QTLs for HRY overlapped with QTLs for regions of pre-broken brown rice kernels, seed density, amylose content (AC), kernel whiteness, and chalkiness on chromosome 6 (Fig. 2A). In addition, clusters defined on chromosome 8 are emphasized as the main genetic basis for the effect of rice chalkiness, amylose, protein, and eating quality of cooked rice (Guo *et al.*, 2007; Liu *et al.*, 2011). These different QTLs co-located on the same region influence various grain quality, cooking, and eating quality traits in rice. Fine-mapping these hotspot QTLs can offer a huge potential to identify high-value genes that could be tapped to improve milling, cooking, and sensory and processing quality. This can be done by molecular breeding to address the demands of the rice industry and rice consumers.

Genetic basis of chalk

More than 140 QTLs were reported for the chalkiness trait across all 12 chromosomes, mostly among Asian cultivars (Tan *et al.*, 2000; Li *et al.*, 2003; Wan *et al.*, 2005). In a recent study, several QTL clusters related to various chalk phenotypes have been identified using five different mapping populations that are stable across two environments (Peng *et al.*, 2014*a*). The *percentage of grain with chalkiness* (*PGWC*) has been genetically mapped on chromosomes 5, 8, and 10, which explains 50.8% of the genetic variation (Liu *et al.*, 2012). Stable QTLs with a reproducible chalky phenotype across many environments have been fine-mapped to regions on chromosomes 8 and 9 (Wan *et al.*, 2004, 2005). Furthermore, using a set of chromosome segment substitution lines developed from a cross between cultivar C-51 (chalky endosperm) and the recurrent parent 93-11 (translucent endosperm), two additional loci that control PGWC located on chromosomes 6 and 7 were detected and designated as *qPGWC-6* and *qPGWC-7*, respectively (Zhou *et al.*, 2009). The chalk QTL *qPGWC-7* contains 13 genes related to several unknown proteins, including COBRA-like proteins. The *qPGWC-6* QTL map position is closer to the *Waxy* gene, and thus variable AC appears to affect chalk (Zhou *et al.*, 2009). Within the proximity of the *Waxy* locus, eight chalk QTLs have been co-located (Peng *et al.*, 2014*a*).

Chalkiness is reported to be influenced by multiple QTLs, though some of these QTLs may not be reproducible because the genetic backgrounds used in some of the studies to generate the bi-parental mapping populations are variable for grain quality parameters such as AC and grain width. So far, fine-mapping studies identified the following as possible candidate genes responsible for chalk: *pyruvate orthophosphate dikinase* (Kang *et al.*, 2005), *starch synthase IIIa* (Fujita *et al.*, 2007*a*), *UDP-glucose pyrophosphorylase* (Woo *et al.*, 2008), *cell wall*
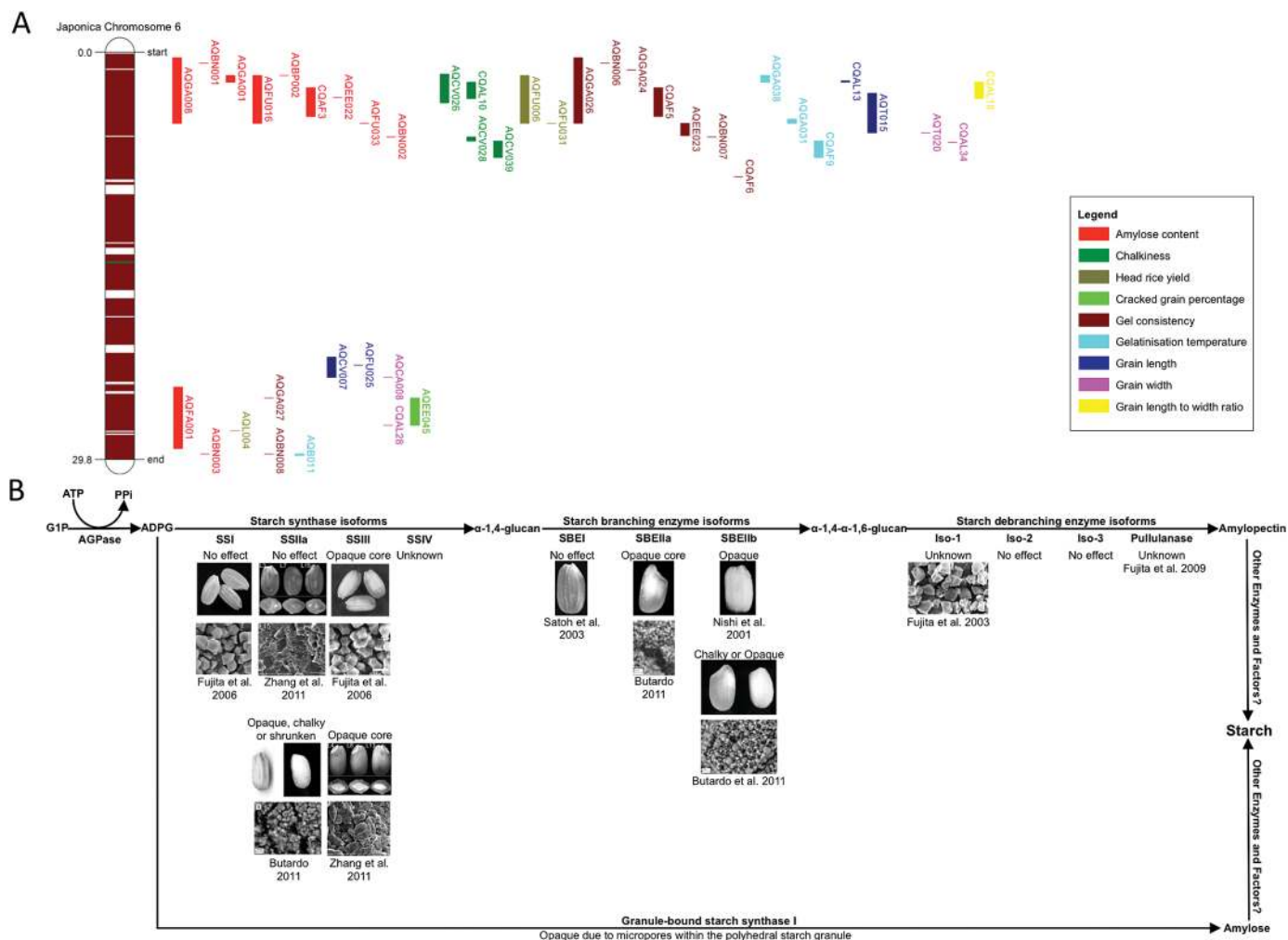
Fig. 2. The grain quality QTL hotspot in rice chromosome 6 and the effect of gene perturbation in the starch biosynthesis pathway. (A) Selected QTLs for grain quality traits on *japonica* chromosome 6 located in syntenic regions between *indica* and *japonica* reference genomes. Chromosome 6 is a grain quality hotspot as multiple grain quality QTLs related to amylose, chalk, head rice yield, gel consistency, gelatinization temperature, and grain dimensions and shape are co-located on both arms of the chromosome. The accession IDs of these grain quality QTLs are listed in Supplementary Table S1 at *JXB* online. (B) Gene perturbation in the starch metabolism pathway. Perturbations in the starch metabolism pathway involving *starch synthase III* and *starch branching enzyme IIa* and *IIb* lead to alterations in amylose–amylopectin composition and starch structure variation (Butardo, 2011). The proposed molecular and physiological mechanisms of chalk formation are a complex system associated with source–sink disturbances. However, the initial genetic evidence points to the disturbance of the storage pathways in developing seeds.

invertase (Wang *et al.*, 2008), and *H+-translocating pyrophosphatase* (Li *et al.*, 2014). How these genes interact to produce the chalky grain phenotype leading to grains susceptible to cracking and reduced milling potential or lowering HRY has not yet been studied.

Novel approaches can be employed to fast-track the design of novel rice ideotypes with superior grain quality and exhibiting chalk-free features under stress. The strengths of a systems genetics approach (combining systems biology and traditional genetics approaches) can be used to unravel structural–functional relationships by tapping the genetic diversity represented by rice core collections or the detailed information that can be obtained from high-density mapping populations. As a proof of concept, we explored rice re-sequencing resources for synteny between *japonica* and *indica* subspecies using the recently cloned chalk gene *H+-translocating pyrophosphatase* (Li *et al.*, 2014) as well as several grain

quality QTLs identified on chromosome 5. We found that they are located in collinear blocks (Fig. 3). Interestingly, hotspots for higher SNP frequency were observed either between the subtypes or within the *indica* re-sequencing genetic region (Fig. 3). Overlaying regulatory networks identified using a top-down systems biology approach using the PLANET database (Mutwil *et al.*, 2011) suggested that the expression variation created between cereal species appears to cause functional divergence for the endosperm-specific *H+-translocating pyrophosphatase* gene responsible for chalk (Fig. 4). The derived gene regulatory network of the *H+-translocating pyrophosphatase* gene in rice seems to control the selective proteolysis pathway (E3.SCF) during 4–6 days after flowering (DAF). On the other hand, its orthologue in wheat (Ta.1322.1.S1_x_at) regulates thousands of genes involved in starch biosynthesis and protease inhibitors during seed filling (Supplementary Table S2 at *JXB* online).
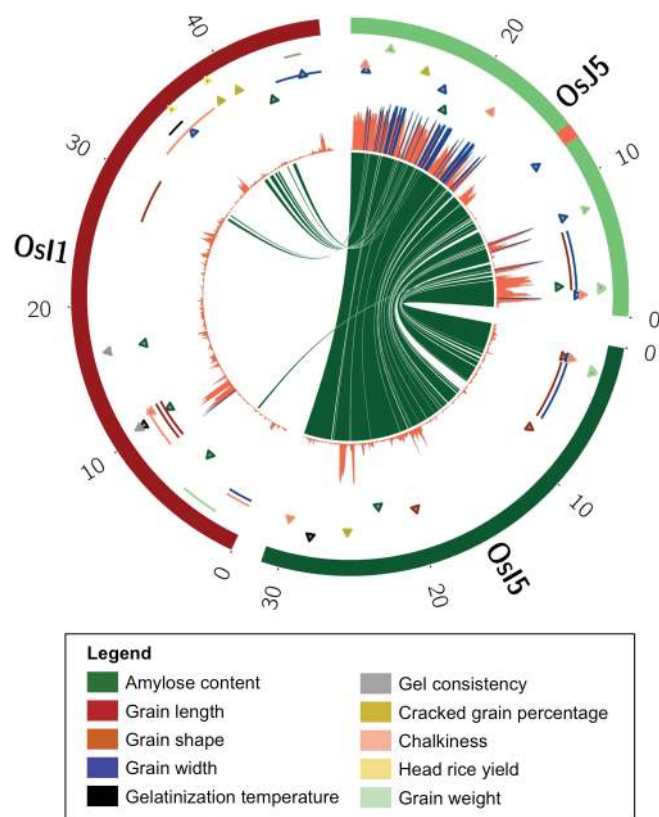
Fig. 3. Synteny between *japonica* chromosome 5 (OsJ5) and related chromosomes in *indica* (OSI1 and OSI5). This figure shows the syntenic blocks between chromosome 5 of *O. sativa japonica* with chromosomes 1 and 5 of *indica* (innermost section of the figure). The bar plot shows SNP density in the genomic region based on SNPs common to five *indica* re-sequenced genomes. Blue lines represent SNP densities ≥5 per kb, while orange lines represent SNP densities <5 per kb. SNP density was calculated separately for every 100 kb. Mapped grain quality QTLs are shown next to the SNP density bar plots. Mapped QTLs whose lengths are between 1 Mb and 5 Mb are represented as lines, while fine-mapped QTLs (size <1 Mb) are represented as triangles. Genomic hotspots that have the highest SNP densities can be found on chromosomes 1 and 5 of *indica* as well as *japonica*. These hotspots coincide with several grain quality QTLs, including the recently cloned chalk gene *H+-translocating pyrophosphatase*.

Such comprehensive information can be mined by combining genome-wide association studies (GWAS) together with gene co-expression networks through systems genetics approaches (Ficklin and Feltus, 2013), to clone both major and minor QTL-encoding genes of grain quality and characterize their functions from diverse populations for stress tolerance.

A molecular understanding of grain chalkiness under elevated day and night temperature and its relationship to seed storage metabolism

High temperature (35/30 °C day/night) reduces grain weight, AC, and flour gel consistency (GC) of rice grains (Lin *et al.*, 2010). If the day temperatures are high beyond the critical levels during the onset of fertilization and post-anthesis period,

flag leaf photosynthesis decreases substantially, resulting in disturbed source–sink assimilate transport. As a result, high temperature not only decreases seed set but also affects grain quality (Jagadish *et al.*, 2010; Lyman *et al.*, 2013) due to reduction in endosperm sink strength and incomplete grain-filling events (Fig. 1). Deterioration of grain quality under high temperature is accompanied by the altered expression of starch metabolism-related genes. Functional analysis of the rice *GRAIN INCOMPLETE FILLING 1* (*GIF1*) gene that encodes a cell wall invertase (CWI) required to cleave sucrose into hexoses for carbon partitioning during early grain filling (Wang *et al.*, 2008) has been successfully targeted to improve grain weight during domestication. However, *GIF1* expression in wild rice is much wider during seed development and results in a grain weight reduction and enhanced chalkiness (Wang *et al.*, 2008). Ectopic expression of *GIF1* with the *35S* or rice *Waxy* promoter resulted in smaller grains. In comparison with sucrose synthase-mediated sucrose cleavage, the *GIF1* gene requires a higher ATP content to convert sucrose into hexoses (Sreenivasulu and Wobus, 2013), and thus it will remain as a rate-limiting step under stress conditions. Because of its role as a sink fine-tuning gene, *GIF1* is an important factor in driving optimum storage and unimpaired quality even under stress.

Many elite rice cultivars are also susceptible to forming chalky grains under elevated temperature. Transcriptome analysis of contrasting lines differing in chalkiness showed that several key starch biosynthesis genes, such as *granule-bound starch synthase I* (*GBSSI*), *branching enzyme IIb* (*BEIIb*), *starch synthases*, and *debranching enzymes*, and a *cytosolic pyruvate orthophosphate dikinase* gene were substantially repressed, while a starch-degrading *alpha amylase gene* was preferentially up-regulated under high temperature (Yamakawa *et al.*, 2007; Liu *et al.*, 2010). In addition, high night temperature affects the activities of many enzymes associated with the conversion of sucrose into starch synthesis during grain filling (Counce *et al.*, 2005; Yamakawa *et al.*, 2007; Yamakawa and Hakata, 2010). Reduced activities of ADP-glucose pyrophosphorylase and starch synthase enzymes have been correlated with reductions in grain weight and starch production at high temperatures (Singletary *et al.*, 1997). Transcriptome analysis, in conjunction with QTL browsing, suggested that many high-temperature-responsive pathways related to starch and storage protein metabolism are co-located along the chalk QTL regions (Yamakawa *et al.*, 2008; Peng *et al.*, 2014a). High temperature increased the accumulation of all classes of storage proteins (glutelins, prolamins, globulins, and protein disulphide isomerase) at the early filling stage, but it decreased prolamin accumulation during maturation (Lin *et al.*, 2010). Accumulation of prolamins and globulins was more sensitive to high temperature than other seed storage proteins (Lin *et al.*, 2010). Stress-responsive elements such as HSE (heat stress responsive element), ARE (anaerobic induction element), ABRE (abscisic acid response element), and MBS (MYB binding site) were found to be enriched in the promoter regions of prolamin and globulin genes. This suggests that high temperature deregulates the temporal patterns of prolamins and
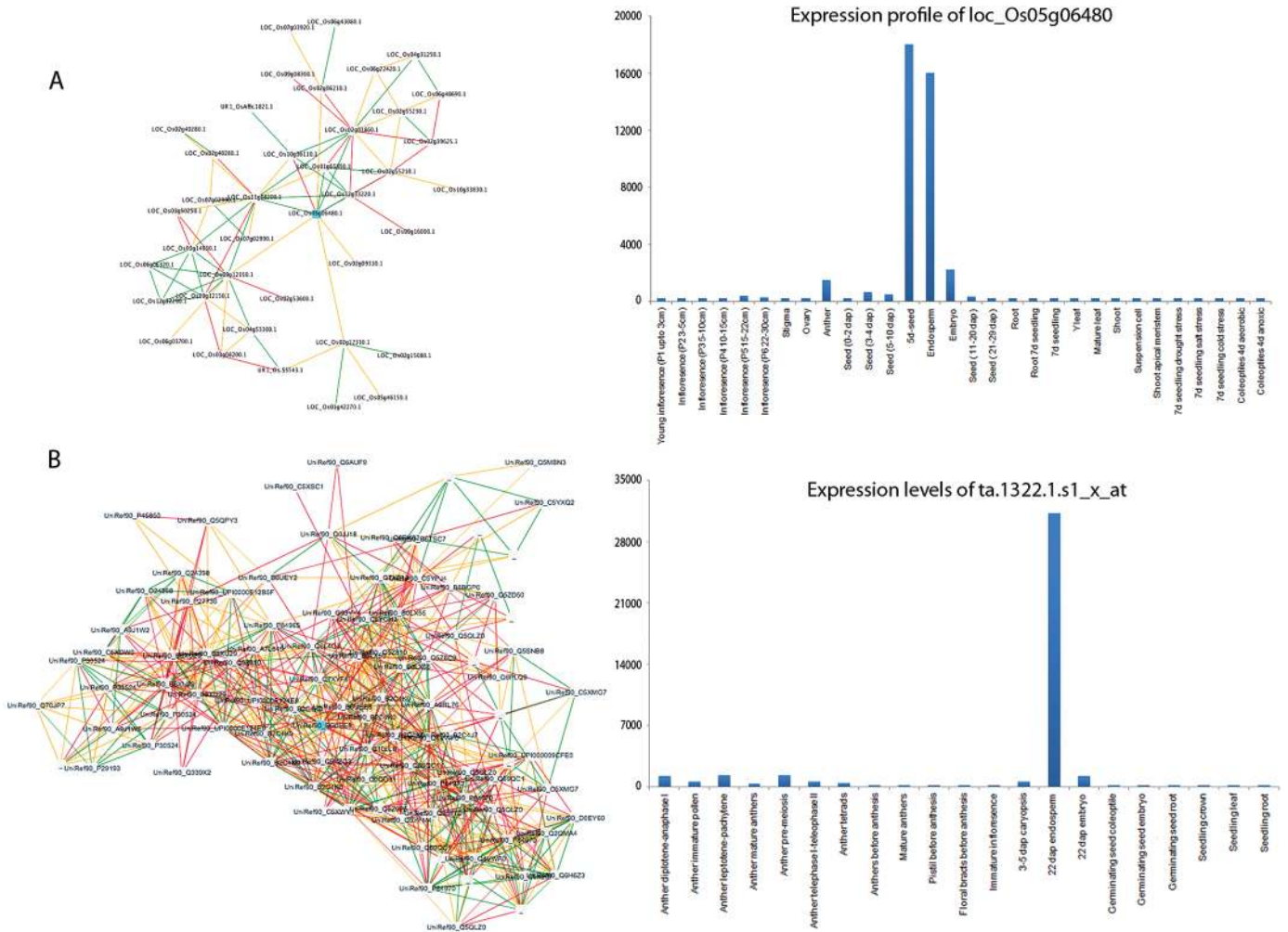
Fig. 4. The gene regulatory networks derived from co-expression data of seed development-specific genes. (A) The gene regulatory networks of the endosperm-specific *H⁺-translocating pyrophosphatase* gene cloned for chalkiness in rice derived using the PLANET database (Mutwil *et al.*, 2011) seem to control the selective proteolysis pathway (E3.SCF) during 3–5 DAF (Supplementary Table S2 at *JXB* online). (B) On the other hand, its orthologue in wheat (Ta.1322.1.S1_x_at) regulates thousands of genes involved in starch biosynthesis and protease inhibitors during seed filling. These divergent expression patterns of *H⁺-translocating pyrophosphatase* genes between rice and triticale members are likely to trigger functional divergence during seed development.

globulins and reduces amylose, leading to the formation of chalk (Li *et al.*, 2011).

It has also been observed that heat stress results in chalky grain production because of changes in starch structure (Yamakawa *et al.*, 2007; Yamakawa and Hakata, 2010; Patindol *et al.*, 2014) which usually results in decreased amylose concentration (Chen *et al.*, 2008; Lanning *et al.*, 2012) and altered gelatinization temperature (GT; Cuevas *et al.*, 2010) in many rice varieties. Alterations in starch structure primarily due to a reduction in AC lead to variation between translucent and chalky grains (Patindol and Wang, 2003). The presence of many air spaces within the loosely packed starch granules in the chalky grain prevents light transmission, which is visible as opaque regions along the translucent grain (Ashida *et al.*, 2009). However, not all opaque grains can be considered as chalky. For example, glutinous rice grains are opaque white due to micropores within the polyhedral starch granules. In contrast, the incidence of chalk in non-glutinous grains is due to air spaces between spherical starch granules (Juliano, 2007). As shown in Fig. 2B, several mutants

characterized from the starch biosynthesis pathway showed a pleiotropic effect of chalk (Fujita *et al.*, 2007b; Yamakawa *et al.*, 2007). For instance, *starch synthase IIIa* mutants (*ss3a-1* and *ss3a-2*) with elevated amylose and reduced amylopectin resulted in loose starch packing with a chalky phenotype (Fujita *et al.*, 2007b). Likewise, many other *substandard starch grain* (*ssg*) mutants affecting the starch granule structure exhibited chalky phenotypes (Matsushima *et al.*, 2014). The chalky to opaque grains were produced when the activity of *starch branching enzyme IIb* (*SBEIIb*) was down-regulated in rice endosperm, and the extent of opacity was found to be directly proportional to the elevation in the proportion of long-chain amylopectin (Butardo *et al.*, 2011). In addition, even minor perturbations in the amylopectin fine structure affected during high night temperature can produce streaks of opacity in rice grains (Patindol *et al.*, 2014).

Hakata *et al.* (2012) reported that the involvement of a starch-hydrolysing enzyme, α-amylase, triggered grain chalkiness at high temperature. In developing seeds, high temperature induced the α-amylase genes *Amy1A*, *Amy1C*, *Amy3A*,
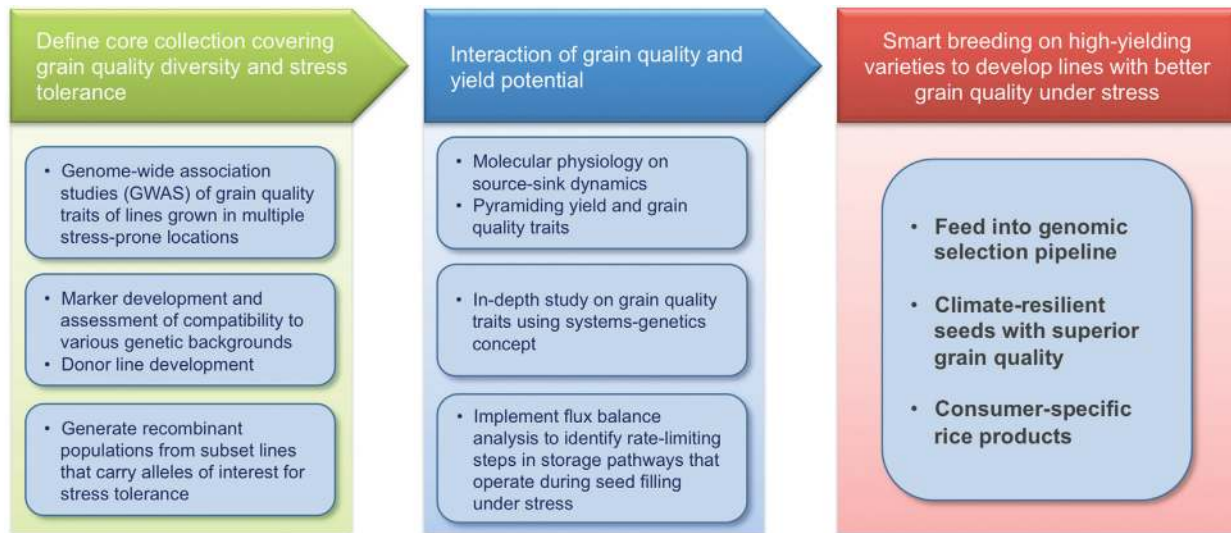
Fig. 5. Schematic representation of the systems genetics approach to explore the potential of existing intraspecific variation for various grain quality traits on a genetic map using GWAS/QTL. Unravelling a holistic view of grain quality perturbation under stress requires the integration of knowledge from systems biology (regulatory networks and flux balance analysis), systems genetics, and comparative genomics to explore the perspectives of a genomics revolution in breeding to develop climate-resilient lines with superior grain quality.

Amy3D, and *Amy3E*, as well as α-amylase activity (Hakata *et al.*, 2012). Through an RNA interference (RNAi) strategy, temperature-induced α-amylase gene expression is suppressed, leading to fewer chalky grains (Hakata *et al.*, 2012). This implies that the degradation of starch by amylase under elevated temperature is another layer of regulation responsible for altering starch structure and grain chalkiness in rice. Furthermore, systematic metabolite profiling of starch mutants and selected lines from *indica* and *japonica* subspecies differing in starch structure and amylose content reveals the importance of phospholipid complexes in starch structure and also emphasizes the mechanisms involved in white cores, opacity, and chalk formation (Kusano *et al.*, 2012; Matsuda *et al.*, 2012).

Based on the inferences drawn from the above studies, we conclude that chalk is the result of poor filling of starch granules in endosperm primarily affecting the amylose to amylopectin pathway and, in addition, imbalances in the finer readjustment with the starch degradation pathway triggered under stress. The formation of chalky grain is also triggered by the disruption of the pH homeostasis in the endomembrane trafficking system during early endosperm development, leading to elevated amounts of small vesicle-like structures and a decrease in the number and size of protein bodies (Li *et al.*, 2014). This results in the formation of air spaces among starch granules and protein bodies, which is responsible for the abnormal shape and spatial rearrangements of these storage compounds, leading to chalky rice grains. Recent additional evidence also suggests that the plant-specific kelch repeat protein interlinks the role of Golgi-associated trafficking involved in the storage protein sorting pathway, leading to the trigger of a floury endosperm and chalky phenotype with a preferential accumulation of glutelin precursor in the mutant grains (Ren *et al.*, 2014). How the altered inter-relationships between storage starch and storage protein biosynthesis affect the formation of the chalky phenotype, leading

to an increase in the susceptibility to cracking and reduced HRY, needs further investigation.

Impact of starch metabolism under stress

Aside from improved HRY and reducing chalk, breeders should also focus on other important traits such as cooking, sensory, and processing quality to address the needs of the food industry and consumers. Up to 90% of the rice grain is starch (on a dry basis) and it is therefore a key contributor to many grain quality attributes. The cooking, sensory, and functional properties of rice are highly influenced by the quality and physicochemical properties of starch (Juliano, 2007). It is therefore important to understand how different abiotic stresses can modify starch composition and accumulation in rice endosperm in an era of abrupt and uncertain environmental fluctuations.

High-temperature stress during the grain-filling stage has deleterious effects on starch quality. Major genes involved in the starch biosynthetic pathway such as *GBSS*, *SBEI*, and *SBEIIb* are down-regulated in grains exposed to high-temperature stress. Grains produced under high-temperature conditions also result in aberrant starch, with small granules and reduced amylose and amylopectin content, reflecting a similar phenotype to that observed in *floury-endosperm* (*flo*) mutants (Kawasaki *et al.*, 1996; Yamakawa *et al.*, 2007; Satoh *et al.*, 2003). The *flo2* mutation in rice affected grain size, with a lower AC, and showed floury features because of the loose filling of starch granules with larger air spaces in the grain compared with their wild-type counterpart (Qiao *et al.*, 2010; She *et al.*, 2010). Mutations in other starch-biosynthesizing enzymes also exhibit similar characters to those of *flo2* mutants (Nishi *et al.*, 2001). She *et al.* (2010) identified the gene responsible for the *flo2* mutant as *OsCEO1*, a novel regulatory cascade of endosperm organogenesis, and it may

have an important role in the response to high-temperature stress. Likewise, the *FLO6* gene codes for an unknown protein that possesses a C-terminal carbohydrate-binding module that binds to the starch molecule, which alters the physico-chemical properties of starch and thus alters complex granule formation (Peng *et al.*, 2014*b*).

Both high night temperature and high day temperature affect grain quality by altering starch and storage protein properties (Li *et al.*, 2011). Apart from *Wx*, numerous QTLs associated with AC and GC have been mapped on different rice chromosomes (Supplementary Fig. S1 at *JXB* online) (Aluko *et al.*, 2004; Sun *et al.*, 2006; Sabouri *et al.*, 2012). High temperatures affect GBSSI activity in rice (Cheng *et al.*, 2005*c*), and the response of GBSSI to temperature is due to a single nucleotide polymorphism (SNP) in the 5' leader intron of the *GBSSI* gene sequence in rice and barley (Hirano and Sano, 1998; Patron *et al.*, 2002). The *GBSSI* intron splicing is high in GT-SNP (AGGTATA) genotypes, resulting in high amylose accumulation (Inukai *et al.*, 2000; Mikami *et al.*, 2008) even if the temperature is altered. Under unfavourable environments, genotypes with alleles containing a TT-SNP (AGTTATA) in the leader sequence of the 5' intron of *GBSSI* are not properly spliced, and hence GBSSI activity is lowered (Bligh *et al.*, 1998). This ultimately results in a low AC in rice grains (Cai *et al.*, 1998; Isshiki *et al.*, 1998; Sato *et al.*, 2002). Thus, *japonica* cultivars appear to be more sensitive to high temperature with regard to amylose synthesis than *indica* cultivars (Inukai *et al.*, 2000; Sun *et al.*, 2011). High-temperature stress also reduced Wx protein expression, leading to lowered amylose with altered starch viscosity and grain quality (Larkin and Park, 2003; Larkin *et al.*, 2003). Some of these QTLs are associated with the stability of AC in various rice varieties grown in high-temperature conditions (Zhang *et al.*, 2014). The chromosome substitution lines carrying the stable major QTLs for amylose (*qHAC8a*, *qHAC8b*, and/or *qHAC4*) have been linked to the high pre-mRNA splicing efficiency of the *Wx* gene. Thus, increasing the pre-mRNA processing of this gene is identified to be the key factor for maintaining stable amylose in rice seeds at high temperature (Zhang *et al.*, 2014). It is therefore possible to combine the correct alleles to ensure proper mRNA splicing of the *GBSSI* gene in rice breeding strategies to maintain stable amylose biosynthesis under increasingly warming climatic conditions.

The effect of heat stress during seed development not only reduces AC but also modifies starch structure and thermal properties by affecting the gelatinization temperature (GT) (Lu *et al.*, 2014). GT is associated with the activity of starch synthase IIa (SSIIa), the enzyme responsible for the elongation of amylopectin chains within the crystalline lamella of degree of polymerization (DP) 12–24 (Umemoto *et al.*, 2004; Nakamura *et al.*, 2005; Waters *et al.*, 2006). Functional SNPs in the gene coding for SSIIa have been identified and can be used to group rice samples into high- and low-GT classes (Nakamura *et al.*, 2005; Waters *et al.*, 2006; Cuevas *et al.*, 2010), which influences cooking quality. Apart from *SSIIa*, QTLs for GT were mapped on other rice chromosomes using different populations (Lanceras *et al.*, 2000; He *et al.*, 2006; Sabouri *et al.*, 2012). Heat stress not only decreases AC but

it also increases the overall proportion of longer amylopectin chains in rice starch (Patindol *et al.*, 2014), possibly because of increased SSI and SSIIa activity (Umemoto *et al.*, 1999; Umemoto and Aoki, 2005; Yamakawa *et al.*, 2007). Although SSI preserves the elongation of A and B chains of amylopectin, reduced activity of SBEIIb and SBEI lowers the branching frequency of amylopectin (Jiang *et al.*, 2003). Thus, reduced GBSSI and increased SSI activities under high temperature contribute to the lower ratio of amylose to amylopectin (Cheng *et al.*, 2005*a*). These results point to the possibility that a decrease in amylose under heat stress may account for the loss of grain weight as well as impaired cooking quality because of the alteration in starch properties. The inferences drawn above can help in engineering starch quality by maintaining an optimum amylose to amylopectin ratio even under stress.

Present state and future perspectives in designing climate-resilient rice with superior quality grain

Rice grain yield has been reported to decline by 6% and HRY by 9–14% for every 1 °C increase in temperature (Peng *et al.*, 2004; Welch *et al.*, 2010; Lyman *et al.*, 2013). This wasted food could have been made available to help the global food requirement considering that rice feeds roughly half of the world's population. High night temperatures greatly influence milling quality by modulating endosperm morphology, grain dimensions, and starch-metabolizing enzymes (Counce *et al.*, 2005). This confounding evidence suggests that the global rise in temperature primarily affects HRY and key grain quality attributes such as seed storage biosynthesis and grain chalkiness. To enhance HRY and reduce susceptibility to chalk-mediated grain breakage under stress conditions, there is a need to (i) select better germplasm with reduced chalk under multienvironments as a source of inbreds; (ii) target both parents to have a similar AC and GT in creating hybrid vigour for stress tolerance with higher HRY as a prime target; and (iii) use marker-assisted selection strategies to reduce chalk in the lines suitable for stress-prone environments. Events leading to chalk formation are associated mostly with reduced sink strength and imbalances in carbon and nitrogen partitioning in a growing sink because of photoassimilate limitation under stress. Thus, besides implementing strategies to enhance yield advantages through elevated grain number per panicle and a change in spike architecture with profuse secondary branches in the panicles (Sreenivasulu and Schnurbusch, 2012), source–sink relationships under stress conditions need to be fine-tuned (Supplementary Fig. S2 at *JXB* online). In addition, strategies need to be developed to synchronize the ability of genotypes to stabilize HRY with reduced chalk under challenging environments.

Based on the inferences drawn from previous published studies, chalk appears to be the result of poor filling of starch granules in the rice endosperm, primarily affecting amylose and amylopectin pathways and disrupting the spatiotemporal packing of

starch granules. Triggering of the chalky phenotype may also be due to imbalances in the finer readjustments with the starch degradation pathway during grain filling. However, susceptibility to cracking because of brittle grains must be overcome to mitigate HRY reduction, and its connected links to the chalky phenotype need to be thoroughly dissected. This is because the rice grain is very sensitive to even minor perturbations in starch structure, which does not necessarily result in brittle grains and a reduction in HRY in some instances. The definitive genetic, environmental, and physiological link between chalkiness and breakage susceptibility needs to be established. In addition, the connection between seed storage protein biosynthesis and starch granule formation has to be empirically tested to determine the role of carbon partitioning and allocation in the formation of brittle chalky grains that significantly reduce HRY.

Grain quality traits are controlled by many major QTLs, implying that the genetic mechanisms underlying quality traits are complex. More than 600 QTLs related to grain quality have been reported (see Supplementary Table S1 at *JXB* onlime) in the Gramene Genome Database (http://www.gramene.org). Additional evidence gathered from meta-QTL mapping studies revealed that stable and major QTL genetic regions identified on chromosomes 3 and 6 have overlapping regions for chalky endosperm, AC, protein content, viscosity properties, and the integrated values of organoleptic evaluation (Supplementary Fig. S1). It appears that this QTL cluster is a novel gene resource for controlling rice grain quality traits. Cloned genes responsible for rice grain quality traits as well as fine-mapped QTLs form a strong base for genomic selection useful in efficient breeding for designing climate-ready lines that are suitable according to regional differences in grain quality preferences. With a much lower sequencing price, genome re-sequencing has been used to accelerate breeding. The genetic and genomic information that can be harnessed from the 3000 whole genomes of cultivated rice relatives (3,000 Rice Genomes Project, 2014) will provide an opportunity to mine different alleles related to grain quality traits involved during adaptation to different climatic conditions at the subspecies level. Because some useful grain quality traits might have been lost during the course of rice domestication, these developments in genotyping technology can also be used to explore the genetic diversity of wild rice (Nock *et al.*, 2011) to find useful genes for starch quality improvement under climate change scenarios. Structural and functional genomics resources available from millet not only offer the prospects of incorporating stress tolerance target traits in other cereals but also offer the value to explore the nutritional benefits (Muthamilarasan and Prasad, 2014). Such comprehensive augmented knowledge obtained that is related to climate-adapted allelic variation can be used to design superior grain quality for stress-prone environments. This can be achieved by exploring valuable alleles that can be targeted to fine-tune grain quality in high-yielding lines that are stable across a variety of stress-prone areas through genomics-assisted selection and marker-assisted breeding. On top of this, employing systems biology strategies such as regulatory networks and flux balance analysis, as well as systems genetics methods, can help to decipher the holistic view of grain quality perturbations to multiple abiotic stress factors (heat stress with drought, humidity, salinity, and elevated carbon dioxide), which co-occur in nature (Fig. 5).

Supplementary data

Supplementary data are available at *JXB* online

Figure S1. Summary of grain quality QTLs identified using bi-parental mapping populations depicted on rice chromosomes 3, 4, and 6.

Table S1. A summary of more than 600 grain quality QTLs.

Table S2. Selected super pfam families which are enriched in the vicinity of networks of H^+ pyrophosphatase in rice and wheat species.

Acknowledgements

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