

The GT1-WRKY53-BZR1 module regulates blast resistance and grain size in rice

Zhuo Li

2677610434@qq.com

Liaoning Academy of Agricultural Sciences

Shuo Yang

State Key Laboratory of Elemental Organic Chemistry

Xinrui Li

State Key Laboratory of Elemental Organic Chemistry

Vikranth Kumar

University of Missouri

Dandan Li

State Key Laboratory of Elemental Organic Chemistry

Wenjing Zheng

Liaoning Academy of Agricultural Sciences

Yuanhu Xuan

State Key Laboratory of Elemental Organic Chemistry

Research Article

Keywords: GT1, BZR1, WRKY53, Blast, grain size, rice

Posted Date: September 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10901407/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Developing disease-resistant rice varieties is a crucial strategy for effective disease management. Both high yield and strong disease resistance are essential targeting traits in rice breeding, yet the signaling pathways governing these traits often exhibit antagonistic interactions. In this study, *grassy tiller 1* (*GT1*) was identified as a positive regulator of rice blast resistance. Overexpression of *GT1* enhanced grain size, whereas *GT1* mutants exhibited normal grain size. Further analysis revealed that *GT1* interacts with *BZR1* and *WRKY53*, two key transcription factors in the brassinosteroid (BR) signaling pathway, both of which positively regulate rice blast resistance. Transcriptome analysis of *GT1* overexpressing (*GT1 OX*), *bzr1-D*, and *WRKY53* overexpressing (*WRKY53 OX*) plants identified *WRKY45*, a master regulator of rice blast resistance, as a potential downstream target of the *GT1*-*WRKY53*-*BZR1* module. Yeast one-hybrid, transient expression, and chromatin immunoprecipitation assays demonstrated that *GT1* and *BZR1* directly activate *WRKY45* by binding to its promoter, while *WRKY53* does not directly bind to *WRKY45* but may enhance its expression through complex formation. The genetic combination of *WRKY53 OX* and *bzr1-D* indicated an additive effect of *BZR1* and *WRKY53* on blast resistance. Additionally, *WRKY53 OX* and *GT1 OX*, but not *bzr1-D*, promoted grain enlargement. Further analysis of downstream genes identified the BR receptor *BRASSINOSTEROID INSENSITIVE1* (*BRI1*) as a positively regulated target of *GT1* and *WRKY53*, which in turn enhances grain size. These findings demonstrate that *GT1* interacts with BR signaling transcription factors to regulate both rice blast resistance and grain size by activating *WRKY45* and *BRI1*.

Introduction

With the continued expansion of the global population, it is estimated that by 2030, over 600 million people will face food crisis (United Nations). Ensuring the stability and productivity of food crops is therefore an urgent priority. Crops are frequently exposed to biotic stresses such as fungal, bacterial, viral, and insect infestations, all of which pose significant threats to global food production (Savary et al., 2019). However, while plant immunity is critical for disease resistance, prolonged immune responses often negatively impact crop yield and quality (Wang et al., 2021). Achieving a balance between immunity and yield is thus a key objective in modern crop breeding (Deng & He, 2024).

Rice yield is predominantly influenced by grain weight, grain number per panicle, and effective tiller number (Li et al., 2021). Several recently documented factors governing grain size and weight, including *GRAIN WIDTH5* (*GW5*), *GRAIN SIZE2* (*GS2*), and *GRAIN SIZE5* (*GS5*), are closely linked to BR signaling. Additionally, BR signaling components such as *BRASSINOSTEROID INSENSITIVE1* (*BRI1*), *BRI1-ASSOCIATED RECEPTOR KINASE* (*BAK1*), *BRASSINOSTEROID DEFICIENT1* (*BRD1*), and *BRD2* influence both grain size and yield (Yamamuro et al., 2000, Li et al., 2009a, Hong et al., 2002). Shoot architectural features, such as tiller angle and plant height, are another major determinant of rice productivity. Overexpression of *MONOCULM1* (*MOC1*), a key switch gene and regulator of tillering, enhances panicle number (Li et al., 2003). *TEOSINTE BRANCHED 1* (*TB1*), a downstream target of *MOC1*, negatively regulates lateral branching (Li et al., 2003, Takeda et al., 2003). Through interactions with *DWARF 53*

(D53), BZR1 forms a transcriptional complex that is capable of inhibiting the expression of *TB1* (Wang et al., 2020). *TB1* can additionally bind the promoter region upstream of *Grassy tiller 1* (*GT1*), activating its expression. Loss of *GT1* function results in significantly increased tillering (Kumar et al., 2021). These studies show that BR signaling influences plant height, leaf angle, tillering number, tiller angle, and grain size. Manipulating BR signaling components can thus improve rice yield by optimizing these traits (Li et al., 2009b).

In this study, the role of *GT1* in the regulation of rice blast resistance and grain size was investigated. The results demonstrate that *GT1* positively modulates rice blast resistance upon *Magnaporthe oryzae* Guy 11 inoculation. *GT1* interacts with *WRKY53* and *BZR1*, two BR-associated transcription factors that enhance blast resistance. This suggests that *GT1*, *WRKY53* and *BZR1* may form a transcriptional complex to regulate disease resistance. Additionally, *GT1* and *WRKY53* were found to promote grain enlargement by upregulating *BRI1*, a key BR receptor. These findings highlight a novel *GT1*-*WRKY53*-*BZR1* module that balances plant growth and immunity, offering new perspectives for rice breeding strategies.

Materials and Methods

Plant materials

Two *Oryza sativa* Japonica cultivars Dongjin (DJ) and Zhonghua 11 (ZH11) were used in this study. The *GT1* overexpression (*GT1 OX*), *GT1* RNA interference (*GT1 RNAi*) and CRISPR-Cas9-edited *gt1* mutant lines were previously described (Yang et al., 2023), as well as the *bzr1-D*, *wrky53*, *WRKY53 OX* and *WRKY53 OX/bzr1-D* lines (Tian et al., 2021). The *BRI1* activation tagging lines (*BRI1 Ac*) and *d61-1* mutants were described previously (Yuan et al., 2018, Je et al., 2010).

Plant growth and *M. oryzae* inoculation

Rice seedlings were grown in greenhouse at Shenyang Agricultural University under natural light conditions with temperatures being maintained between 23–30°C and 60% relative humidity (RH). Plants were cultivated until the three-leaf stage, then continued to grow for an additional week with a minimal nitrogen supplement. *M. oryzae* Guy11 was activated on potato dextrose agar (PDA) medium and subsequently cultured on tomato-oat medium under low light conditions for approximately two weeks to induce spore production. The spores were harvested using sterile water containing 0.28% Tween-20, and the spore concentration was adjusted to $1.5\text{--}2 \times 10^5$ spores/mL using a hemocytometer. For inoculation, rice leaves were excised into 5 cm segments, and uniform scratches were made on the adaxial surface. The spore suspension was then applied to the wounds, and samples were incubated in the dark for 24 h. Subsequently, a 12 h light/12 h dark cycle was employed, and RH was kept at ~ 75% to prevent excessive moisture accumulation. Lesion lengths on the leaves were measured 5–7 days post-inoculation. All experiments were set up with three independent biological replicates. In each experiment, six plants with consistent growth were selected, and parallel operations were carried out on the leaves at the same leaf

position of each plant. Additionally, *Nicotiana benthamiana* plants were grown under a 14 h light/10 h dark cycle at 22–24°C with 70% RH in a controlled chamber for four weeks before use.

Yeast two-hybrid assay

The open reading frames (ORFs) of *GT1*, *WRKY53*, and *BZR1* were amplified by PCR and verified by sequencing. The DNA fragments were cloned into pGADT7 and pGBKT7 vectors and transformed into *Saccharomyces cerevisiae* strains Y2HGold and Y187 (Clontech, <http://www.clontech.com/>). Transformed yeast cells were grown on SD/-Trp/-Leu selection plates and subsequently transferred to SD/-Trp/-Leu/-His plates to assess protein interactions. Primers for cloning *GT1*, *WRKY53*, and *BZR1* are listed in Table S1.

Bimolecular fluorescence complementation (BiFC) assay

For the BiFC assay, *GT1-cCFP*, *BZR1-nYFP*, *WRKY53-cCFP*, and *WRKY53-nYFP* fusion constructs were generated and co-transformed into *Agrobacterium tumefaciens* strain GV3101. Agroinfiltration was performed in *N. benthamiana* leaves (Kim et al., 2009). To study their interactions, the constructs were co-infiltrated with the nuclear marker H2B-mRFP1 and the silencing suppressor P19 in a 1:1:1:1 ratio. Fluorescence signals were visualized 48–72 h post-injection using a confocal microscope (Olympus FV1000, Japan). All primers are listed in Table S1.

Co-immunoprecipitation (Co-IP)

The ORFs of *GT1*, *WRKY53* and *BZR1* were cloned into the pGD3GGm and pGD3G3Flag vectors to generate *GT1*-GFP, *GT1*-3×Flag, *WRKY53*-GFP, *WRKY53*-3×Flag *BZR1*-GFP and *BZR1*-3×Flag fusion constructs, respectively. Forty-eight hours after agroinfiltration, total protein extracts were prepared using an extraction buffer (150 mM NaCl, 2.5 mM Tris-HCl, 1 mM EDTA, 10% glycerol, 0.1% NP-40, 2% PVPP, 10 mM DTT, and 1× protease inhibitor cocktail). Immunoprecipitation was performed using anti-Flag magnetic beads (Sigma-Aldrich, MO, USA), followed by Western blotting using anti-GFP and anti-Flag (EASYBIO, Beijing, China) to confirm protein interactions.

Yeast one-hybrid (Y1H) assay

To investigate *GT1* and *BZR1* binding to the *WRKY45* promoter, three DNA fragments containing *GT1* binding motifs (3.5–2.0 kb upstream) and *BZR1* binding motifs (1.0 kb upstream) were separately cloned into the pHISi-1 vector. The ORFs of *GT1* and *BZR1* were inserted into the pGAD424 vector. The *pHISi-WRKY45* (*GT1*), *pHISi-WRKY45* (*BZR1*), *pGAD424-GT1*, *pGAD424-BZR1*, or empty *pGAD424* (negative control) vectors were transformed into *S. cerevisiae* (YM4271). Transformants were plated on SD/-Leu

and SD/-Leu/-His media and incubated for 48–72 h to monitor yeast growth. Utilized primers are compiled in Table S1.

Dual Luciferase Reporter assay

The 3500 bp and 1000 bp promoter regions of *WRKY45* were cloned into the pGreenII 0800-LUC vector via infusion cloning and transformed into *A. tumefaciens* GV3101 (pSoup). Transformed colonies were cultured in LB medium supplemented with rifampicin and kanamycin for 12–14 h. Agrobacterium suspensions were prepared and infiltrated into *N. benthamiana* leaves. To ensure consistency, control, and experimental bacterial suspensions were injected into different sections of the same leaf. Luciferase activity was observed 48–60 h post-inoculation. All experiments were set up with three independent biological replicates. Utilized primers are compiled in Table S1.

Chromatin immunoprecipitation (ChIP)

For ChIP assays, samples from 10-day-old seedlings of *35S:GT1-GFP* and *35S:bzr1-D-Flag* transgenic lines were cross-linked in 37% formaldehyde under vacuum. Chromatin was extracted and immunoprecipitated using magnetic beads conjugated to GFP (*35S:GT1-GFP*) and Flag (*35S: bzr1-D-Flag*) antibodies. Immunoprecipitated complexes were washed, and DNA was purified for ChIP-PCR analysis as described previously (Je et al., 2010). Utilized primers are compiled in Supplementary Table S1.

Statistics analyses

All analyses were performed with Prism 8.0 (GraphPad, CA, USA). Data are presented as means $\pm$ standard error (SE). Data were compared using one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. $P < 0.05$ was deemed significant.

Results

GT1 functions as a positive regulator of rice blast resistance

Previous studies have demonstrated that the mutation of *GT1* significantly increases the number of tillers in rice (Kumar et al., 2021). However, the role of *GT1* in resistance to rice blast disease remains unclear. *GT1* overexpressing (*GT1 OX*) and RNA interference (*GT1 RNAi*) plants were inoculated with *M. oryzae* Guy 11. The *GT1 RNAi* plants exhibited increased susceptibility to rice blast compared to wild-type (WT) plants, whereas *GT1 OX* plants (Yang et al., 2023) were more resistant (Fig. 1A, 1B). To further confirm the role of *GT1* in rice blast resistance, CRISPR-Cas9-edited *gt1* mutant plants were analyzed (Yang et al., 2023). Consistently, *gt1* mutants on the ZH11 background displayed heightened

susceptibility to rice blast relative to ZH11, supporting the notion that GT1 positively regulates rice blast resistance (Figs. 1C, 1D). Additionally, *GT1 OX* plants produced significantly larger grains than WT (ZH11), whereas *GT1 RNAi* plants exhibited no substantial differences from WT (Figs. 1E-H). This suggests that GT1 plays a dual role in promoting rice blast resistance and grain size enhancement.

GT1 interacts with BR signaling-related transcription factors

Previous research has established that GT1 interacts with WRKY53 to negatively regulate rice sheath blight resistance (Yang et al., 2023) (Figs. 2A-C). Notably, WRKY53 has been shown to enhance both rice blast resistance and plant growth through BR signaling (Meng et al., 2024, Hao et al., 2022). WRKY53 interacts with BZR1, forming a transcriptional complex that modulates target gene expression (Tian et al., 2021) (Figs. 2D-F). Furthermore, the *bzr1-D* mutant demonstrated increased resistance to rice blast compared to WT plants (He et al., 2024). Given that GT1 interacts with WRKY53, a key BR signaling transcription factor (Yang et al., 2023), we investigated whether GT1 also interacts with BZR1. Our results confirmed that GT1 interacts with BZR1 in the nucleus (Figs. 2G-I). This suggests that GT1, WRKY53 and BZR1 may form a transcriptional complex involved in the control of rice blast resistance.

GT1 and BZR1 interact to activate WRKY45 and regulate rice blast resistance

To explore the molecular mechanism underlying GT1-mediated rice blast resistance, transcriptome data from WT vs. *GT1 OX* (Kumar et al., 2021), WT vs. *WRKY53 OX* (Tian et al., 2021), and WT vs. *bzr1-D* (Ren et al., 2020) samples were evaluated. This analysis identified 13 genes co-regulated by GT1, WRKY53 and BZR1, including two WRKY family transcription factors, WRKY1 and WRKY45, which were upregulated in *GT1 OX* and downregulated in *WRKY53 OX* and *bzr1-D*. Specifically, *WRKY1* (*Os01t0246700*) and *WRKY45* (*Os05t0322900*) exhibited increased expression in *GT1 OX* samples compared with WT controls (Fig. 3A). WRKY45 is a well-characterized transcription factor known to enhance rice blast resistance (Shimono et al., 2007). RT-qPCR analysis further confirmed that *WRKY45* expression was reduced in *GT1 RNAi* plants and elevated in *GT1 OX* plants (Fig. 3B). In contrast with these transcriptomic data, *WRKY45* expression was decreased in the *BZR1 RNAi* line and *wrky53* mutants, whereas it was elevated in *bzr1-D* and *WRKY53 OX* plants relative to the WT controls (Fig. 3C, 3D).

To determine whether GT1, WRKY53, and BZR1 directly regulate *WRKY45*, we analyzed the 3.5 kb promoter region upstream of *WRKY45*. The analysis identified putative GT1-binding motifs, BR response elements (BRREs), and G-box motifs but no W-box elements, which are recognized by WRKY53 (Fig. 3E, 3H). Yeast one-hybrid assays demonstrated that GT1 and BZR1 directly bind the *WRKY45* promoter (Figs. 3E, 3H). Dual-luciferase reporter assays further confirmed that *WRKY45* transcription was activated by GT1 and BZR1 but not by WRKY53 (Figs. 3F, 3G, 3J, 3K, 3M, 3N). ChIP-PCR analyses revealed that BZR1 bound to the G-box and BRRE motifs in the P1, P2 and P3 regions of the *WRKY45* promoter but not to the P4, P5 or P6 regions (Fig. 3H). Similarly, GT1 was found to be associated with P1 and P2 but not with P3, P4 or P5 (Fig. 3L). Finally, to evaluate the functional relevance of the GT1-

WRKY53-BZR1 complex, rice blast resistance was assessed in *WRKY53 OX/bzr1-D* plants. The results showed that *WRKY53 OX/bzr1-D* plants were more resistant to rice blast than either *bzr1-D* or *WRKY53 OX* plants alone (Fig. 3O-3R). These indicate that GT1 interacts with BZR1 to form a transcriptional complex that activates *WRKY45*, thus enhancing rice blast resistance.

GT1 interacts with WRKY53 to modulate grain size through the control of BR signaling

The balance between plant growth and immunity is often regulated by shared molecular components that coordinate developmental and defense responses(He et al., 2024). Notably, grain size in *bzr1-D* mutants was comparable to WT, whereas *WRKY53* overexpression resulted in increased grain length, grain width, and leaf angle(Tian et al., 2021). To determine whether GT1 and WRKY53 regulate genes involved in grain size modulation, transcriptomic data from *GT1 OX* and *WRKY53 OX* plants were analyzed, identifying 166 co-regulated genes, including those associated with plant growth, hormone biosynthesis, and signal transduction pathways (Figure S1). Given the close relationship between BR signaling and grain size, further examination revealed that GT1 modulates the expression of four BR signaling-associated genes, including a BRI1 precursor gene, *Os07g0597200* (Fig. 4A). Intriguingly, WRKY53 transcriptomic data included multiple BRI1-related genes, such as *BRI1* itself and its associated receptor kinases (Fig. 4B). Phenotypic analysis showed that the grain length of *BRI1* activation-tagged plants (*BRI1 Ac*) was greater than that of WT controls, whereas *d61-1*, a *BRI1* mutant, exhibited reduced grain length compared to its WT counterpart (Fig. 4C-F). These results suggest that GT1 and WRKY53 likely regulate grain size by activating *BRI1*.

Discussion

Previous studies have demonstrated that upon pathogen infection, plants prioritize hormone signaling pathways that activate defense responses over those that promote growth(He et al., 2022, Yang et al., 2012, Yang et al., 2013). However, this shift often results in inhibited growth and developmental retardation, leading to significantly reduced crop yield(Brown, 2003). This trade-off mechanism presents a major challenge in crop breeding, as achieving both enhanced resistance and increased yield simultaneously has proven difficult.

Rice blast is one of the three most devastating rice diseases, affecting all developmental stages and substantially impacting both yield and grain quality(Yin et al., 2021). With advancements in molecular biology, an increasing number of blast resistance genes have been identified and cloned in recent years(Liao et al., 2025, Gao et al., 2021). For example, the *ROD1* gene encodes a novel calcium receptor that promotes catalase CatB localization to the plasma membrane by recognizing calcium ion signals and binding to lipids. This mechanism allows the catalase to degrade reactive oxygen species (ROS) in membrane regions, thereby suppressing immune responses in the absence of pathogen infection. However, this suppression leads to delayed panicle primordium development, ultimately affecting grain yield(Gao et al., 2021). Here, we identified the homeobox transcription factor GT1 as a positive regulator

of rice blast resistance. Notably, *GT1 OX* plants exhibited larger grain size than WT plants, suggesting that *GT1* plays a crucial role in circumventing the trade-off mechanism to simultaneously bolster disease resistance and yield. Recent findings indicate that *GT1* interacts with *WRKY53* to activate downstream sugar signaling genes, thereby enhancing sheath blight resistance (Yang et al., 2023). Furthermore, *WRKY53* overexpression enhances blast resistance, increases grain length and width, and positively regulates BR signaling (Tian et al., 2021, Meng et al., 2024). Given that BR signaling is central to the trade-off between plant immunity and growth (Meng et al., 2024, Hao et al., 2022, He et al., 2024), our findings that *GT1* interacts with both *WRKY53* and *BZR1* (Fig. 2) suggest that *GT1* enhances blast resistance and grain size through BR signaling.

These results further demonstrated that *GT1* enhances rice blast resistance by interacting with *BZR1* to activate downstream *WRKY45* expression (Fig. 3). *WRKY45* is a key transcription factor involved in rice blast resistance. The E3 ubiquitin ligase *IPI7* has been shown to specifically enhance the transcriptional activity of *IPA1* (S163D), promoting *WRKY45* expression through K29 ubiquitination and enhancing disease resistance (Shi et al., 2024). In addition to *WRKY45*, several other *WRKY* family transcription factors are known to regulate rice blast resistance (Kim et al., 2000, Cheng et al., 2015, Shi et al., 2024). cDNA library screening of blast fungus-infected rice revealed that *WRKY1* is blast-inducible (Kim et al., 2000). Notably, *WRKY1* expression is co-regulated by *GT1*, *WRKY53*, and *BZR1* (Fig. 3A). Other *WRKY* transcription factors, including *WRKY13* and *WRKY42*, function in a transcriptional cascade involved in the rice blast resistance. *WRKY42* negatively regulates rice blast resistance by suppressing jasmonic acid (JA) signaling-related genes, while *WRKY13* inhibits *WRKY42* transcription, thereby enhancing resistance (Cheng et al., 2015). Additionally, *WRKY67* directly activates the pathogenesis-related genes *PR1a* and *PR10*, thereby modulating resistance to both blast and bacterial blight in rice (Liu et al., 2018). These findings further support the role of *GT1* in regulating blast resistance through *BZR1*-mediated activation of *WRKY45*, a key *WRKY* family transcription factor.

GT1 overexpression resulted in increased grain size, whereas *BZR1* did not influence grain size. Transcriptome analysis identified 166 genes co-regulated by *GT1* and *WRKY53*, including those involved in plant growth, hormone biosynthesis, and signal transduction pathways, such as auxin, gibberellin, ethylene, JA, and receptor-like cytoplasmic kinases (RLCKs) (Figure S1). Notably, both *GT1* and *WRKY53* were found to regulate *BRI1*-related genes (Figs. 4A, 4B). Overexpression of *BRI1* resulted in significantly larger grains compared to WT, whereas the *BRI1* allele mutant *d61-1* exhibited reduced grain size (Fig. 4). These findings align with previous studies indicating that *BRI1* regulates grain size in various plant species (Yuan et al., 2022). For instance, in semi-dwarf wheat, *ZnF*, a positive regulator of BR signaling, promotes the interaction between *TaBRI1* and *TaBKI1*, thereby activating BR signaling and increasing yield (Song et al., 2023). In *Setaria italica*, *SiBRI1* interacts with an E3 ubiquitin ligase without undergoing degradation, thereby increasing yield (Tang et al., 2023). Similarly, in potato, activated *StBRI1* enhances BR-mediated cell expansion by activating a plasma membrane proton pump, leading to increased tuber yield (Deng et al., 2024). These findings suggest that *GT1* and *WRKY53* may regulate grain size by modulating downstream *BRI1* expression.

In summary, our study elucidates how GT1 interacts with BR signaling transcription factors to regulate both rice blast resistance and grain size. These findings reveal the multifunctional regulatory mechanisms of the GT1-WRKY53-BZR1 transcriptional complex in rice blast resistance and provide valuable genetic resources for breeding high-yield, disease-resistant rice varieties. However, further research is necessary to fully elucidate the pathogenic mechanisms of rice blast and the molecular basis of resistance, which will contribute to the development of new strategies to ensure global food security.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The manuscript has been approved by all authors.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (32372482 and 32072406).

Author Contribution

Z.L.: literature survey, visualization, and writing–original draft. S.Y, X.R.L., V.K. and D.D.L: review and editing. W.J.Z: conceptualization. Y.H.X. : funding acquisition, resources, writing–review and editing.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (32372482 and 32072406). We appreciate very much Prof. Chang-deok Han from Gyeongsang National University for providing GT1 RNAi and GT1 OX seeds.

Data Availability

The data and materials supporting the findings of this study are available upon request.

References

1. Brown JKM (2003) A cost of disease resistance: paradigm or peculiarity? *Trends Genet* 19:667–671
2. Cheng H, Liu H, Deng Y, Xiao J, Li X, Wang S (2015) The WRKY45-2 WRKY13 WRKY42 Transcriptional Regulatory Cascade Is Required for Rice Resistance to Fungal Pathogen. *Plant Physiol* 167:1087–1099
3. Deng R, Huang S, Du J, Luo D, Liu J, Zhao Y et al (2024) The brassinosteroid receptor StBRI1 promotes tuber development by enhancing plasma membrane H⁺-ATPase activity in potato. *Plant Cell* 36:3498–3520
4. Deng Y, He Z (2024) The seesaw action: balancing plant immunity and growth. *Sci Bull* 69:3–6
5. Gao M, He Y, Yin X, Zhong X, Yan B, Wu Y et al (2021) Ca(2+) sensor-mediated ROS scavenging suppresses rice immunity and is exploited by a fungal effector. *Cell* 184:5391–5404e5317
6. Hao Z, Tian J, Fang H, Fang L, Xu X, He F et al (2022) A VQ-motif-containing protein fine-tunes rice immunity and growth by a hierarchical regulatory mechanism. *Cell Rep* 40:111235
7. He Y, Zhao Y, Hu J, Wang L, Li L, Zhang X et al (2024) The OsBZR1–OsSPX1/2 module fine-tunes the growth–immunity trade-off in adaptation to phosphate availability in rice. *Mol Plant* 17:258–276
8. He Z, Webster S, He SY (2022) Growth–defense trade-offs in plants. *Curr Biol* 32:R634–R639
9. Hong Z, Ueguchi-Tanaka M, Shimizu-Sato S, Inukai Y, Fujioka S, Shimada Y et al (2002) Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase, prevents the organized arrangement and polar elongation of cells in the leaves and stem. *Plant J* 32:495–508
10. Je BI, Piao HL, Park SJ, Park SH, Kim CM, Xuan YH et al (2010) RAV-Like1 Maintains Brassinosteroid Homeostasis via the Coordinated Activation of BRI1 and Biosynthetic Genes in Rice. *Plant Cell* 22:1777–1791
11. Kim C, Lee S-H, Park H, Bae C, Cheong Y-H, Ju C et al (2000) Identification of Rice Blast Fungal Elicitor-Responsive Genes by Differential Display Analysis. *Mol Plant Microbe Interact* 13:470–474
12. Kim JG, Li X, Roden JA, Taylor KW, Aakre CD, Su B et al (2009) Xanthomonas T3S Effector XopN Suppresses PAMP-Triggered Immunity and Interacts with a Tomato Atypical Receptor-Like Kinase and TFT1. *Plant Cell* 21:1305–1323
13. Kumar V, Kim SH, Adnan MR, Heo J, Jeong JH, Priatama RA et al (2021) Tiller Outgrowth in Rice (*Oryza sativa* L.) is Controlled by OSGT1, Which Acts Downstream of FC1 in a PhyB-Independent Manner. *J Plant Biology* 64:417–430
14. Li D, Wang L, Wang M, Xu Y-Y, Luo W, Liu Y-J et al (2009a) Engineering OsBAK1 gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnol J* 7:791–806
15. Li D, Wang L, Wang M, Xu YY, Luo W, Liu YJ et al (2009b) Engineering OsBAK1 gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnol J* 7:791–806
16. Li G, Tang J, Zheng J, Chu C (2021) Exploration of rice yield potential: Decoding agronomic and physiological traits. *Crop J* 9:577–589

17. Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D et al (2003) Control of tillering in rice. *Nature* 422:618–621
18. Liao H, Fang Y, Yin J, He M, Wei Y, Zhang J et al (2025) Rice transcription factor bHLH25 confers resistance to multiple diseases by sensing H₂O₂. *Cell Research*
19. Liu Q, Li X, Yan S, Yu T, Yang J, Dong J et al (2018) OsWRKY67 positively regulates blast and bacteria blight resistance by direct activation of PR genes in rice. *BMC Plant Biol* 18:257
20. Meng F, Zheng X, Wang J, Qiu T, Yang Q, Fang K et al (2024) The GRAS protein OsDLA involves in brassinosteroid signalling and positively regulates blast resistance by forming a module with GSK2 and OsWRKY53 in rice. *Plant Biotechnol J* 22:363–378
21. Ren Y, Tian X, Li S, Mei E, He M, Tang J et al (2020) *Oryza sativa* mediator subunit OsMED25 interacts with OsBZR1 to regulate brassinosteroid signaling and plant architecture in rice. *J Integr Plant Biol* 62:793–811
22. Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A (2019) The global burden of pathogens and pests on major food crops. *Nat Ecol Evol* 3:430–439
23. Shi H, Yin J, Zhao Z, Yu H, Yi H, Xu L et al (2024) Fine-tuning of IPA1 transactivation activity by E3 ligase IPI7-mediated non-proteolytic K29-ubiquitination during *Magnaporthe oryzae* infection. *Nat Commun* 15:7608
24. Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S et al (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* 19:2064–2076
25. Song L, Liu J, Cao B, Liu B, Zhang X, Chen Z et al (2023) Reducing brassinosteroid signalling enhances grain yield in semi-dwarf wheat. *Nature* 617:118–124
26. Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M et al (2003) The OsTB1 gene negatively regulates lateral branching in rice. *Plant J* 33:513–520
27. Tang S, Zhao Z, Liu X, Sui Y, Zhang D, Zhi H et al (2023) An E2-E3 pair contributes to seed size control in grain crops. *Nat Commun* 14:3091
28. Tian X, He M, Mei E, Zhang B, Tang J, Xu M et al (2021) WRKY53 integrates classic brassinosteroid signaling and the mitogen-activated protein kinase pathway to regulate rice architecture and seed size. *Plant Cell* 33:2753–2775
29. Wang F, Han T, Song Q, Ye W, Song X, Chu J et al (2020) The Rice Circadian Clock Regulates Tiller Growth and Panicle Development Through Strigolactone Signaling and Sugar Sensing. *Plant Cell* 32:3124–3138
30. Wang H, Li Y, Chern M, Zhu Y, Zhang L-L, Lu J-H et al (2021) Suppression of rice miR168 improves yield, flowering time and immunity. *Nat Plants* 7:129–136
31. Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S et al (2000) Loss of Function of a Rice brassinosteroid insensitive1 Homolog Prevents Internode Elongation and Bending of the Lamina Joint. *Plant Cell* 12:1591–1605

32. Yang D-L, Yang Y, He Z (2013) Roles of Plant Hormones and Their Interplay in Rice Immunity. *Mol Plant* 6:675–685
33. Yang DL, Yao J, Mei CS, Tong XH, Zeng LJ, Li Q et al (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc Natl Acad Sci USA* 109:E1192–1200
34. Yang S, Fu Y, Zhang Y, Yuan P, Li D, Kumar S, V. et al (2023) Rhizoctonia solani transcriptional activator interacts with rice WRKY53 and grassy tiller 1 to activate SWEET transporters for nutrition. *J Adv Res* 50:1–12
35. Yin J, Zou L, Zhu X, Cao Y, He M, Chen X (2021) Fighting the enemy: How rice survives the blast pathogen's attack. *Crop J* 9:543–552
36. Yuan DP, Zhang C, Wang ZY, Zhu XF, Xuan YH (2018) RAVL1 Activates Brassinosteroids and Ethylene Signaling to Modulate Response to Sheath Blight Disease in Rice. *Phytopathology®* 108:1104–1113
37. Yuan H, Xu Z, Chen W, Deng C, Liu Y, Yuan M et al (2022) OsBSK2, a putative brassinosteroid-signalling kinase, positively controls grain size in rice. *J Exp Bot* 73:5529–5542

Figures

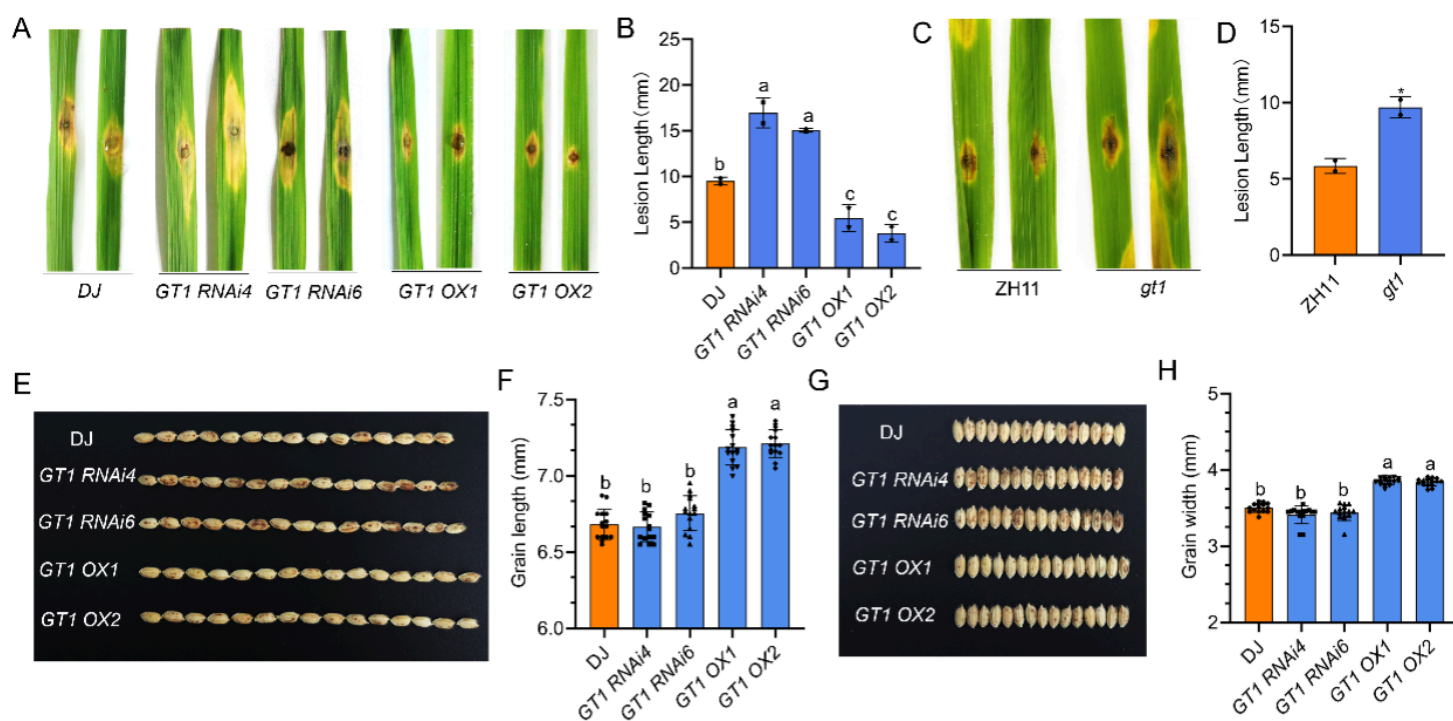


Figure 1

GT1 is a positive regulator of rice sheath blight resistance and grain size. A. Blast responses in *GT1 RNAi* and *GT1 OX* plants. B. Comparisons of lesion length between the *GT1 RNAi*, *GT1 OX*, and WT plants. Data are means $\pm$ standard error ($n > 10$). Different letters indicate significant differences (Tukey's HSD test, $P < 0.05$). C. Blast responses in *gt1* plants. D. Comparisons of lesion length between *gt1* and WT plants.

Data are means $\pm$ standard error ($n > 10$). Different letters indicate significant differences (Tukey's HSD test, $P < 0.05$). E-G. Images (E, G) of WT (DJ), *GT1 RNAi*, and *GT1 OXs* grains, with corresponding quantification of grain length (F) and width (H). Different lowercase letters indicate significant differences ($*P < 0.05$).

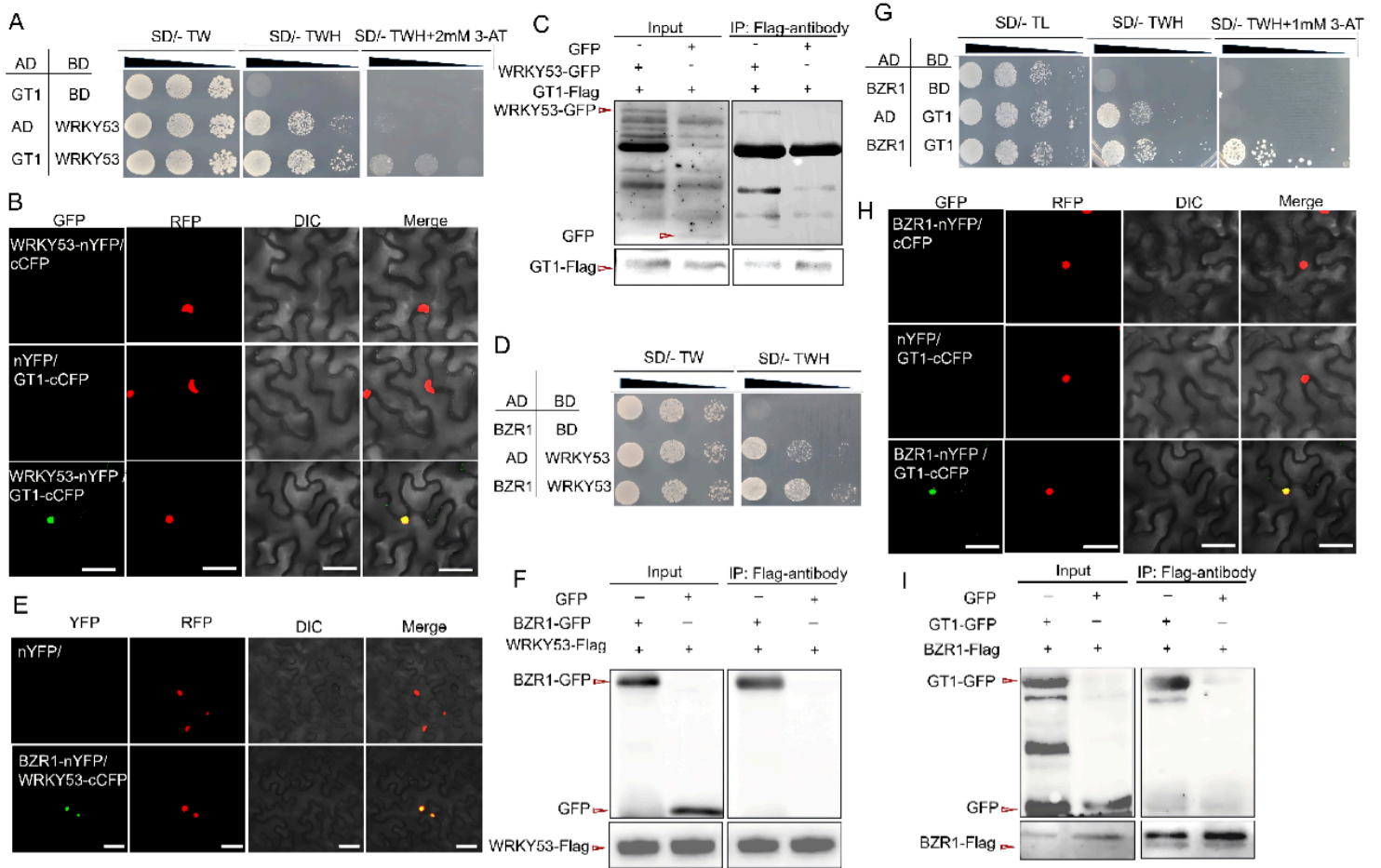


Figure 2

Interactions between GT1 and WRKY53 or BZR1. **A**. Yeast two-hybrid (Y2H) assay results demonstrating interactions between GT1 and WRKY53. Diploid yeast cells containing BD-WRKY53 and AD-GT1 were grown on SD/-Trp -Leu, SD/-Trp -Leu -His, or SD/-Trp -Leu -His + 2 mM 3-amino-1,2,4-triazole (3-AT). **B**. BiFC assay results showing the interaction of GT1 with WRKY53 in the nucleus. *WRKY53-nYFP*, *GT1-cCFP*, and *H2B-mRFP1* co-expression in tobacco leaves led to the reconstruction of functional YFP which co-localized with *H2B-mRFP1*. No yellow fluorescence was evident in the control group. Bar: 20 μ m. **C**. Co-immunoprecipitation (Co-IP) assay demonstrating interactions between GT1 and WRKY53. *GT1-3xFlag* and *WRKY53-GFP* were co-expressed in tobacco leaves. Following total protein extraction, anti-Flag beads were used for immunoprecipitation, followed by Western blotting with anti-GFP and anti-Flag antibodies. **D**. Y2H assay results demonstrating the interaction between WRKY53 and BZR1. Diploid yeast cells containing both BD-WRKY53 and AD-BZR1 were grown on SD/-Trp -Leu and SD/-Trp -Leu -His. **E**. BiFC assay results demonstrating the interaction between WRKY53 and BZR1 in the nucleus. *BZR1-nYFP*, *WRKY53-cCFP*, and *H2B-mRFP1* co-expression in tobacco leaves led to the reconstruction of

functional *YFP* which co-localized with *H2B-mRFP1*. No yellow fluorescence was evident in the control group. Bar: 20 μ m. F. Co-IP results showing the interaction between WRKY53 and BZR1. *BZR1-GFP* and *WRKY53-3xFlag* were co-expressed in tobacco leaves. Following total protein extraction, immunoprecipitation was performed with anti-Flag beads, followed by Western blotting with anti-GFP and anti-Flag antibodies. G. Y2H assay results showing the interaction between GT1 and BZR1. Diploid yeast cells containing both BD-GT1 and AD-BZR1 were grown on SD/-Trp -Leu, SD/-Trp -Leu -His, or SD/-Trp -Leu -His + 1 mM 3-AT. H. BiFC assay results demonstrating the interaction between GT1 and BZR1 in the nucleus. *BZR1-nYFP*, *GT1-cCFP*, and *H2B-mRFP1* reconstruction of functional *YFP* in tobacco leaves and co-localization with *H2B-mRFP1*. No yellow fluorescence was evident in the control group. Bar: 20 μ m. I. Co-IP assay showing the interaction between GT1 and BZR1. *GT1-GFP* was co-expressed with *BZR1-3xFlag* in tobacco leaves. Following total protein extraction, anti-Flag beads were used for immunoprecipitation, followed by Western blotting with anti-GFP and anti-Flag antibodies. IP: immunoprecipitation. EV: empty vector.

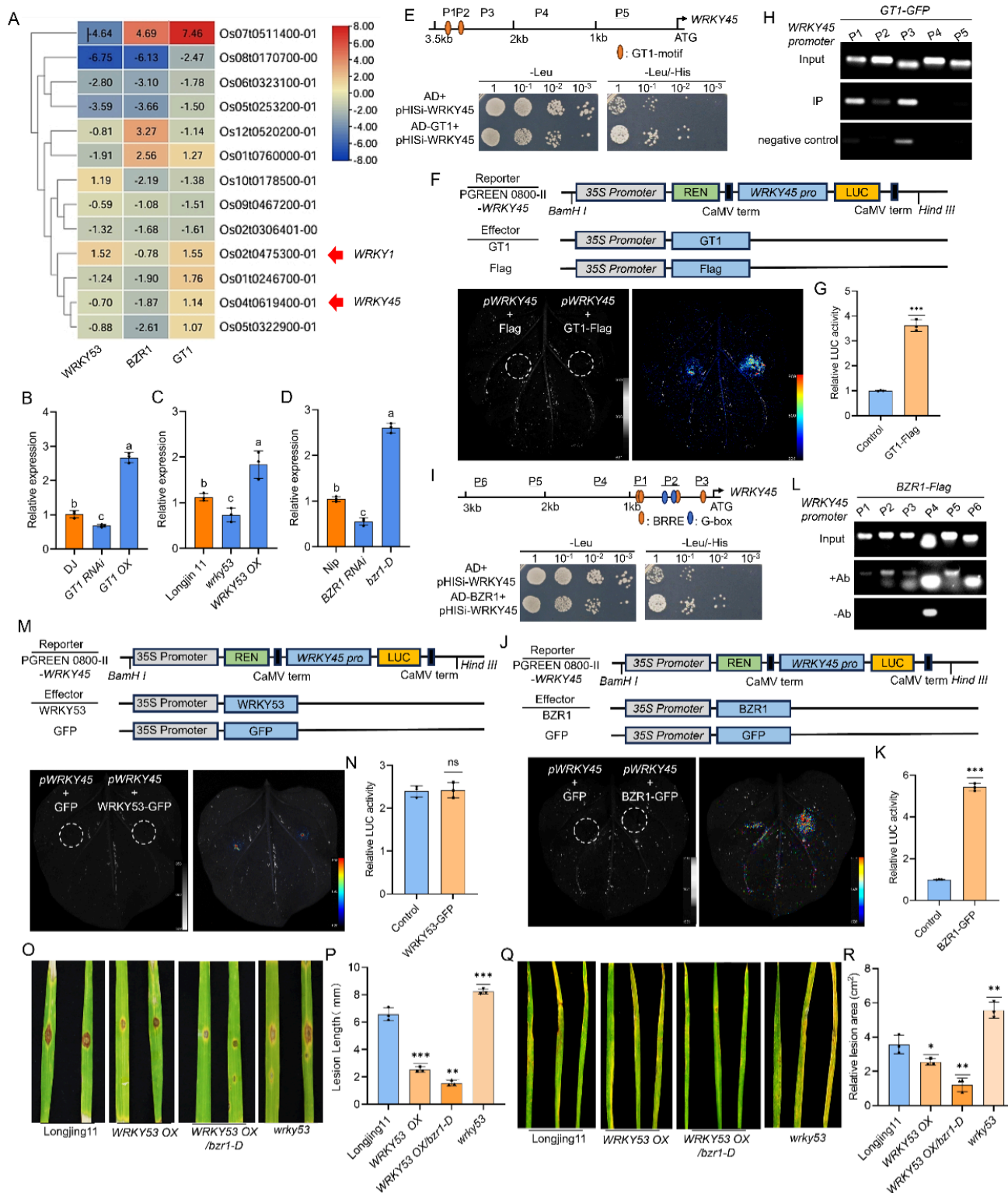


Figure 3

GT1 and BZR1 directly activate *WRKY45*. **A**. Heatmap showing the expression levels of genes regulated by GT1, WRKY53 and BZR1. DEGs for the *WRKY53* OX vs. WT (left), *bzr1-D* vs. WT (middle), and *GT1* OX vs. WT (right) comparisons are shown. Variations in expression are presented with different colors. Red arrows denote *WRKY1* and *WRKY45*. **B-D** qRT-PCR analysis of *WRKY45* in different *GT1*, *WRKY53*, and *BZR1* transgenic plants relative to WT controls. Data are means $\pm$ SE from three replicate experiments. **E**.

Overview of the *WRKY45* promoter. Orange ovals correspond to the two putative GT1-binding motifs within 3.5 kb upstream of the *WRKY45* start codon (ATG). P1 to P5 denote the positions used for ChIP-PCR assays. Yeast one-hybrid assay results revealing the ability of GT1 to activate the *WRKY45* promoter. H. ChIP-PCR results revealing direct GT1 binding to the GT1-binding motifs within the *WRKY45* promoter. The *WRKY45* promoter fragment containing the GT1-binding motifs (P1, P2) was enriched in the ChIP-PCR analysis but not in the negative control (P3, P4, P5). -Ab: Negative control without anti-GFP; +Ab: anti-GFP added. Data are means $\pm$ SE from three replicate experiments. *35S::GFP* plants served as a negative control. I. Overview of the *WRKY45* promoter region. Orange and blue ellipses correspond to the BRRE and G-box motifs, respectively, within 1 kb upstream of the *WRKY45* start codon (ATG). P1 to P6 correspond to the probes used for ChIP-PCR assays. Yeast one-hybrid assay results revealed the activation of the *WRKY45* promoter by BZR1. J, K. Dual-luciferase reporter assay results revealing that BZR1 activates the *WRKY45* promoter. L. ChIP-PCR results revealing direct binding to the BZR1-motif within the *WRKY45* promoter. ChIP-PCR results revealed the enrichment of the *WRKY45* promoter fragment containing the BRRE and G-box motifs (P1, P2, P3) while the same was not evident in the negative control (P4, P5, P6). -Ab: Negative control without anti-Flag; +Ab: anti-Flag added. Data are means $\pm$ SE from three replicates. *35S::Flag* plants served as the negative control. M, N. Dual-luciferase reporter assay results revealed that *WRKY53* did not activate the *WRKY45* promoter. O-R. Assessment of *WRKY53 OX/bzr1-D* responses to blast (O, Q) with corresponding comparisons of relative lesion area (P) and lesion length (R) for the *WRKY53 OX*, *WRKY53 OX/bzr1-D*, and *wrky53* lines relative to the WT (Longjing11). Data are means $\pm$ standard error ($n > 10$). Different lowercase letters indicate significant differences (* $P < 0.05$).

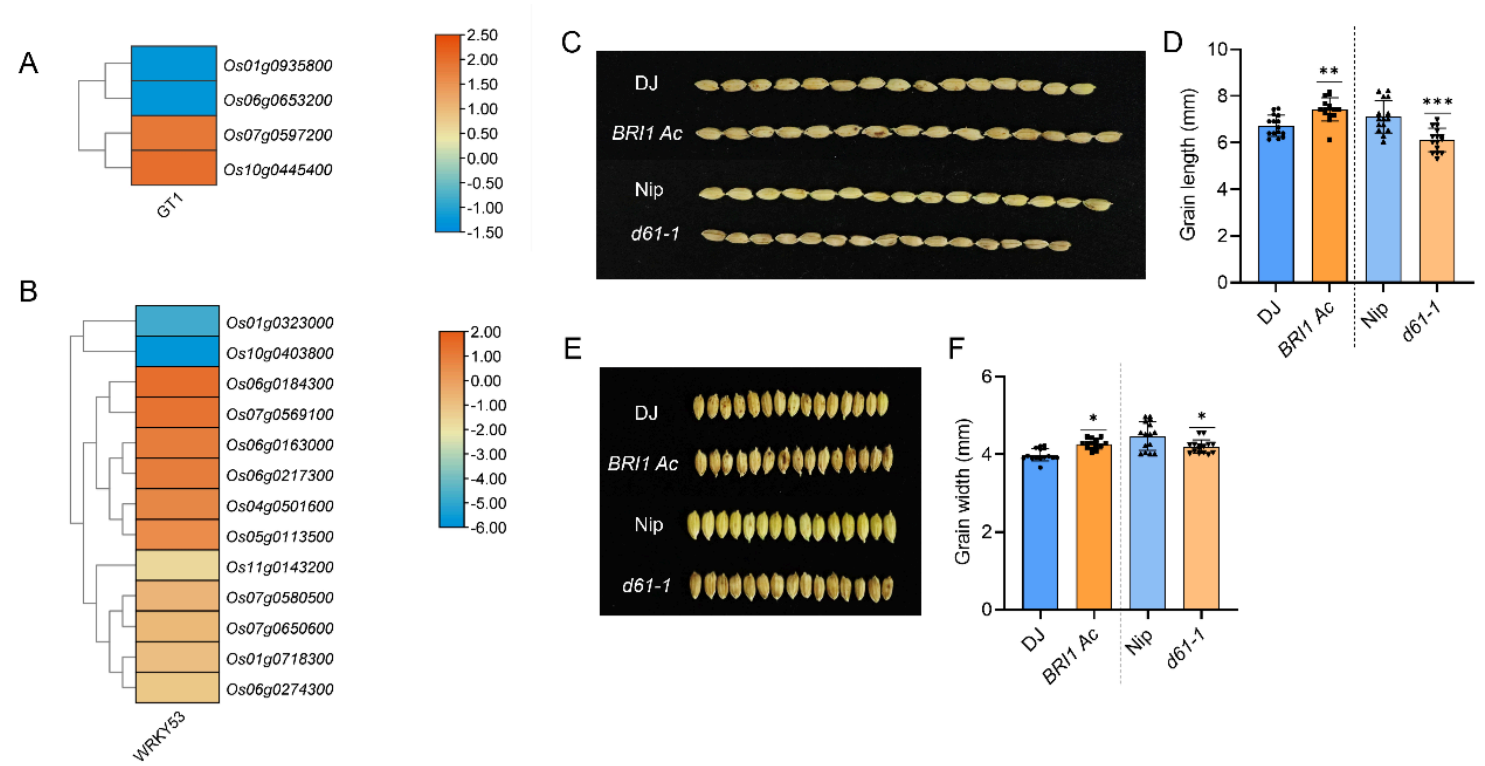


Figure 4

Grain size analyses for *BR1* activation and mutant plants. A, B. GT1 (A) and WRKY53 (B) regulate BR signaling-related gene expression. C-F. Images of Nip, *BR1-Ac*, DJ and *d61-1* grains (C, E) with corresponding quantification of grain length (D) and width (F) (n=10). In total, 15 grains from each line were analyzed. * $P < 0.05$; ns, non-significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplemaentalmaterials.docx](#)
- [SupplementaltableS1.xlsx](#)
- [Completeuncroppedoriginalimagesofgelsandblots.docx](#)