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A natural gene on-off system confers field thermotolerance for grain quality and yield in rice

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SUMMARY

Rising global temperatures threaten crop grain quality and yield; however, how temperature regulates grain quality and how to achieve synergistic thermotolerance for both quality and yield remain unknown. Here, we identified a rice major locus, *QT12*, which negatively controls grain-quality field thermotolerance by disrupting endosperm storage substance homeostasis through over-activating unfolded protein response (UPR). Natural variations in *QT12* and an NF-Y complex form a natural gene on-off system to modulate *QT12* expression and thermotolerance. High temperatures weaken NF-YB9/NF-YC10 interactions with NF-YA8, releasing *QT12* suppression and triggering quality deterioration. Low *QT12* expression confers superior quality and increases elite rice yield up to 1.31–1.93 times under large-scale high-temperature trials. Two trait regulatory haplotypes (TRHs) from co-selected variations of the four genetically unlinked genes in NF-Ys-*QT12* were identified for subspecies thermotolerance differentiation. Our work provides mechanistic insights into rice field thermotolerance and offers a proof-of-concept breeding strategy to break stress-growth and yield-quality trade-offs.

INTRODUCTION

Global warming intensifies heat stress damage in agriculture globally.[1–6] The global surface temperature rose by 1.1°C in 2011–2020, compared with 1850–1900, reaching a surge to 1.5°C in 2021–2040.[4] Such a 1°C global average temperature increase has already reduced mean yield of major crops by 3.1%–7.4%, posing a significant threat to global food security.[2] Global food security is not only closely related to yield but also to quality, which determines human nutrition, market value, and farmer income, especially in Asia and Africa, where high temperatures are more frequent and populations are heavily reliant on cereals.[3,7] High temperatures deteriorate grain appearance, milling, cooking, and eating and nutrition qualities in cereals.[8–10] These problems highlight improving crop high-temperature resistance for quality and yield for more sustainable agriculture. The fundamental biological mechanisms underlying grain quality thermotolerance and how to achieve synergistic thermotolerance for both quality and yield remain unknown.

Grain chalkiness, characterized by a chalky texture of endosperm, is a universal grain quality trait in cereals (called opaque in maize and grain hardness/softness in wheat), and high chalkiness greatly deteriorates appearance, cooking, and eating and nutritional qualities as well as milled head rice yield/rate, thus representing a major problem for rice market value and grain food consumption globally.[3,9,11–13] Grain chalkiness, as a consequence of complex endosperm development, is a quantitative trait controlled by multiple quantitative trait loci (QTL) and is highly susceptible to high temperatures.[9,11] The increase of grain chalkiness has long been known to be the most direct and sensitive indicator of grain quality deterioration caused by high temperature.[9,11] The income of rice farmers around the world, especially where high temperatures occur frequently, largely depends on the grain quality trait.[3,7] Furthermore, endosperm development and grain chalkiness formation are driven by the filling of endosperm storage substances during grain filling stage, which directly determines grain quality and yield and is greatly affected by high temperatures.[14,15] However, the mechanisms by which high temperatures regulate endosperm development and control grain chalkiness/quality variation remain elusive.

The development of superior-quality rice in Asian and African countries faces enormous challenges from frequent high temperatures, as evidenced by both low proportion and weak market competitiveness of high-quality rice worldwide, due to

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low thermotolerance for grain quality of most modern rice varieties.[7,9,11,16] Without attractive prices driven by superior quality, farmers' enthusiasm in rice production is often dampened. To improve crop thermotolerance, it is crucial to identify germplasms that are stably thermotolerant in multiple natural high-temperature environments.[17–19] Traditional methods of identifying thermotolerant phenotypes and the underlying QTL/genes, typically focused on seedling survival rate, seed-setting rate, or yield traits within the controlled environments in greenhouses, fail to accurately mimic natural high temperatures in field, often resulting in an incomplete catalog of genuinely thermotolerant germplasms.[10,17] Consequently, germplasms and QTL that possess authentic thermotolerance suitable for breeding purposes remain scarce. Because of domestication and breeding, two major rice subspecies, *indica* and *japonica*, have diverged to suit their geographical locations and field temperatures, endowing *indica* with enhanced thermotolerance and *japonica* with greater cold tolerance.[20–22] The underlying mechanisms for such subspecies differences in temperature tolerance remain to be elucidated.

Here, we identified a key grain quality-thermotolerant gene, *QT12*, through multiple-year field high-temperature trails. Natural variations in *QT12* promoter and NF-Y transcription factors responsible for *QT12* differential expression in response to high temperatures form a natural gene on-off system. High temperature disrupts NF-Y interactions, activates the switch system, and leads to increased *QT12* expression, which disrupts storage substance homeostasis during endosperm development, thereby reducing rice thermotolerance. A co-selection pattern of natural variations in *QT12* and *NF-Ys* between subspecies establishes the concept of trait regulatory haplotypes (TRHs), providing mechanisms for subspecies thermotolerance differentiation and offering genes for breeding thermotolerant rice.

RESULTS

The *QT12* locus controls grain quality thermotolerance in rice

High temperatures seriously deteriorate grain quality in a rice mini-core collection of 533 accessions worldwide (Figures 1A–1C). To study the genetic basis of grain-quality thermotolerance variation in rice, we used grain chalkiness as an indicator of grain developmental consequence in response to high temperatures. We investigated various grain chalkiness phenotypes of the accessions over 12 years, including 9-season natural high and 5-season normal temperatures during grain filling stage, and identified 7 accessions that are highly and stably resistant and 8 elite accessions that are sensitive to all 9-season field high temperatures, all of which belong to superior quality under normal temperatures (Figure 1D). We found that both *indica* Chenghui448 and OM1723 exhibited superior quality and similar grain width under normal temperatures (Figures 1E, S1A, and S1B). When planted in natural high-temperature conditions, OM1723 showed significantly inferior quality, and Chenghui448 still maintained superior quality, exhibiting great thermotolerance (Figures 1E and S1B). To identify genes underlying the natural variation in thermotolerance for grain quality, an F_2 genetic population from crosses between the two accessions was planted under different natural temperatures (Figures S1C and S1D). Using RapMap,[23] we identified a single-locus major QTL, *grain quality-thermotolerant QTL on chromosome 12* (*QT12*), controlling grain quality thermotolerance (Figures S1C, S1E, and S1F). Using the phenotypes of 57 recombinants from progenies, we further fine-mapped *QT12* to a 14-kb region, where only one open reading frame, *LOC_Os12g07490* (Figure 1F), encoding a putative Sec61 translocon β subunit that facilitates the co-translational translocation of newly generated peptide chains or unfolded proteins,[24,25] was identified. The gene was constitutively expressed (Figures S1G and S1H), and its encoded short-peptide protein with 84 amino acids was highly conserved in monocots and dicots (Figures S1I and S1J). Hence, *LOC_Os12g07490* was selected as the candidate gene for *QT12*.

Comparative sequencing of *QT12* between two parents revealed multiple mutations in the 3-kb promoter but no mutation in coding region (Figure 1G). To verify the allelic effect, we developed a pair of near-isogenic lines (NILs) in Chenghui448 background, NIL^{CH} and NIL^{OM}, which showed no significant difference in *QT12* expression under normal temperatures. However, under high temperatures, *QT12* expression was significantly increased in NIL^{OM}, while remaining unchanged in NIL^{CH} (Figure 1H). Additionally, NIL^{CH} is also more thermotolerant than NIL^{OM} at seedling stage, suggesting its thermotolerant function at vegetative phase (Figure S1K). Based on all SNPs in *QT12* promotor between 2 parents, the 533 accessions are grouped into 2 major haplotypes, Hap^{CH} and Hap^{OM} (Table S1); also, Hap^{CH} is more thermotolerant, and *QT12* expression is lower in Hap^{CH} than Hap^{OM} (Figure 1I). Using the most resistant and sensitive accessions for grain chalkiness identified under multiple-year high temperatures, we found that *QT12* expression was significantly lower in resistant accessions than sensitive accessions under high temperatures, but there was no difference under normal temperatures (Figure S1L). Thus, the grain quality thermotolerance is generally negatively correlated with *QT12* expression. Among the 533 accessions, the thermotolerant haplotype of *QT12* with the SNP variation A ($QT12^A$) in Hap^{CH} (Figure 1G) is predominantly found in *indica*, with an allelic frequency of 18.0% (Figure S1M). The G/A SNP in the CCAAT-box divided 249 *indica* accessions into two haplotypes, $QT12^A$ and $QT12^G$. $QT12^A$ exhibits higher grain quality thermotolerance and lower *QT12* expression than $QT12^G$ in *indica* (Figure S1N), consistent with the observations in the NILs and mini-core collection. Collectively, these results suggest that *QT12* expression is negatively correlated with grain quality thermotolerance and that the G/A variation in *QT12* promotor explains the thermotolerance difference within *indica*.

QT12 confers thermotolerance for superior grain quality by maintaining endosperm storage substance homeostasis

To confirm QT12's role in grain quality thermotolerance, we generated complementation lines (*QT12*-Com) in thermotolerant Chenghui448. *QT12*-Com exhibited low grain chalkiness at normal temperatures but significantly higher grain chalkiness than wild type under high temperatures (Figures 2A, S2A, and S2B). Compared with normal temperatures, we found a

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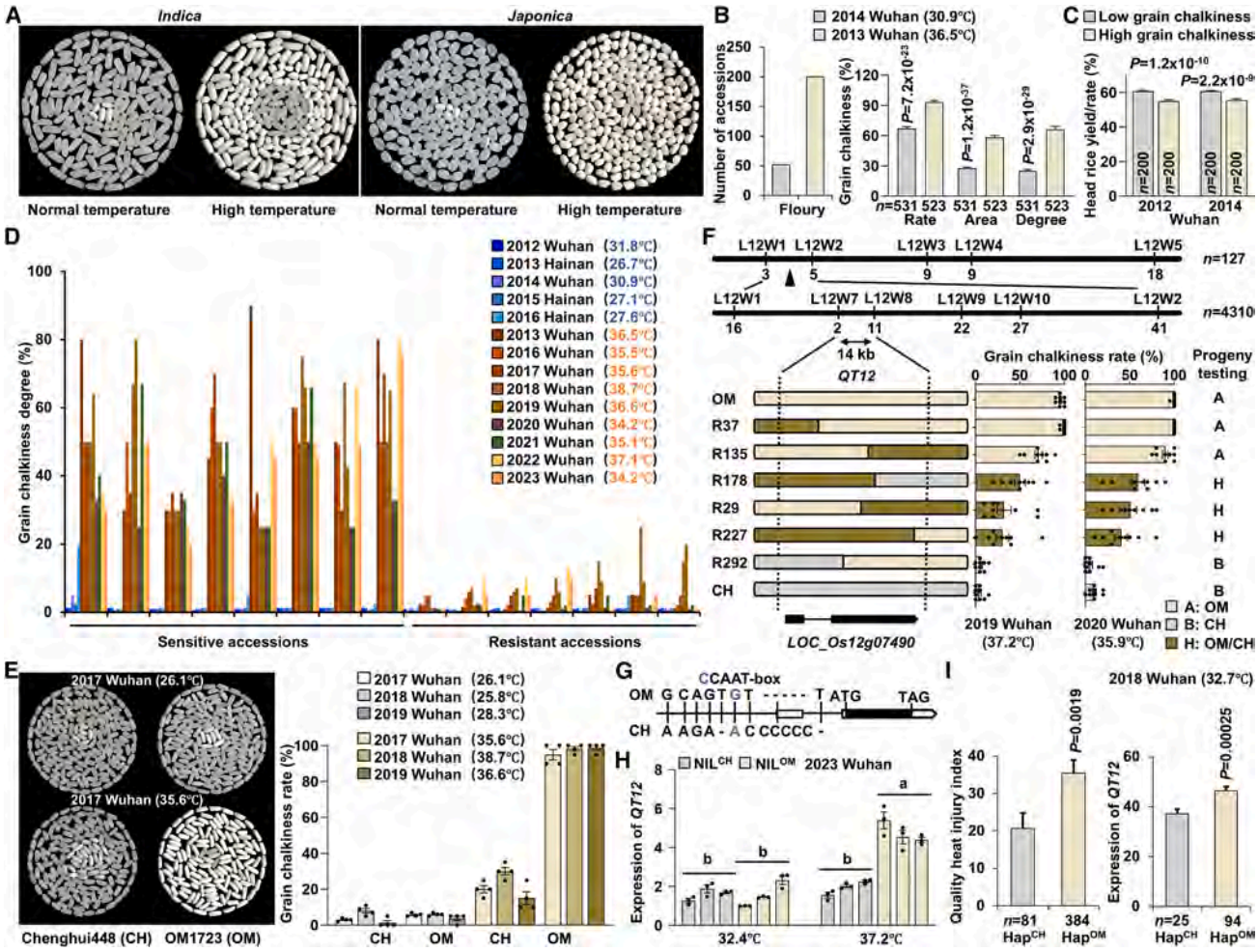

Figure 1. Isolation and characterization of *QT12*

(A) The effect of field high temperature on grain quality of rice.

(B) The number of floury endosperm accessions and grain chalkiness phenotypes in a rice mini-core collection under different field temperatures.

(C) The head rice yield/rate in 200 accessions with high and low grain chalkiness.

(D) Grain chalkiness of identified germplasms that are highly resistant and sensitive to high temperatures under 14 natural temperature seasons. Temperature values marked in blue and orange indicate the normal and high temperatures, respectively.

(E) Grain chalkiness of Chenghui448 and OM1723 planted under six different natural temperatures.

(F) Fine-mapping and cloning of *QT12*.

(G) Natural variations of *QT12* between two parents. The important *cis*-element variation caused by the G/A SNP was marked in blue and green.

(H) Comparative expression of *QT12* alleles using 5-days after flowering (5-DAF) endosperms of two NILs under different field temperatures.

(I) Quality heat injury index and expression of *QT12* in 5-DAF endosperms between Hap^{CH} and Hap^{OM} in a mini-core collection.

Significant differences indicated by different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.

See also Figure S1 and Table S1.

significant decrease in storage protein content and an increase in amylose content in *QT12*-Com at high temperatures (Figures 2A and S2C–S2E). This decrease in the ratio of storage protein to amylose/starch content causes an imbalance of storage substance, causing grain chalkiness formation. In contrast, the thermotolerant wild type maintained such balance, resulting in low grain chalkiness (Figure 2A). Further, we found that the $QT12^{G}$ mRNA and protein levels were induced by high temperatures in *QT12*-Com, while those of $QT12^{A}$ did not change (Figures S2F and S2G). In the *QT12* overexpression lines, the amylose and starch contents were significantly increased, and storage protein was greatly reduced even planted under normal temperatures, leading to a great decrease in the ratios between protein and amylose/starch and finally more grain chalkiness (Figures S2H–S2K). Thus, *QT12* overexpression rendered higher grain chalkiness, mimicking high-temperature effects on thermosensitive lines. We also generated CRISPR lines (*QT12*-CR) in the thermosensitive OM1723 (Figure S2L). Compared with normal temperatures, *QT12*-CR lines exhibited little change in protein and amylose/starch contents at high temperatures,

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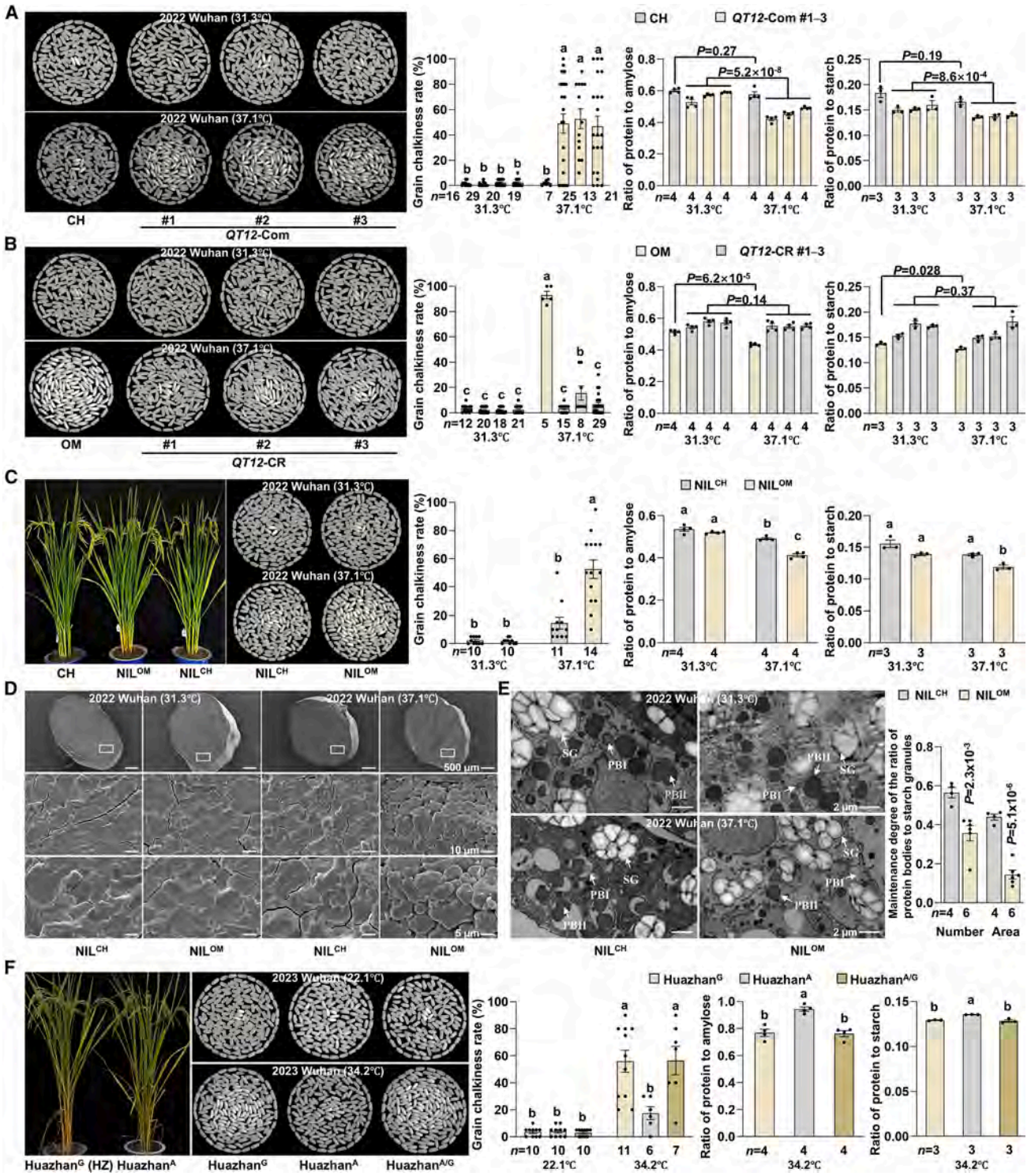

Figure 2. *QT12* negatively confers grain quality thermotolerance by balancing storage substance

(A–C) Grain chalkiness, ratios of storage protein to amylose/starch content of complementation lines (A), CRISPR lines (B), and NILs (C) for *QT12* under different natural temperatures.

(D) Transverse sections of mature endosperm bellies of NILs by SEM. Scale bars: 500 μm (top), 10 μm (middle), 5 μm (bottom).

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thus maintaining superior quality (Figures 2B and S2M–S2P), suggesting that mutation or low expression of *QT12* confers thermotolerance for superior quality by maintaining storage substance homeostasis. Collectively, these results indicate that grain chalkiness formation in response to high temperature is positively regulated by *QT12* and is likely due to the disruption of storage substance balance.

NIL^{CH} displays a lower grain chalkiness with lower amylose and higher protein contents under high temperatures (Figures 2C, S2Q, and S2R). Compared with normal temperatures, NIL^{CH} maintains lower grain chalkiness by keeping protein and amylose/starch ratio under balance at high temperatures. However, NIL^{OM} showed a significant decrease in the ratios, causing chalkiness formation (Figures 2C and S2Q–S2T). To further understand the effects of *QT12* on grain storage substances, we examined their subcellular structures in NILs using scanning electron microscopy (SEM). When grown under high temperatures, the chalky endosperms of NIL^{OM} contained loosely packed spherical storage granules with large air spaces, whereas the non-chalky grains from NIL^{CH} had densely and regularly packed polyhedral storage granules, consistent with both NIL^{CH} and NIL^{OM} grown under normal temperatures (Figure 2D). To find the subcellular causes on the structure changes, we compared the ultra-structures of 10-days after flowering (10-DAF) endosperm cells in NIL^{CH} and NIL^{OM} under different temperatures using transmission electron microscopy (TEM) (Figure 2E). Compared with normal temperatures, the number and area of protein bodies in both NILs decreased under high temperatures (Figure S2U), but the decreased extent of protein bodies in NIL^{CH} is much less than that of NIL^{OM}, resulting in a higher maintenance degree of the ratio of protein bodies to starch granules in NIL^{CH} and thus non-chalky grains (Figures 2E, S2V, and S2W). These subcellular pieces of evidence further confirm that the balance and imbalance between storage protein and starch result in resistant and sensitive phenotypes of grain quality under high temperatures, respectively.

We found that the *QT12* allele of the elite cultivar Huazhan, the most popular restoring line currently widely used in high-yield hybrid rice breeding in China, was a high-temperature-sensitive genotype ($QT12^G$) (Figure 2F). To improve the thermotolerance of Huazhan, we introgressed $QT12^A$ from Chenghui448 into Huazhan ($Huazhan^G$) by backcrossing and generated a NIL, $Huazhan^A$ (Figure 2F). Under natural high temperatures, $Huazhan^A$ has superior quality and lower amylose content as well as a higher protein content than $Huazhan^G$ (Figures 2F, S2X, and S2Y). There is no significant difference in these quality traits between the heterozygous line $Huazhan^{A/G}$ and the homozygous line $Huazhan^G$ (Figures 2F, S2X, and S2Y), indicating that $QT12^G$ is a dominant allele. Altogether, *QT12* has a significant potential for improving elite rice grain quality under high temperatures.

NF-YA8 and *NF-YB9/NF-YC10* oppositely regulate thermotolerance for grain quality by controlling *QT12* expression

To identify the upstream regulators that cause *QT12* differential expression, we discovered seven variations in its promotor that lead to *cis*-element changes that may affect the binding of *trans*-factors (Table S2). The G-to-A SNP-containing CCAAT-box is a binding site for Nuclear Factor Y (NF-Y) complexes that participates in plant heat stress response.[26–28] *NF-YC10*, an endosperm-specific gene, showed the highest negative correlation with *QT12* expression (Figures S3A–S3C; Table S1). Thus, we produced CRISPR and overexpression lines of *NF-YC10* in ZH11 background to analyze its function on *QT12* expression. Compared with ZH11 and *NF-YC10*-CR lines under high temperatures, the *NF-YC10*-OE lines exhibited lower grain chalkiness with a higher storage substance maintenance degree (Figures 3A and S3D–S3G). Compared with ZH11 under normal temperatures, there was no change in grain chalkiness in *NF-YC10*-OE lines, while the *NF-YC10*-CR lines showed a mild increase in grain chalkiness (Figures 3A and S3D–S3G). Moreover, *QT12* expression was much lower in *NF-YC10*-OE lines but significantly higher in *NF-YC10*-CR lines than in ZH11 (Figure 3B). These results indicate that *NF-YC10* promotes thermotolerance for grain quality by negatively regulating *QT12* expression.

Genome-wide association study (GWAS) for grain chalkiness, using 533 accessions, identified *NF-YC10* near QTL1 peak point (Figure 3C), indicating that *NF-YC10* may have functional natural variations. Indeed, we found that the four indels and three SNPs in the coding region, which lead to frameshift and stop codon mutations, respectively, are non-functional haplotype (*nf-yc10*), while the variations that do not clearly affect the protein function are functional haplotype (*NF-YC10*) (Figure 3D). Grain chalkiness of *NF-YC10* accessions was significantly lower than *nf-yc10* under high temperatures, but there was no difference under normal temperatures (Figure S3H). Phenotypic analysis using the four haplotypes derived from *NF-YC10* and QT12 showed that only in the $QT12^G$ haplotype background under high temperatures, grain chalkiness of *NF-YC10* was significantly lower than that of *nf-yc10*, suggesting a significant epistatic interaction between the two genes (Figures 3E and S3I). These results indicate that *NF-YC10* negatively regulates *QT12* expression likely through the CCAAT-box *cis*-element.

To identify other subunits that interact with NF-YC10, we found that *NF-YA8* and *NF-YB9* were specifically and highly expressed in endosperms and co-expressed with *NF-YC10* (Figure S3J). Additionally, expression of *NF-YA8* and *NF-YB9* in NILs was induced by high temperatures (Figures S3K and S3L), suggesting their high-temperature response. The three proteins interacted with one another by split-luciferase (LUC) assays *in vivo*, pull-down assays *in vitro*, and co-immunoprecipitation (coIP) *in vivo* (Figures 3F–3H). At high temperatures, *NF-YA8*-CR lines showed significantly lower grain chalkiness with

(E) Ultrastructure of cells in 10-DAF endosperms of NILs by TEM. PBI and PBII, protein bodies I and II; SG, starch granule. Scale bars, 2 μm.
(F) Plant architecture, grain chalkiness, and ratios of protein to amylose/starch content of NILs improved in Huazhan under different natural temperatures. Significant differences with different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.
See also Figure S2.

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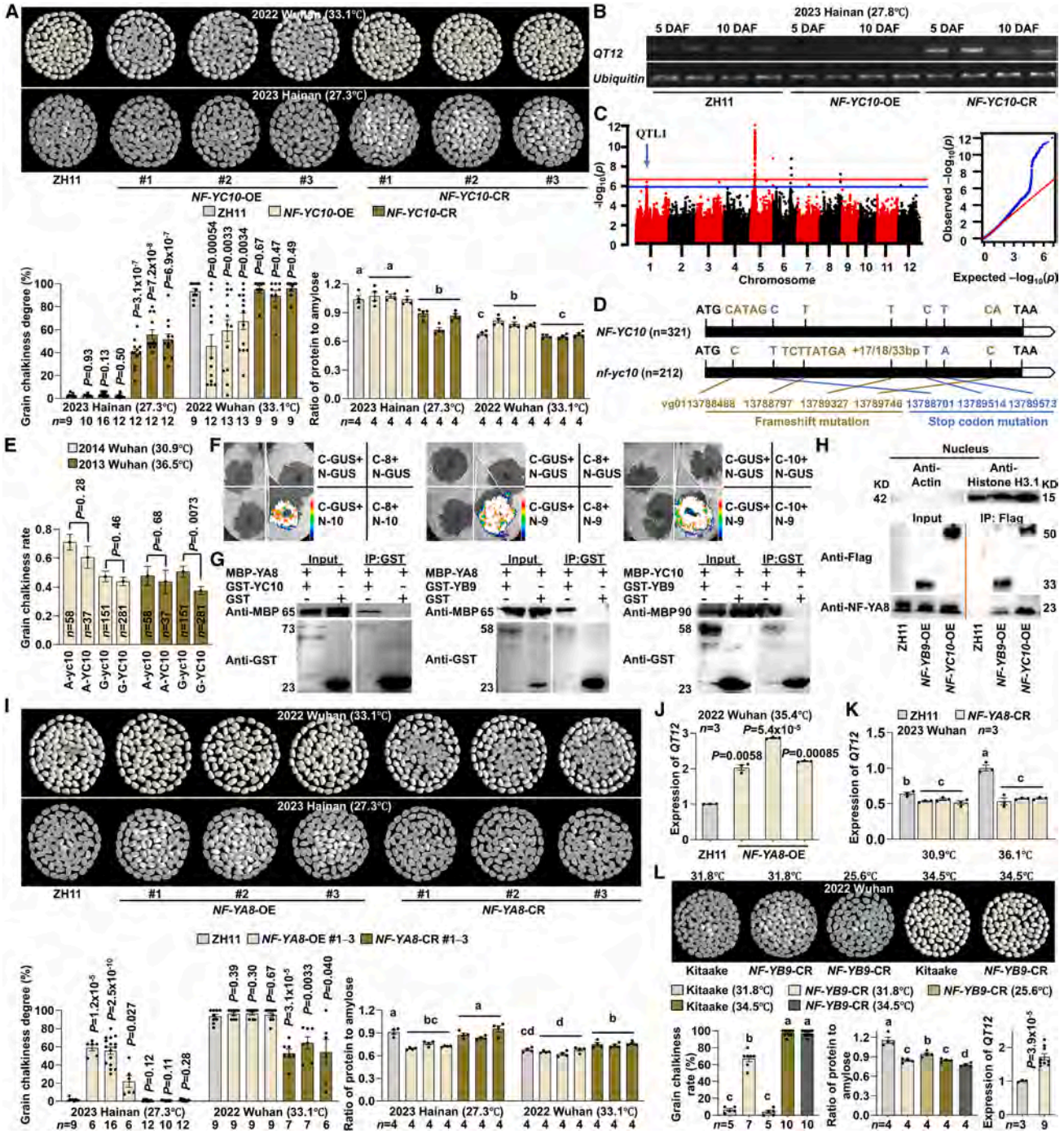

Figure 3. *NF-YA8* negatively regulates and *NF-YB9/NF-YC10* positively regulate grain quality thermotolerance through controlling *QT12* expression

(A) Grain chalkiness and ratios of protein to amylose content of *NF-YC10* overexpression and CRISPR lines under different natural temperatures.

(B) Expression of *QT12* in 5-DAF and 10-DAF endosperms of *NF-YC10* overexpression and mutant lines.

(C) GWAS of grain chalkiness under normal temperatures. The most prominent peak point on chromosome 1 named QTL1 contains *NF-YC10*.

(D) Natural variations of *NF-YC10* and *nf-yc10* in rice mini-core collection.

(E) Grain chalkiness rate of four haplotype combinations of *NF*-YC10 and *QT12* under different temperatures. "A-yc10," "A-YC10," "G-yc10," and "G-YC10" are genotypic combinations of $QT12^{A}$ and *nf-yc10*, $QT12^{A}$ and *NF-YC10*, $QT12^{G}$ and *nf-yc10*, as well as $QT12^{G}$ and *NF-YC10*, respectively.

(F–H) Split-LUC (F), pull-down (G), and coIP (H) assays for interactions among NF-YA8, NF-YB9, and NF-YC10. Nucleus proteins of 5-DAF endosperms were used for coIP.

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a more stable storage substance ratio than ZH11 and *NF-YA8*-OE lines, suggesting that *NF-YA8* positively regulates chalkiness formation (Figures 3I and S3M–S3P). Consistently, at normal temperatures, *NF-YA8*-OE showed an increase in grain chalkiness, and *NF-YA8*-CR lines showed no change, compared with ZH11 (Figure 3I). Furthermore, *QT12* expression was much higher in developing endosperms of *NF-YA8*-OE and lower in *NF-YA8*-CR, compared with ZH11 (Figures 3J and 3K), with a similar trend in leaves of *NF-YA8*-OE (Figure S3Q). Significantly, compared with normal temperature, *QT12* was upregulated by high temperature in ZH11, but it did not show significant change in *NF-YA8*-CR lines (Figure 3K), indicating that NF-YA8 is required for *QT12* expression in response to high temperature. As for *NF-YB9*, at high temperatures (34.5°C), both Kitaake and *NF-YB9*-CR lines exhibited high grain chalkiness with extremely floury endosperms (Figures 3L, S3R, and S3S). At normal temperatures (31.8°C), the *NF-YB9*-CR line showed a higher grain chalkiness than Kitaake, but no grain chalkiness change was observed at lower temperatures (25.6°C) (Figure 3L). *QT12* expression significantly increased in *NF-YB9*-CR plants (Figure 3L) yet significantly dropped in *NF-YB9*-OE lines (Figure S3T), indicating negative regulation of *QT12* by NF-YB9. Taken together, *NF-YA8* negatively and *NF-YB9*/*NF-YC10* positively regulate grain quality thermotolerance by oppositely controlling *QT12* expression.

High temperature enhances NF-YA8 activation on *QT12* by releasing the inhibition of NF-YB9 and NF-YC10

Electrophoretic mobility shift assay (EMSA) revealed that NF-YA8 directly binds the CCAAT motif in $QT12^G$ promoter, but its binding to the altered motif in $QT12^A$ was very weak (Figures 4A and S4A). Although NF-YC10 and NF-YB9 themselves do not bind the CCAAT motif, the binding affinity of NF-YA8 on $QT12^G$ was reduced when NF-YC10 or NF-YB9 was added (Figure 4A), suggesting that NF-YC10 and NF-YB9 inhibited the binding affinity of NF-YA8 on *QT12*, which was further confirmed by chromatin immunoprecipitation (ChIP)-qPCR *in vivo* (Figure 4B). *In vivo* dual-LUC assays demonstrated that NF-YA8 could activate $QT12^G$ promoter but not $QT12^A$ (Figure 4C). After adding NF-YB9, NF-YC10, or both, $QT12^G$ promoter activities driven by NF-YA8 significantly decreased (Figure 4C). Therefore, NF-YB9 and NF-YC10 inhibit the binding ability and transcriptional activity of NF-YA8 on *QT12*.

Next, we tested the effect of high temperatures on NF-Y function in regulating *QT12*. In EMSA, NF-YA8 exhibited increased binding affinity on $QT12^G$ when temperature increased, but no significant change was observed on $QT12^A$ (Figures 4D, S4B, and S4C). After adding NF-YB9 and NF-YC10 alone or together, the binding affinities significantly decreased, and the decreased level was gradually weakened by increasing temperatures (Figures 4D and S4B). ChIP-qPCR confirmed that the binding affinities of NF-YA8 on *QT12* increased after high-temperature treatment *in vivo* (Figure 4E). Dual-LUC assays showed that the activity of $QT12^G$ promoter alone is not induced by high temperatures but increased when co-transformed with NF-YA8 (Figure 4F). After adding NF-YB9 or NF-YC10, the promoter activity decreased but significantly increased when temperature increases (Figure 4F). By assaying the activities of four full-length promoters with variations at the G/A site caused by NF-YA8, we found that NF-YA8 can only activate promoters containing G, which was further upregulated by high temperatures (Figure S4D), further confirming the G/A functional variation of *QT12*. We next found that NF-Ys' interactions were significantly weakened by high temperature in split-LUC assays (Figures 4G and S4E), which was further confirmed by coIP *in vivo* and pull-down *in vitro* (Figures 4H and 4I). These results indicate that high temperature enhances the binding and transcriptional activity of NF-YA8 on $QT12^G$ by weakening its interactions with NF-YB9 and NF-YC10.

To confirm the genetic relationship between *QT12* and *NF-YA8*, we overexpressed *QT12* in *NF-YA8*-CR background and found a significant increase in grain chalkiness, coupled with a great decrease of protein-to-amylose ratio (Figures 4J and S4F). This rescued the *NF-YA8* mutant phenotype of low chalkiness, confirming that *NF-YA8* acts upstream of *QT12*. Further analysis using the 533 core-germplasms revealed that accessions with low *QT12* or *NF-YA8* expression have higher thermotolerance, compared with those with high expression (Figure S4G). Among accessions with low *QT12* expression, no significant thermotolerance difference was observed between those with high and low *NF-YA8* expression; however, among accessions with relatively higher *QT12* expression, those with low *NF-YA8* expression demonstrated higher thermotolerance, compared with those with high *NF-YA8* expression (Figure S4G). These results demonstrate that the thermotolerance regulated by *NF-YA8* is important and depends on *QT12*, suggesting that *QT12* is a major downstream target of NF-YA8. Furthermore, simultaneous overexpression of *NF-YC10* and *NF-YA8* significantly inhibited the high chalkiness caused by *NF-YA8* overexpression only and maintained storage substance balance (Figures 4K, S4H, and S4I), indicating that NF-YC10 genetically inhibits NF-YA8. These results indicate that high temperature weakens NF-YA8 interaction with NF-YB9 and NF-YC10, releasing the NF-YA8 binding and transcriptional activation on $QT12^G$, ultimately leading to the disruption of storage substance homeostasis during endosperm development.

Altogether, the G/A variation in *QT12* functions as the primary switch controlling its differential expression. The NF-Y regulatory system acts as the secondary and high-temperature-dependent switch to modulate *QT12* expression and grain quality thermotolerance. Thus, the cascaded NF-Ys-*QT12* module forms a natural gene on-off system of thermotolerance, which achieves the step-by-step and differential regulation of high-temperature signals, resulting in field thermotolerance differences for grain quality.

(I) Grain chalkiness and ratios of protein to amylose content of *NF-YA8* overexpression and CRISPR lines under different temperatures.
(J and K) *QT12* expression in 5-DAF endosperms of *NF-YA8* overexpressing (J) and mutant (K) lines under different temperatures.
(L) Grain chalkiness, ratios of protein to amylose content, and *QT12* expression in *NF-YB9* CRISPR lines under different temperatures.
Significant differences indicated by different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.
See also Figure S3 and Tables S1 and S2.

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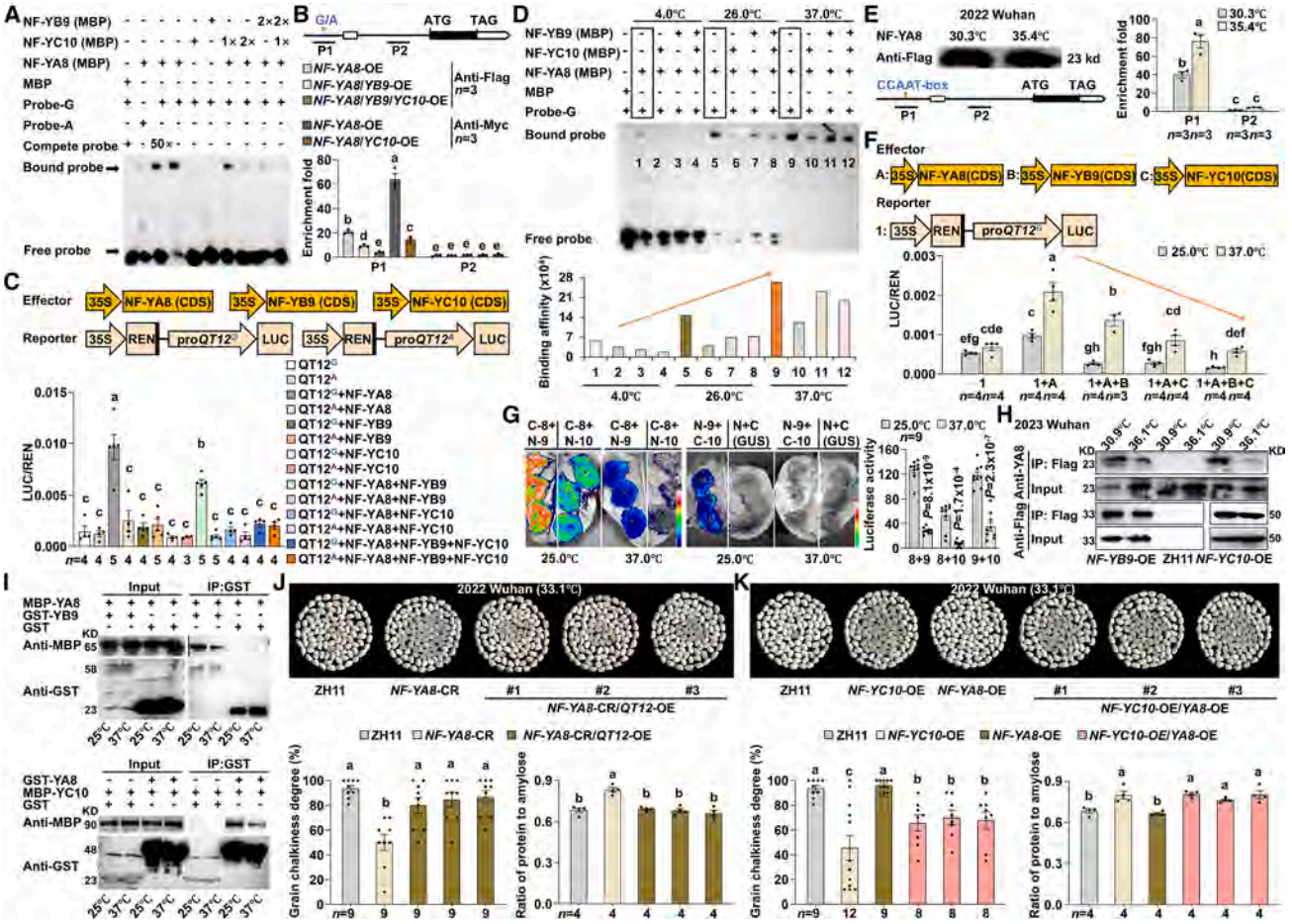

Figure 4. High temperature enhances the binding and transcriptional activities of NF-YA8 on *QT12* by inhibiting its interactions with NF-YB9 and NF-YC10

(A) EMSA for the binding of NF-YA8, NF-YB9, and NF-YC10 on DNA fragments containing the CCAAT motif (probe G) or the variable one (probe A) in *QT12* promoter.

(B) Binding of NF-YA8 on the CCAAT-box region of *QT12* by ChIP-qPCR using different single, double, or triple overexpression plants. No-antibody fragment and the P_2 fragment in *QT12* intron were used as negative controls.

(C) Dual-luciferase assays of the transcriptional activity of NF-Ys on the G/A site of *QT12* promoter.

(D) EMSA for the binding of NF-Ys with DNA fragments containing the CCAAT motif at different temperatures.

(E) Binding of NF-YA8 on the CCAAT-box of *QT12* by ChIP-qPCR.

(F) Dual-luciferase assays of NF-Ys-*QT12* under different temperatures.

(G–I) Split-LUC (G), coIP (H), and pull-down (I) assays for interactions among NF-Ys under different temperature treats.

(J and K) Grain chalkiness, ratios of protein to amylose content of *QT12* overexpression lines in *NF-YA8* mutant (J), and single and double overexpression lines of *NF-YA8* and *NF-YC10* (K) under natural high temperatures.

Significant differences with different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.

See also Figure S4.

TRHs synergistically contribute to thermotolerance differentiation in rice subspecies

To identify the effects of the NF-Ys-*QT12* module on thermotolerance differentiation between *indica* and *japonica*, we conducted haplotype analysis of the 4 genes in 4,726 accessions worldwide. The representative variations in *QT12*, *NF-YA8*, *NF-YB9*, and *NF-YC10* (Table S3) were classified into 7, 9, 14, and 11 haplotypes, respectively, and each haplotype showed significant *indica-japonica* differentiation (Figures 5A and S5A). Furthermore, a 6-bp indel in the 5′ untranslated region (UTR) of *QT12* was identified as a key marker distinguishing *indica* from *japonica* (Figures 5B and 5C). We found that *QT12* promoter activity in *japonica* was significantly higher than that in *indica* at 25°C, and it further increased under 37°C (Figure 5D). Additionally, the three major haplotypes of *QT12* determined by the 6-bp indel between *indica* and *japonica* and the functional G/A variation present only in *indica* can significantly separate *QT12* expression in 120 accessions into a stepwise expression pattern (Figure 5E), suggesting the importance of the 2 variations in thermotolerance differentiation. To further confirm the functional

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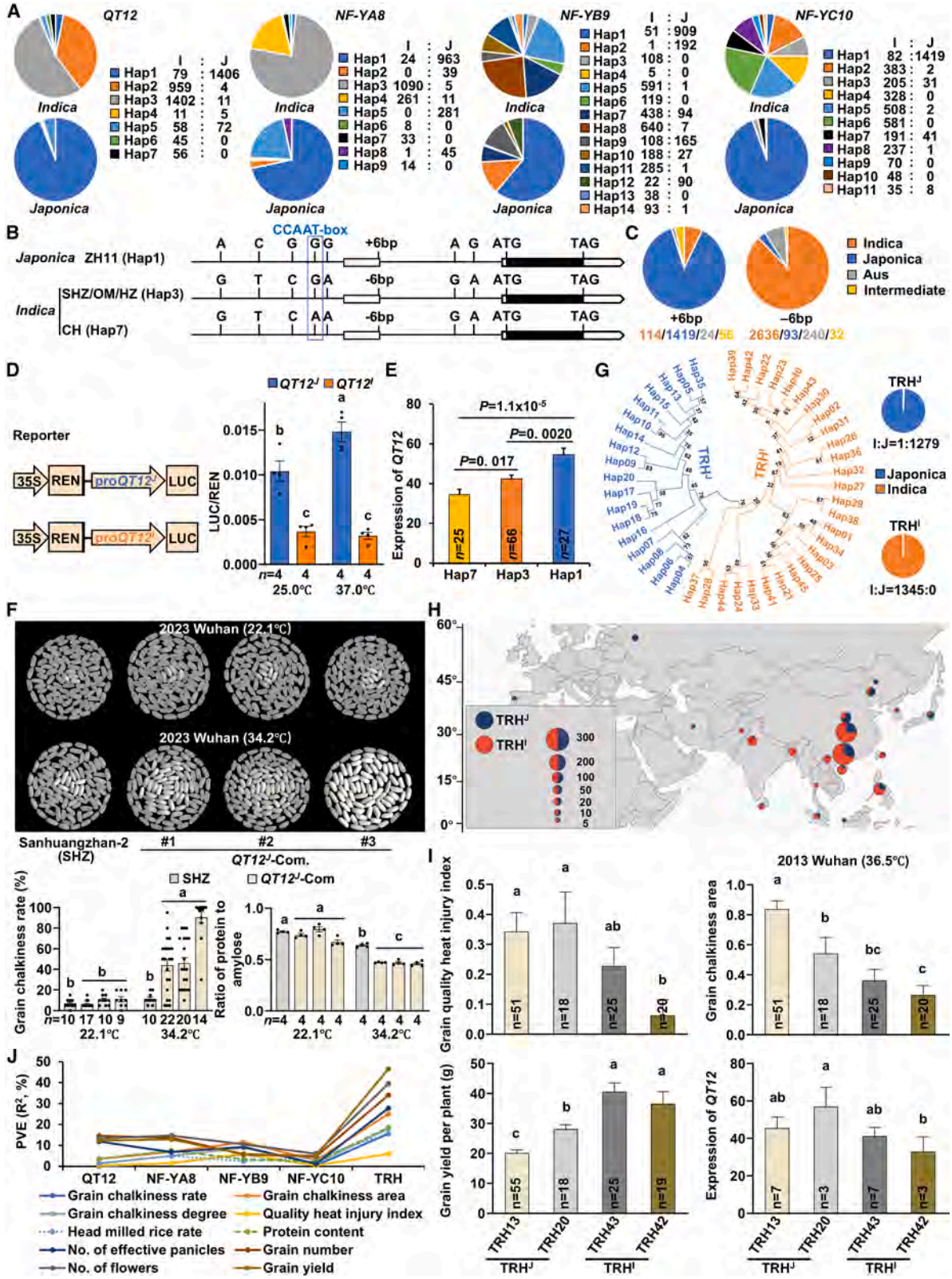

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difference between $QT12^J$ and $QT12^I$ in grain quality thermotolerance, the $QT12^J$ allele of the *japonica* ZH11 was introduced into the moderate-resistance *indica* Sanhuangzhan-2 (SHZ) (Figures 5B–5E). Compared with normal temperatures, *QT12* expression in all complementation lines was significantly induced by high temperatures (Figure S5B), which disrupted storage substance balance and finally promoted grain chalkiness formation, while wild type maintained better homeostasis and thus superior quality (Figures 5F and S5C). This result indicates that $QT12^J$ significantly reduces grain quality under high temperature and confirms the subspecies differentiation function of *QT12* in thermotolerance. Meanwhile, *NF-YB9* expression induced by high temperature was completely different between *indica* and *japonica* (Figure S5D)—it increased in *indica* but decreased in *japonica*. In addition, *NF-YB9* expression in *indica* was significantly higher than that in *japonica* (Figure S5E). Further thermotolerance analysis of the four main haplotypes of *NF-YB9* revealed that Hap7 and Hap8 exhibited lower grain-quality injury index and grain chalkiness degree under higher temperatures than Hap5 and Hap1 (Figure S5F). Similarly, Hap3 from the five major haplotypes of *NF-YA8* has the lowest grain-quality injury index and grain chalkiness degree under high temperatures (Figure S5G). Altogether, we demonstrated that natural variations in each component of NF-Ys-*QT12* have significant thermotolerance differentiation between subspecies.

Since the four genes function together to participate in *indica-japonica* thermotolerance differentiation, we performed a combined haplotype analysis using the G/A SNP and the 6-bp indel of *QT12*, functional and non-functional haplotypes of *NF-YC10*, and representative variations of *NF-YA8* and *NF-YB9* across 4,726 germplasms (Table S4). Remarkedly, the natural variations of the four genetically unlinked genes, located on different chromosomes, were co-selected with one another other. These co-selections collectively formed multiple haplotype combinations, termed TRHs, which may efficiently execute diverse functions and contribute to trait genetic diversity in nature. In total, 45 TRHs were identified and classified into two major TRH types based on phylogenetic analysis, TRH^J (18 TRHs) and TRH^I (27 TRHs), accounting for nearly 100% *japonica* and *indica*, respectively (Figure 5G). Subsequently, we found that *QT12* expression in TRH^J was much higher than that in TRH^I (Figure S5H). The geographical distribution showed that TRH^I was enriched in lower latitude regions, whereas TRH^J was mainly distributed in higher latitudes, with no difference in longitudes (Figures 5H, S5I, and S5J). These data suggest that two major TRHs are highly differentiated and generally explain the differentiation in both *QT12* expression and geographical distribution between *indica* and *japonica*.

To verify whether TRHs can be applied for thermotolerance improvement, four main TRHs with the same $QT12^G$ genotype were identified to access their potential breeding value (Figure 5I). TRH42 and TRH43 have lower grain-quality injury index and grain chalkiness under high temperature, along with higher grain yield and lower *QT12* expression than TRH13 and TRH20. These results suggest that different *NF-YA8*, *NF-YB9*, and *NF-YC10* haplotypes, with the same $QT12^G$ genotype, lead to diverse differences in thermotolerance by regulating *QT12* expression, highlighting the significant role of three NF-Ys in the NF-Ys-*QT12* module. Next, we used TRH^J and TRH^I to analyze their genetic contribution to major grain quality and yield traits in 533 accessions (Figures 5J and S5K). Compared with TRH^J, TRH^I accessions exhibited lower grain chalkiness degree and quality heat injury index (Figure S5K), indicating that *indica* has higher thermotolerance. In addition, compared with TRH^J, TRH^I has lower protein content, gel consistency, and alkali spreading value and higher amylose content, head rice rate, and fatty acid C16:0 (Figure S5K). We then selected two main haplotypes of each gene and TRHs to evaluate their genetic contribution to grain quality and yield traits, and we found that the genetic contribution of TRHs was much higher than each single gene in the module (Figure 5J). These results further indicate that the TRHs synergistically contribute to the variation of grain quality and yield traits in rice.

QT12 promotes ER stress by inhibiting the UPR sensor IRE1 to disturb storage substance balance

The Sec61 translocon in endoplasmic reticulum (ER) membrane participates in ER stress and unfolded protein response (UPR) in animals.[29,30] During heat stress, the accumulation of misfolded/unfolded proteins in ER triggers UPR, leading to upregulation of

Figure 5. Two major trait regulatory haplotypes of *QT12* and *NF-Ys* genes synergistically contribute greatly to *indica-japonica* differentiation in thermotolerance

(A) Haplotype distribution of the four genes in 4,726 rice accessions worldwide. The ratio is the corresponding frequency distribution between *indica* (I) and *japonica* (J).

(B) Representative natural variations of three major *QT12* haplotypes with typical varieties.

(C) Distribution of the 6-bp indel in *QT12*.

(D) Dual-luciferase assays of two *QT12* promoters from *indica* and *japonica* under different temperatures.

(E) *QT12* expression among the three haplotypes determined by the 6-bp indel and G/A variation.

(F) Grain chalkiness and ratios of protein to amylose content of the complementation lines by introducing $QT12^J$ from ZH11 into $QT12^I$ background of SHZ under different natural temperatures. Both of ZH11 and SHZ are of the $QT12^G$ genotype.

(G) Phylogenetic analysis and distribution of 45 TRHs.

(H) Geographical distribution of TRH^J and TRH^I.

(I) Thermotolerance, grain chalkiness, yield and *QT12* expression in four main TRHs with the same $QT12^G$ genotype.

(J) Genetic contributions of the four genes and TRHs to grain quality and yield traits in the mini-core collection. Grain chalkiness phenotypes were obtained under high temperatures in 2013 Wuhan.

The phenotype variance explanation (PVE) was determined by one-way ANOVA. Significant differences indicated by different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.

See also Figure S5 and Tables S3 and S4.

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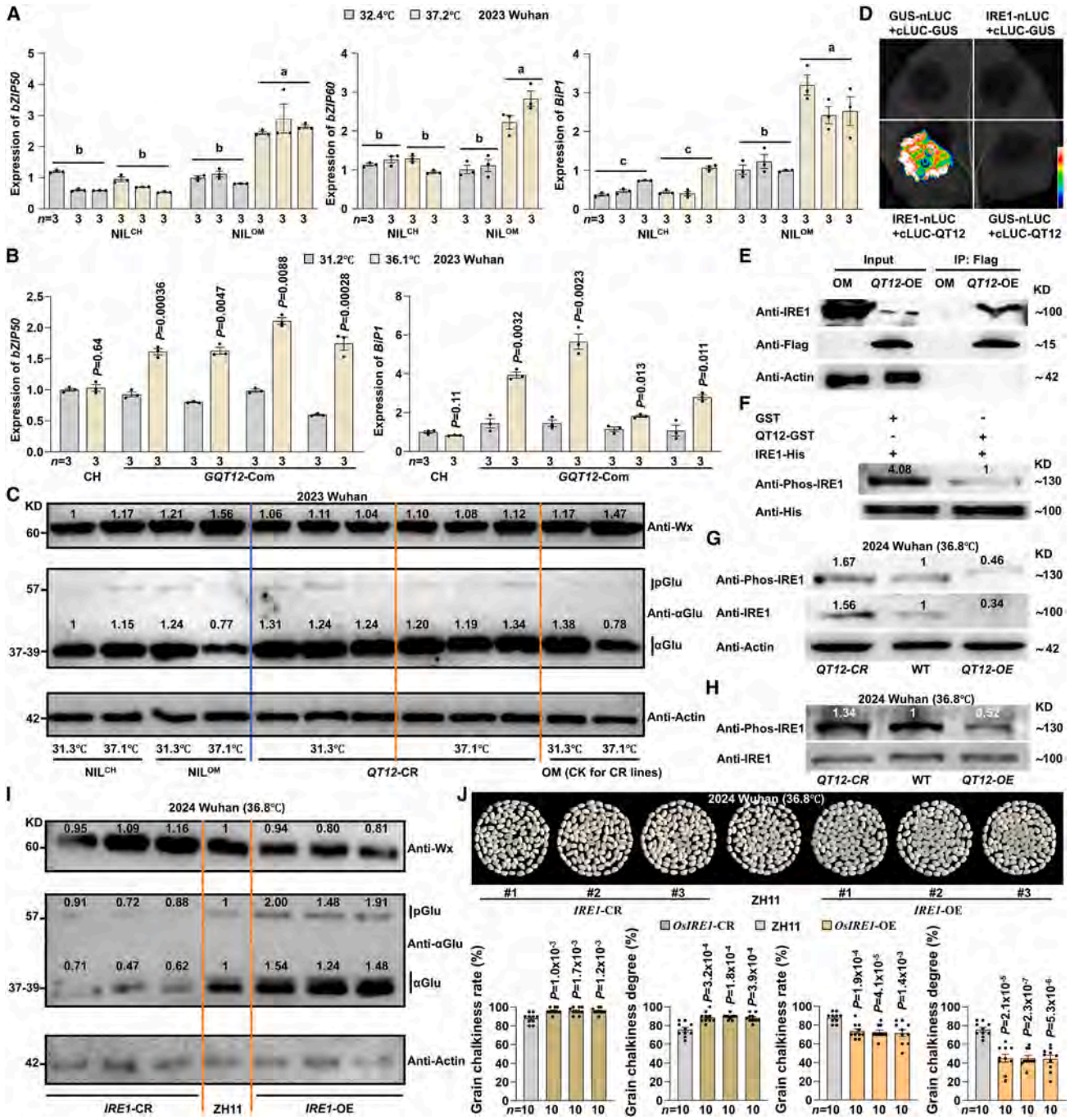

Figure 6. *QT12* inhibits the UPR sensor IRE1 and promotes ER stress to disturb storage substance balance

(A and B) Expression of three UPR marker genes (*bZIP50*, *bZIP60*, and *BiP1*) in 5-DAF developing endosperms of *QT12* NILs (A) and complementation lines (B) under different temperatures.

(C) Immunoblot analysis of Wx and glutelins in mature seeds from *QT12* NILs and transgenic lines. pGlu represents proglutelins, αGlu represents α-glutelins.

(D and E) Split-LUC (D) and coIP (E) assays for the interaction between QT12 and IRE1.

(F) The autophosphorylation activity of IRE1 after co-expression with QT12, using the specific anti-Phos-IRE1 antibody.

(G and H) The autophosphorylation activity and protein abundance of IRE1 in 5-DAF developing endosperms of *QT12*-OE and knockout lines (G) and when maintaining consistent IRE1 protein abundance in them (H) under high temperatures.

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marker genes, including *bZIP50*, *bZIP60*, and *BiP1*.[31–33] In this study, the expression of these genes in NIL^{OM} and *QT12*-Com were indeed significantly induced by high temperature, while NIL^{CH} and CH maintained stable expression, thereby preserving ER homeostasis (Figures 6A and 6B). UPR-induced transcriptional factors like bZIP60 and bZIP50 directly bind to the promoters of storage protein and starch synthesis-related genes.[31–33] In NIL^{OM}, the expression of storage protein synthesis-related genes was significantly decreased under high temperatures, while the expression of the major amylose synthesis gene *Wx* was significantly increased, accompanied by changes of storage starch metabolism-related genes (Figures S5L–S5N). In contrast, these genes showed no significant expression change in NIL^{CH} (Figures S5L–S5N), consistent with the trends of *bZIP50*, *bZIP60*, and *BiP1* expression. Similar trends were observed for glutelins and Wx protein by immunoblotting in NILs and CRISPR lines (Figure 6C). Thus, the misregulation of the key genes for major storage substance metabolisms contributed to the increase of grain chalkiness under high temperatures. Compared with normal temperatures, glutelins, prolamins, and globulins in mature endosperms of NIL^{OM} were significantly reduced at high temperatures (Figure S5O). The overexpression lines showed similar results. On the contrary, NIL^{CH} and CRISPR lines exhibited no change in storage proteins under different temperatures (Figure S5O). Overall, high-temperature-induced QT12 enhanced ER stress and activated UPR, thereby disrupting the expression of genes responsible for storage substance synthesis.

IRE1 (Inositol Requiring Enzyme 1), a key sensor of UPR with kinase activity precisely inhibits UPR through a negative feedback loop to relieve ER stress and restore ER homeostasis.[34–36] To further explore the molecular mechanism of QT12 in activating UPR, we found that QT12 can interact with IRE1 *in vivo*, and overexpression of *QT12* resulted in a significant decrease in IRE1 protein abundance (Figures 6D and 6E). Furthermore, the autophosphorylation activity of IRE1 significantly decreased after the addition of QT12 *in vitro* (Figure 6F). *In vivo* experiments showed that the protein abundance and autophosphorylation activity of IRE1 significantly increased when knocking out *QT12* and decreased when overexpressing *QT12* (Figures 6G and 6H). These results suggest that QT12 inhibited IRE1, thereby releasing the negative feedback inhibition on UPR, leading to the sustained ER stress, activation of UPR, and UPR-induced transcriptional factors, eventually disrupting the expression balance of key storage substance synthesis genes.

To verify whether IRE1 affects the expression of storage protein/starch synthesis-related genes, we analyzed the protein levels of Wx and glutelins in *IRE1*-OE and knockout lines (Figures S5P and S5Q). We found that the *IRE1*-OE lines had lower Wx abundance than ZH11 under high temperature, while the expression of glutelins was higher (Figure 6I). The opposite trend was observed in the *IRE1* knockout lines, indicating that IRE1 negatively regulates Wx expression and positively regulates glutelin expression under high temperatures (Figure 6I), contrasting with QT12's role. Overexpression of *IRE1* significantly reduced grain chalkiness under high temperature, while knockout lines of *IRE1* still exhibited a higher chalkiness phenotype (Figure 6J), demonstrating that *IRE1* positively regulates grain quality thermotolerance, as opposed to *QT12*. Thus, these results suggest that QT12 promotes ER stress by inhibiting IRE1, thereby continuously activating UPR to disrupt storage substance balance by regulating their key gene expression, thus negatively controlling thermotolerance.

Low expression of *QT12* synergistically improves grain quality and yield under field high temperatures

To investigated the impact of *QT12* on grain yield under natural high temperatures, phenotypic analysis showed that the seed-setting rate of the *QT12*^{A} haplotype was significantly higher than *QT12*^{G} in rice core collection and *indica* accessions (Figure S6A), and TRH^{I} has a higher grain yield (+38.9%) than TRH^{J} (Figure S6B), indicating the potential application of *QT12* and TRHs for grain yield. Then, we found that there was no significant difference in seed-setting rate and yield of NILs under normal temperatures; however, under high temperatures, NIL^{CH} exhibited higher seed-setting rate and yield per plant (+18.1%) than NIL^{OM} (Figures 7A and S6C). Additionally, the taste value of cooked rice of NIL^{CH} was consistently higher than that of NIL^{OM} under both temperature conditions (Figures 7A and S6C). These results indicate that *QT12* has significant potential for improvement of both grain yield and quality under high temperatures.

Seed-setting rate and taste value of cooked rice of *QT12*-Com lines significantly decreased, compared with CH under high temperatures, with a 40.7% decrease in grain yield per plant (Figures 7B and S6D). For CRISPR lines, seed-setting rate significantly increased, resulting in a great increase of grain yield per plant by 1.7 times, showing great thermotolerance for grain yield (Figures 7C and S6E). Meanwhile, the taste values of cooked rice significantly improved under high temperatures (Figure 7C). Complementation of *QT12*^{J} into the *QT12*^{I} variety SHZ significantly reduced seed-setting rate and grain taste values/quality, with a 31.9% decrease in grain yield, compared with SHZ (Figures S6F and S6G). However, under normal temperatures, there were no change in both yield and quality traits of various genetic lines (Figures 7A–7C, S6C, S6F, and S6H–S6J).

Large-scale field trials using various genetic lines were conducted in three major cities in China's Yangtze River Basin, a major rice-producing region that has constantly experienced record-breaking extreme high temperatures for the past 20 years, particularly in 2024 (Figure 7D). Grain yield per plot of *QT12*-Com lines significantly decreased by 49.7%, 52.1%, and 40.3% in Wuhan, Hangzhou, and Changsha, respectively, accompanied by a significant increase in grain chalkiness,

(I) Immunoblot analysis of Wx and glutelins in mature seeds from the *IRE1* transgenic lines.
(J) Grain chalkiness of *IRE1*-OE and knockout lines under high temperatures.
Quantification of band intensity relative to controls is shown by numbers on each gel using ImageJ. Significant differences with different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.
See also Figure S5.

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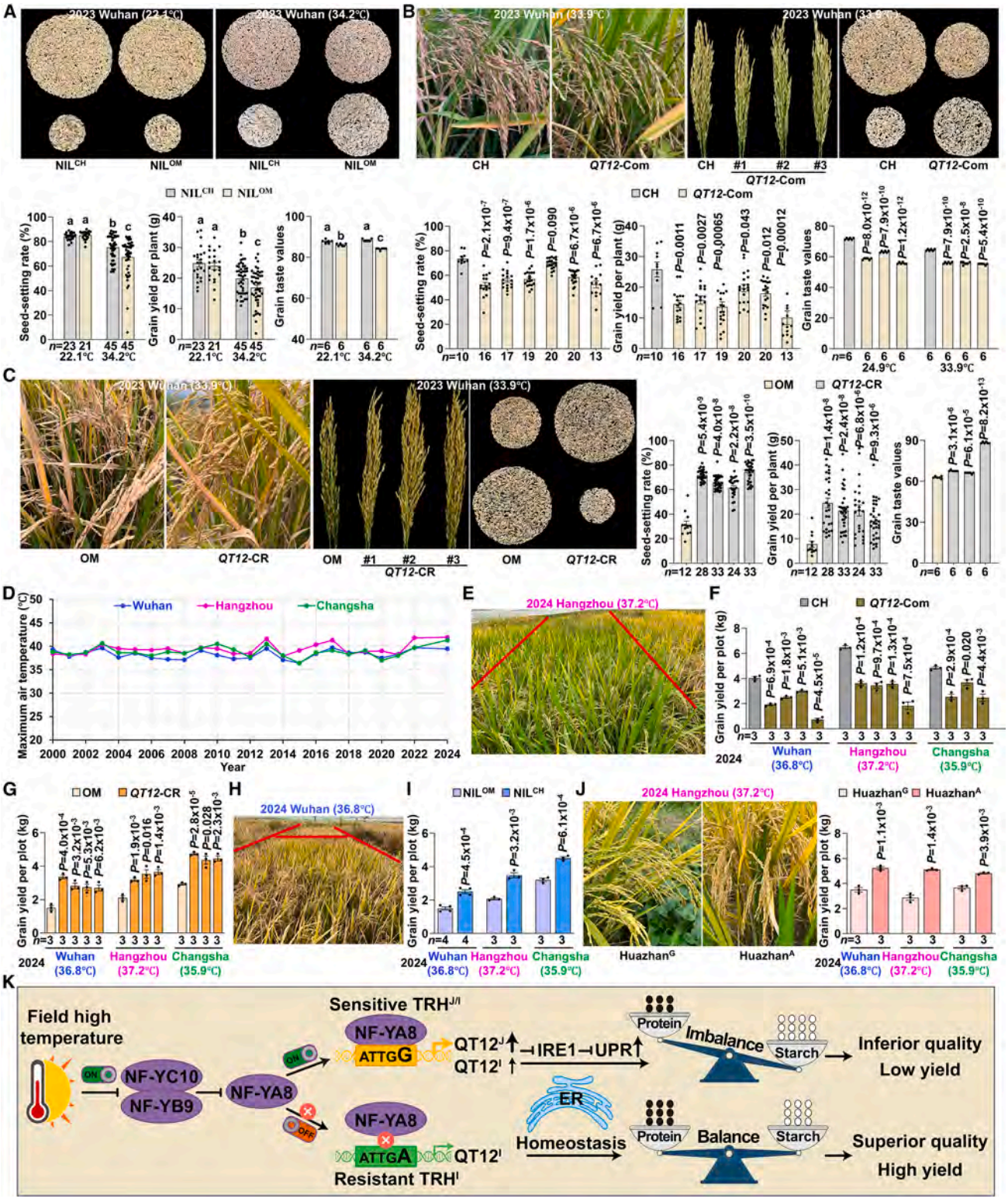

Figure 7. Low expression of *QT12* synergistically confers thermotolerance for grain yield and quality and a model for the NF-Ys-*QT12* module acting as a natural gene on-off system conferring thermotolerance in rice

(A–C) Seed-setting rate, grain yield, and taste values of *QT12* NILs (A), complementation (B), and mutant lines (C) under different natural temperatures.

(D) Maximum air temperatures at rice flowering and grain filling stages in Wuhan, Hangzhou, and Changsha from July to September during 2000 to 2024.

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compared with CH (Figures 7E, 7F, and S7A). In contrast, grain yield per plot increased significantly in both CRISPR lines (by 92.5%, 64.1%, and 54.7%, compared with OM) and NIL^{CH} (by 67.4%, 68.1%, and 40.6%, compared with NIL^{OM}) in Wuhan, Hangzhou, and Changsha, respectively, while grain chalkiness was significantly reduced in both lines (Figures 7G–7I and S7B–S7D). Importantly, compared with $Huazhan^{G}$, $Huazhan^{A}$ significantly improved seed-setting rate (by 13.6%, 32.5%, and 10.9%), grain yield per plant (by 46.9%, 80.8%, and 28.0%), and grain yield per plot (by 49.1%, 77.9%, and 31.2%) in Wuhan, Hangzhou, and Changsha, respectively, while exhibiting a much lower grain chalkiness degree than $Huazhan^{G}$ (Figures 7J and S7E). Notably, other agronomic traits of all *QT12*-related genetic materials showed no change (Figures S7F–S7L). Taken together, these results demonstrate that low-expression or non-functional *QT12* can simultaneously increase grain yield and improve quality of elite rice varieties planted in field high-temperature environments, breaking the breeding bottleneck of stress-growth and yield-quality trade-offs in crops.

DISCUSSION

As global warming intensifies, crops are experiencing more heat damage, causing major declines in both yield and quality, threatening global food security and farmer income.[1–4] Here, we advance the understanding of how grain quality and endosperm development develop in response to field high temperatures by identifying *QT12* as a key gene that synergistically maintains grain quality and yield under natural high temperatures. We also elucidated an operative natural gene on-off system for the molecular mechanism behind high-temperature-induced grain quality deterioration during rice grain filling stage. We finally demonstrated how this discovery can be leveraged to improve grain quality of elite varieties while increasing yield in large-scale field high-temperature trials in multiple regions, thereby enhancing agricultural sustainability worldwide and improving the income of numerous farmers in Asia and Africa.

The discovery of the natural gene on-off system of NF-Ys-*QT12* establishes a regulatory model that explains the thermotolerance difference among *indica* and between *indica* and *japonica* subspecies (Figure 7K). In high-temperature-sensitive *indica* and all *japonica* under normal temperatures, NF-YA8 binds directly the CCAAT-box in *QT12* promoter to upregulate its expression, while NF-YB9 and NF-YC10 inhibit the binding and transcriptional activity of NF-YA8, turn off the gene on-off system, and thus repress *QT12* expression and maintain ER homeostasis, conferring superior quality and high yield by balancing storage substance content. Under high temperatures, the NF-YA8 interactions with NF-YB9 and NF-YC10 are weakened, resulting in higher binding affinity of NF-YA8 and turning on the gene on-off system to activate *QT12*. Activated QT12 induced ER stress and UPR by inhibiting the UPR sensor IRE1, thus inhibiting storage protein accumulation coupled with increased starch by UPR-induced marker transcriptional factors, ultimately leading to a shift in the homeostasis between storage protein and starch and thereby to inferior grain quality (Figure 7K). In thermotolerant *indica*, the G/A natural variation as the primary switch disrupts the CCAAT-box element; consequently, regardless of temperature conditions, NF-YA8 cannot bind *QT12* promoter, thus keeping the gene on-off system off and maintaining basal *QT12* expression. This disruption activates IRE1 or probably slows down the process of co-translational translocation of unfolded proteins induced by high temperatures,[24] maintaining ER homeostasis and storage substance balance in endosperms, thereby conferring high-temperature resistance (Figure 7K). Due to the co-differentiation of natural variations in *QT12* and NF-Ys between *indica* and *japonica*, *QT12* expression in *indica* is significantly lower than in *japonica*, conferring a higher thermotolerance of *indica* than *japonica*. The two switches of the gene on-off system exist in nature probably due to or responsible for *indica* and *japonica* differentiation and thus naturally evolved in rice. We therefore proposed a concept, TRHs (Figure 5G; Table S4), combinations of haplotypes from these genes that form a regulatory module with diverse thermotolerance. This concept establishes the genetic basis for thermotolerance differentiation between *indica* and *japonica* at grain filling stage (Figures 5G–5J). How *QT12* regulates seed-setting rate and grain yield and synergistically improves both yield and quality under high temperatures remains to be further studied.

Little is known about the complete NF-Y ternary complex in crops. In rice, most mutants of NF-Y subunit genes preferentially expressed in seeds exhibit a high chalkiness, indicating that they generally and negatively regulate grain chalkiness,[37–46] while the positive regulation in this study is rarely reported. Meanwhile, some NF-Y subunits are involved in abiotic stresses[27]; however, how they are regulated by high temperature to control endosperm development and grain quality remains unclear. Furthermore, we discovered a unique inhibition mechanism among NF-Y subunits, differing from the canonical NF-Y complexes where NF-YB and NF-YC subunits usually promote NF-YA activity.[26,28] The complex cascaded regulatory pattern in NF-Ys-*QT12* forms a natural gene on-off system, highlighting the complexity and flexibility of the coordination that finely transduces high-temperature signals to fine-tune *QT12* expression. How the NF-Y-*QT12* switch system senses high-temperature signals remains to be investigated.

The Sec61 translocon, consisting of three subunits, α, β, and γ, mediates the entry of newly generated peptide chains or unfolded proteins from cytosol into ER lumen.[25] The α and γ subunits are essential for channel function and cell viability, while the β subunit function is still poorly understood.[24,25] Glutelin and prolamin, two major storage proteins, are secretory

(E–J) Grain yield per plot (5 m^2) of *QT12* complementation lines (E and F), CRISPR lines (G), NILs (H and I), and introgression lines of *QT12* in Huazhan (H and J) in Wuhan, Hangzhou, and Changsha in 2024.

(K) A working model for the natural gene on-off system of NF-Ys-*QT12* conferring field thermotolerance in rice (see detailed explanations in the second paragraph of discussion).

Significant differences indicated by different letters via one-way ANOVA and Duncan's test. *p*, two-tailed t test. Means ± SEM.

See also Figures S6 and S7.

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proteins that must be translocated and rightly folded in ER before being transported into protein storage vacuoles in developing endosperms, thereby forming protein bodies II and I, respectively.[8] Sec61 also acts as an ER Ca^{2+} leakage channel, and inhibition of Sec61 complex protects cells from apoptosis by inhibiting Ca^{2+} leakage during ER stress.[47,48] When the Ca^{2+} signal induced by high temperature is weakened, rice exhibits enhanced thermotolerance.[49] Therefore, we speculate that *QT12* as a Sec61 β subunit induces UPR to inhibit the co-translational translocation of storage proteins for ER homeostasis under high temperatures, thereby hindering storage protein accumulation in endosperm cells and disrupting final storage substance balance (synthesis of storage protein and starch is competitive for energy and matter, resulting into the general trade-off between them[50]). Meanwhile, *QT12* may also regulate UPR and ER homeostasis by modulating Ca^{2+} signaling or IRE1 activity in ER at high temperatures, thereby affecting thermotolerance. How *QT12* regulates Ca^{2+}, IRE1, and differential translation or transport of storage protein, starch, or their metabolic enzymes under high temperatures is unknown.

Abundant natural variation combinations form diverse TRHs likely to efficiently execute diverse functions, resulting in rich genetic diversity of a trait. As more molecular modules underlying QTL interactions are identified, applying the TRH concept will allow accurately explaining phenotype variation and fully defining the underlying molecular mechanisms. Different superior combinations of TRHs offered a strategy for the fine regulation of diverse *QT12* levels to achieve an optimal and robust balance between high yield and superior quality under high temperatures. It could also be employed for breeding other thermotolerant crops for a warmer future, owing to its highly conserved nature. Furthermore, high temperatures naturally inhibit the burst of various pests and diseases in crops,[51,52] and thus thermotolerance is usually coupled with resistance to pests and diseases for further maintaining grain yield and quality, which has comprehensive application prospects for addressing the crisis caused by global climate changes.

Limitations of the study

In this study, we identified the major grain quality-thermotolerant gene *QT12* that confers field thermotolerance simultaneously for quality and yield in rice, and we discovered a natural gene on-off system of NF-Ys-*QT12* for thermotolerance variation among subspecies. However, the specific biochemical function of QT12 as a translocon subunit for protein transport or as a Ca^{2+} channel for Ca^{2+} leakage at high temperatures remains to be fully explored, and the detailed molecular and cellular mechanisms by which QT12 or UPR regulates the trade-off/homeostasis between storage protein and starch under high temperature have not yet been fully elucidated.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yibo Li (liyibo@mail.hzau.edu.cn).

Materials availability

Materials and further information are available from the lead contact upon completing material transfer agreement.

Data and code availability

- All data are available in our paper or at public databases. Original phenotype data for grain chalkiness and yield traits in this study have been deposited at Mendeley (https://doi.org/10.17632/kxs54cfp67.1) and are publicly available at the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

W.L. completed most experiments and data analysis and wrote the draft manuscript. K.Y. completed *IRE1* experiments. C.H., W.A., Jian Zhang, W. Y., A.S., Q.L., and B.L. participated in some experiments in genetic materials. P.X., B.C., Juncheng Zhang, and Y.H. provided GWAS or some grain-quality data. X.L., L.X., L.Q., and Q.Z. provided assistance in editing the manuscript. Y.L. designed and supervised the study and wrote and thoroughly revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Phosphorylation assays

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2025.04.011.

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