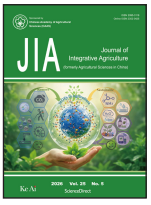
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LETTER

A weak *OsBRI1* allele in Zhonghua 11 as a genetic tool for brassinosteroid signaling research in rice

Yanzhao Feng[1*], Qingfeng Zhu[1*], Qiuyue Yuan[1, 2], Pei Chen[1], Xielian Tan[1, 3], Ning Huang[1, 2], Jiao Xue[1#], Yang Yu[1#]

[1] *Guangdong Key Laboratory of Crop Germplasm Resources Preservation and Utilization/Agro-biological Gene Research Center, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China*
[2] *College of Agriculture, South China Agricultural University, Guangzhou 510642, China*
[3] *College of Life Sciences, South China Agricultural University, Guangzhou 510642, China*

Highlights

- Zhonghua 11 and Taichung 65 exhibit distinct physiological and molecular responses to brassinosteroids despite their close *japonica* relationship.
- A near-isogenic line, *d61-zh11*, was developed to transfer the classical brassinosteroid (BR)-insensitive mutation into a modern functional genomics background.
- *d61-zh11* provides a standardized reference mutant for the reproducible and system-level dissection of BR signaling in rice.

Brassinosteroids (BRs) are essential phytohormones that shape rice architecture, grain development, and stress resilience by modulating leaf angle, stem elongation, tillering, and spikelet growth, all of which collectively influence light interception and yield formation (Manghwar *et al.* 2022; Yuan *et al.* 2022; Hou *et al.* 2025; Li *et al.* 2025). Although partial manipulation of BR signaling has recently gained attention as a strategy for ideotype breeding, the translation of BR biology into crop improvement remains hindered by an incomplete understanding of how BR responsiveness varies among elite *japonica* backgrounds. In this study, we uncovered pronounced differences in BR morphological responsiveness and transcriptional feedback between two widely used *japonica* cultivars, Taichung 65 (TC65) and Zhonghua 11 (ZH11), indicating that genetic background has substantial impacts on BR perception and signaling efficiency. Motivated by this finding, we developed a non-transgenic near-isogenic BR-insensitive line, *d61-zh11*, by introgressing the classical weak *OsBRI1* allele (*d61-1*) from TC65 into the modern, genome-enabled ZH11 background. This line retains agronomic viability while enabling robust BR pathway interrogation. By coupling comparative physiological and molecular analyses with the establishment of a standardized BR-deficient platform in ZH11, our study reveals previously overlooked cultivar-dependent differences in BR signaling and provides an urgently needed resource to unify BR functional genomics and accelerate ideotype-driven rice improvement.

The genetic dissection of brassinosteroid biosynthesis and signaling pathways has largely depended on the identification and characterization of BR-related mutants. Among these, mutants defective in the BR receptor gene *OsBRI1*, notably the *d61* series, have proven particularly valuable. As the primary receptor of BRs, OsBRI1 triggers the entire signaling cascade, and its hypomorphic alleles show BR-insensitive phenotypes such as dwarfism, erect leaves, and reduced grain size (Nam and Li 2002; Nakamura *et al.* 2006; Zhang *et al.* 2014). The *d61* mutants, especially the *d61-1* allele in the TC65 background, have become the cornerstone for validating novel components of the BR signaling pathway (Yamamuro *et al.* 2000). A substantial proportion of newly identified BR signaling elements, from upstream kinases to downstream transcription factors, require genetic epistasis analysis with *d61* to determine their functional position within the BR regulatory network. This rigorous validation standard highlights the indispensable role of *d61* mutants in establishing the BR signaling hierarchy and elucidating its integration with other developmental pathways.

Despite sharing the same subspecies classification, different *japonica* rice cultivars exhibit marked variation in BR-related traits due to their distinct genetic backgrounds

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[#]Correspondence Jiao Xue, E-mail: xuejiao@gdaas.cn; Yang Yu, E-mail: yuyang@gdaas.cn
[*] These authors contributed equally to this study.

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and breeding histories. TC65, developed in the 1920s in Taiwan of China, represents a traditional *japonica* variety characterized by lax leaves, photoperiod hypersensitivity, and moderate BR responsiveness. In contrast, ZH11 is a modern *japonica* cultivar bred for high-density planting, and it features compact architecture, rapid flowering, and stable performance across diverse environments. These phenotypic differences are likely to reflect underlying divergence in BR perception, signaling efficiency, or feedback regulation mechanisms. With ZH11 increasingly becoming the genotype of choice for functional genomics due to its superior transformation efficiency, well-annotated telomere-to-telomere and gap-free genome (Hou *et al.* 2025), and extensive mutant resources, there is a pressing need to establish key BR genetic tools like *d61* in this background. Although our previous work (Yu *et al.* 2024) successfully generated *OsBRI1* RNA interference (RNAi) lines in ZH11, the presence of transgenic elements and the severe BR-insensitive phenotypes caused by strong RNAi suppression limit their utility. Therefore, it is necessary to develop a non-transgenic, weak *OsBRI1* mutant in the ZH11 background. Such a genetic resource would enable direct comparisons within the expanding ZH11 mutant collection and promote the integration of classical BR genetics with modern high-throughput screening approaches.

In this study, we systematically compared the brassinosteroid (BR)-related phenotypes between two *japonica* rice cultivars, TC65 and ZH11. TC65 was developed in the pre-Green Revolution era and exhibits a relatively lax plant architecture, whereas ZH11 was bred in the 1980s for higher planting density and shows a more erect growth habit (Fig. 1-A). In terms of grain traits, ZH11 had significantly longer grains than TC65 (Fig. 1-B and D), while their grain widths showed no notable difference (Fig. 1-C and E). As a result, ZH11 displayed a higher thousand-grain weight ((27.11±0.87) g) compared to TC65 ((24.62±1.23) g; **, P<0.01) (Fig. 1-F). These results indicate substantial differences in BR-related morphological traits between the two cultivars.

We next assessed the physiological responses to brassinosteroids in ZH11 and TC65. Under normal growth conditions, the second leaf of one-week-old ZH11 seedlings exhibited significantly smaller leaf angles ((20.08±2.64)°) compared to TC65 ((30.44±4.55)°; **, P<0.01) (Fig. 1-G and H), suggesting a potential difference in BR sensitivity between the two cultivars. To further investigate this possibility, we performed a lamina joint inclination assay. Rice seeds were germinated and grown on 1/2 Murashige and Skoog (MS) medium for 8 days, followed by treatment with various concentrations of epi-brassinolide (epi-BL; 0, 0.1, 1.0, and 5.0 μmol L^{-1}) for 3 days. The epi-BL treatment significantly increased the leaf angle in TC65 in a dose-dependent manner, whereas ZH11 showed only a modest response (Fig. 1-I and J), indicating that TC65 is more sensitive to BR signaling than ZH11.

To further investigate the differential BR sensitivity between TC65 and ZH11, we examined the expression levels of representative genes involved in BR biosynthesis and signaling using quantitative real-time PCR (qRT-PCR), and the primer sequences are listed in Appendix A. The BR signaling genes *OsBRI1*, *BZR1*, and *BZR2*, as well as the BR biosynthesis genes *CPD1* and *CPD2*, were all expressed at higher levels in TC65 than in ZH11 (Fig. 1-K), indicating stronger BR signaling feedback in TC65. To determine whether this transcriptional difference translates into altered metabolic output, we quantified the endogenous BR metabolites in 10-day-old seedlings. In rice, castasterone (CS) is considered the predominant bioactive BR (Joo *et al.* 2015), whereas brassinolide (BL) is rarely detected. BR profiling showed that TC65 accumulated higher levels of the downstream intermediates 6-deoxocastasterone (6-deoxoCS) and CS, while the BL levels remained negligible in both genotypes (Fig. 1-L). To explore potential genetic factors contributing to this difference, we performed whole-genome resequencing of both cultivars, which revealed 431,632 SNPs and 85,185 InDels (Appendix B). Notably, a missense SNP (Chr8:4,350,638) in the *BAK1* gene causes an amino acid substitution in TC65 from arginine (positively charged) to glutamine (uncharged) in ZH11. Structural prediction and alignment suggested that this change might weaken a local electrostatic interaction (Appendix C), potentially affecting BAK1 conformation and BR signal transduction efficiency. Collectively, these results point to cultivar-specific genomic and transcriptional differences as potential contributors to the varied BR responsiveness between TC65 and ZH11.

These findings underscore that even closely related *japonica* cultivars can vary significantly in plant architecture, grain traits, and BR responsiveness, which highlights the necessity of using BR mutants in a consistent genetic background when dissecting BR regulatory networks. *OsBRI1*, the gene encoding the BR receptor, contains a single exon and a 3,366-bp coding sequence that produces a 1,121-amino acid receptor kinase. The classic *d61-1* allele carries a C-to-T transition at amino acid position 988, resulting in a threonine-to-isoleucine substitution, and this allele has been widely used to validate key regulators in the BR signaling pathway. Given the growing use of ZH11 as a model for BR-related studies due to its genomic resources and transformation efficiency, we sought to introduce the *d61-1* mutation into this background. Although initial attempts using prime editing failed to generate edited plants, we successfully developed a near-isogenic line (NIL) through a molecular marker-assisted backcrossing strategy. Specifically, F_1 progeny from a cross between the *d61-1* mutant (in the TC65 background) and ZH11 were backcrossed to ZH11 for four generations, followed by two generations of selfing to produce the BC_4F_3 population. Using an *OsBRI1*-specific molecular marker as the foreground selection tool (Fig. 2-A), we identified a ZH11 near-isogenic line harboring the *d61-1* mutation, which we designated as *d61-zh11*. Sanger sequencing confirmed the homozygous Thr988Ile mutation in *OsBRI1* (Fig. 2-B and C). Whole-genome resequencing was performed to evaluate the genome recovery of *d61-zh11*. Analysis of the SNPs revealed that all *d61-zh11* individuals had more than 90% recovery of the ZH11 background, indicating that the BC_4F_3 population was largely restored to ZH11 (Appendix D).

To determine the effects of the weak allelic mutation of *d61* in ZH11, the key agronomic traits of plant height, leaf angle,

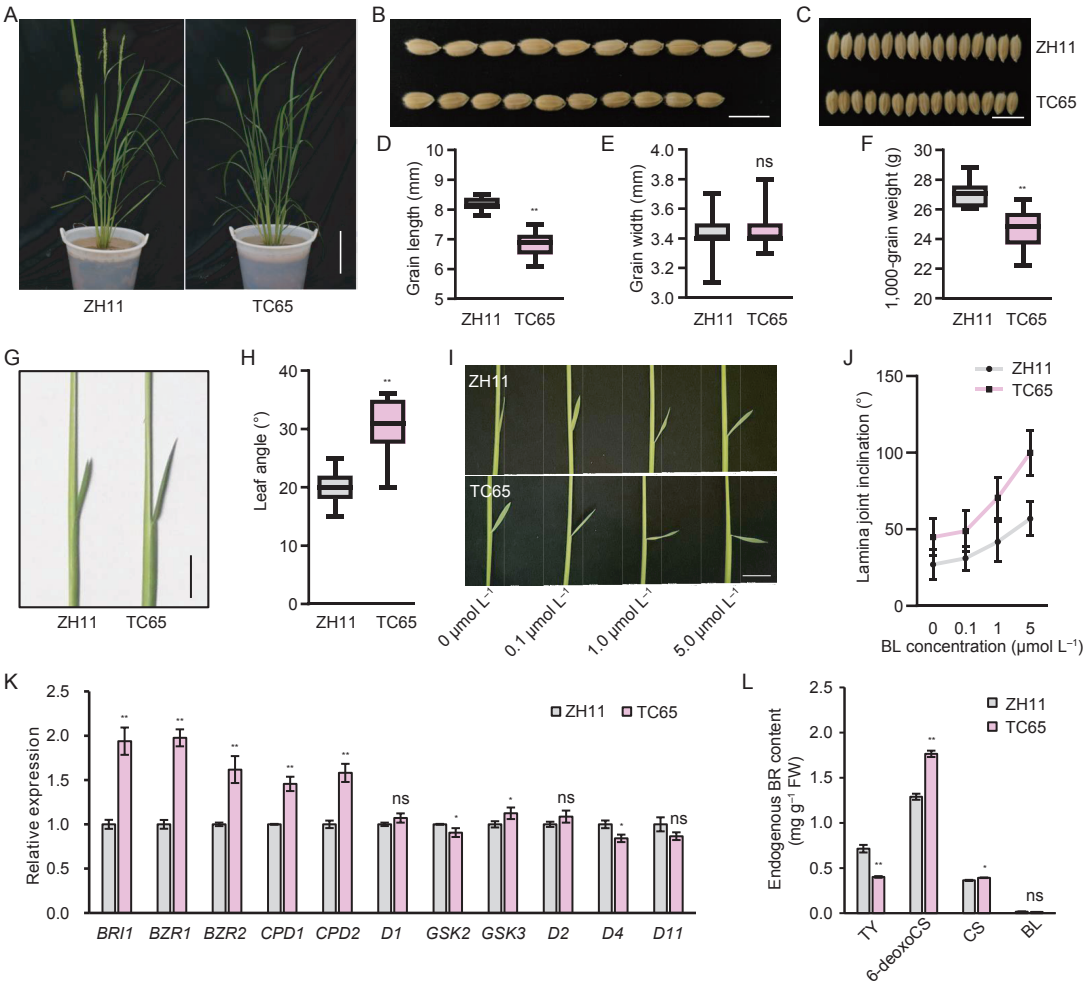


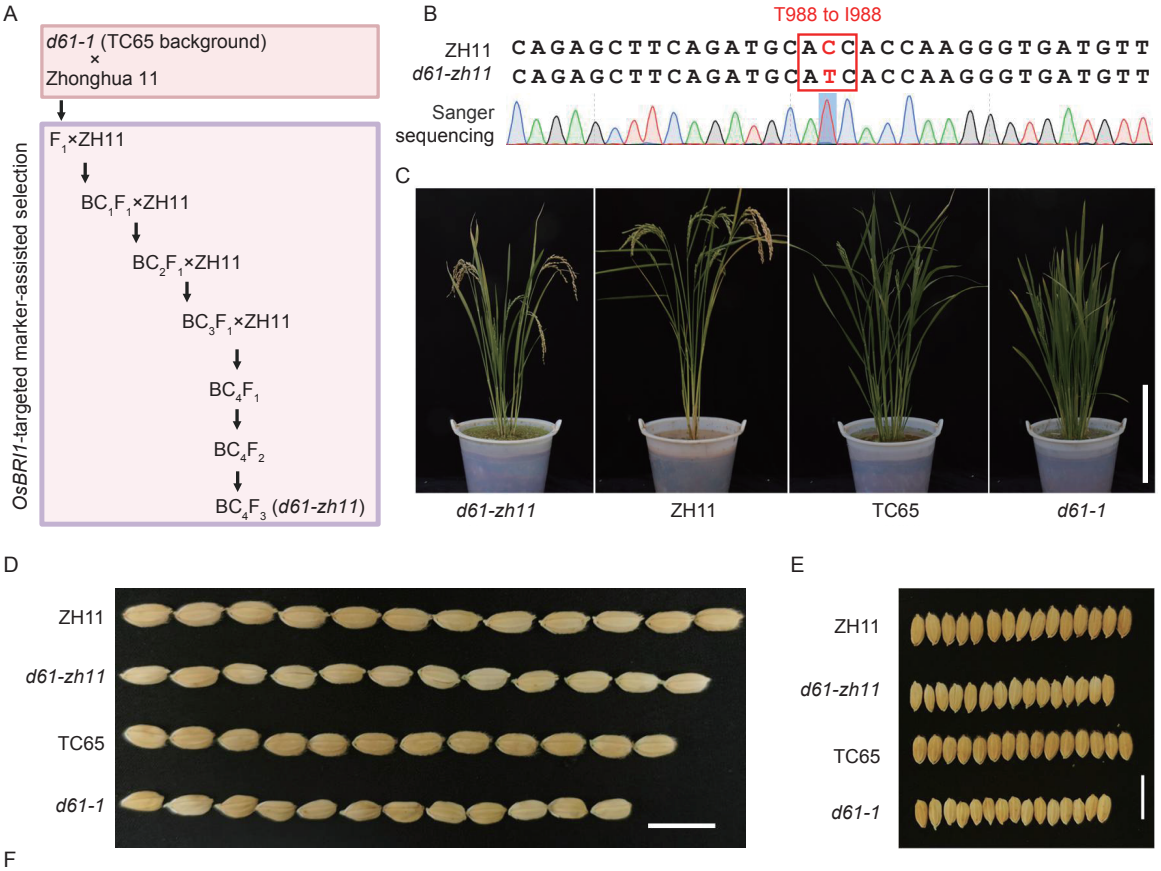
Fig. 1 Comparison of brassinosteroid (BR)-associated traits in Zhonghua 11 (ZH11) and Taichung (TC65). A, representative plant architectures of ZH11 and TC65 at 65 days after germination, showing contrasting leaf angles and tillering habits. Scale bar=20 cm. B and C, grain morphology comparison between ZH11 and TC65. Scale bars=1 cm. D and E, data for grain length (D) and grain width (E), presented as mean±SD (*n*=30). Asterisks indicate statistically significant differences compared with ZH11, as determined using Student's *t*-test (**, *P*<0.01; ns, not significant). F, 1,000-grain weight of ZH11 and TC65. Values are shown as mean±SD (*n*=10 biological replicates). Asterisks indicate statistically significant differences compared with ZH11, as determined using Student's *t*-test (**, *P*<0.01). G, leaf angle of the second leaves under normal growth conditions. Scale bar=1 cm. H, quantification of the leaf angles shown in G, represented by the mean±SD (*n*=15 plants per cultivar). Asterisks indicate statistically significant differences compared with ZH11, as determined using Student's *t* test (**, *P*<0.01). I, lamina joint inclination assay results showing leaf angle responses to 0, 0.1, 1.0, and 5.0 μmol L^{-1} epi-BL treatment for 3 days. Scale bar=1 cm. J, quantification of lamina joint inclination under epi-BL treatment, represented by the mean±SD (*n*=10 plants per treatment). K, expression levels of representative BR biosynthesis genes and signaling genes in seedlings of ZH11 and TC65 under normal conditions. Expression was normalized to *FhaB* and is presented relative to ZH11. Data are shown as mean±SD (*n*=3). Asterisks indicate statistically significant differences compared with ZH11 by Student's *t*-test (*, *P*<0.05; **, *P*<0.01; ns, not significant). L, endogenous BR contents in ZH11 and TC65, presented as mean±SD (*n*=3). Asterisks indicate statistically significant differences compared with ZH11, as determined using Student's *t*-test (*, *P*<0.05; **, *P*<0.01; not significant).

tiller number, grain size, and grain weight were evaluated in both genetic backgrounds. Compared with their respective wild types, *d61-zh11* and *d61-1* showed significantly reduced plant height and leaf angle, retaining 81.35 and 16.63% for *d61-zh11*, and 68.89 and 16.33% for *d61-1*, respectively, while tiller number remained largely unchanged. Grain length and width were also moderately reduced in both mutants. Moreover, *d61-zh11* retained 83.01% of the 1,000-grain weight of ZH11, whereas *d61-1* retained only 79.84% of it in TC65, indicating that the phenotypic effect of the *OsBRI1* mutation is less severe in the ZH11 background (Fig. 2-D–F). This result is consistent with the relatively weaker BR responsiveness previously observed in ZH11 at both morphological and transcriptional levels.

To explore the potential mechanisms underlying this genotype-dependent difference, we examined the expression of several BR-responsive and metabolic genes (*BZR1*, *BZR2*, *GSK2*, *GSK3*, *CPD1*, *CPD2*, *D2*, *D11*, *BU1*, and *D4*). Most of those genes showed comparable expression tendencies between the two backgrounds, while a subset of biosynthetic genes (e.g., *D4* and *CPD2*) displayed stronger induction in *d61-1* (Appendix E), consistent with a more pronounced negative-feedback response under severe BR deficiency. To broaden our understanding of these transcriptional

differences, we performed a comparative RNA-seq analysis. Consistent with the targeted gene expression results, the global transcriptomic response differed markedly between the two backgrounds. In the TC65 background, *d61-1* caused a strong bias towards gene upregulation (1,036 up-regulated genes *vs*. 475 down-regulated genes) and a massive enrichment of "Plant hormone signal transduction" and "MAPK signaling pathway" genes (Appendix F). This suggests that the mutation triggers an intense, systemic feedback oscillation in TC65. In contrast, *d61-zh11* displayed a more balanced transcriptional change (897 up- *vs*. 833 down-regulated genes) with enrichment focused on metabolic processes such as "fatty acid elongation" rather than hormone signaling networks. Combined with the endogenous BR measurements suggesting a lower basal BR level in ZH11, these data implied that the relatively milder phenotype of *d61-zh11* is attributable to the distinct signaling homeostasis of the ZH11 background. This background appears to buffer the pathway disruption, thereby promoting metabolic adaptation rather than suffering from the systemic signaling collapse observed in TC65.

Integrating these phenotypic observations with the molecular and physiological evidence presented above, we cautiously propose that BR responsiveness is inherently lower in ZH11 than in TC65, resulting in a narrower phenotypic response range upon BR signaling disruption. This genotype-



F

Plant line ID	Plant height (cm)	Leaf angle (°)	Grain length (mm)	Grain width (mm)	1,000-grain weight (g)
ZH11	88.65±3.60	30.31±15.60	7.94±0.17	3.46±0.09	26.98±0.86
d61-zh11	72.12±5.41	5.04±2.79	7.36±0.32	3.29±0.17	22.39±1.68
P-value	¨<0.01	¨<0.01	¨<0.01	¨<0.01	¨<0.01
Variation rate	81.35%	16.63%	92.25%	95.09%	83.01%
TC65	92.11±3.40	33.53±11.76	6.84±0.19	3.51±0.12	24.50±0.49
d61-1	63.45±7.16	5.48±2.28	6.44±0.36	3.19±0.18	19.56±0.76
P-value	¨<0.01	¨<0.01	¨<0.01	¨<0.01	¨<0.01
Variation rate	68.89%	16.33%	94.15%	91.08%	79.84%

Fig. 2 Development and characterization of *d61* hypomorphic alleles in the Zhonghua 11 (ZH11) background. A, schematic diagram of the development of the *d61-zh11* near-isogenic line (NIL) through marker-assisted selection using *OsBRI1*-specific molecular markers across the backcross generations. B, sanger sequencing chromatograms confirming the homozygous C-to-T transition (Thr988Ile) in the *OsBRI1* gene in *d61-zh11*, consistent with the *d61-1* allele in the Taichung 65 (TC65) background. C, plant morphology of wild-type (WT) ZH11, *d61-zh11*, WT TC65, and *d61-1*. Scale bar=30 cm. D and E, grain morphology showing the grain length (D) and width (E) of *d61* mutants and wildtypes in both backgrounds. Scale bar=2 cm. F, analysis of the agronomic traits of *d61-zh11*, with comparison to *d61-1*. Values are mean±SD (n≥10). The P values were calculated by Student's t-test.

dependent variability suggests that manipulating BR pathways may not produce uniform outcomes across different rice backgrounds, underscoring the need for background-tailored and fine-tuned BR regulation strategies in breeding applications. To further uncover the factors contributing to genotype-dependent BR sensitivity, future high-throughput strategies may be useful. For instance, chemical genomics or CRISPR-based modifier screens could help to identify genetic or regulatory components that modulate BR responsiveness. Such efforts may provide broader mechanistic insights and support more predictable, background-tailored BR manipulation strategies in rice breeding.

In conclusion, this study demonstrates that even closely related *japonica* cultivars can exhibit substantial differences in brassinosteroid pathway regulation, with ZH11 showing reduced morphological sensitivity compared to TC65. To bridge classical BR genetics with modern functional genomics, we developed *d61-zh11*, a near-isogenic line in the ZH11 background carrying the *d61-1* mutation. This line fulfills a critical need by offering a standardized genetic platform for validating BR signaling components, thereby enabling seamless integration with ZH11's expansive mutant resources for systematic investigations, while also enhancing experimental reproducibility through ZH11's stable environmental performance. Strong loss-of-function mutations in core BR-signaling genes often cause severe developmental defects (Yamamuro *et al.* 2000), which may mask additional regulatory inputs and also limit their suitability for breeding applications. By contrast, weak alleles such as *d61-zh11* retain relatively normal agronomic performance while still exhibiting reduced BR responsiveness, so they offer a more favorable background for uncovering subtle BR regulatory factors and exploring breeding-compatible BR modulation. As BR research advances toward dissecting complex, multi-gene regulatory networks, *d61-zh11* will serve as a pivotal reference mutant for elucidating BR signaling hierarchies in rice. We are making this resource available to the research community to support broader collaborative efforts.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 5.2 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of this article.

Data availability

All data generated or analyzed during this study are included in this article and its supplementary information files. The raw RNA-seq data have been deposited in the National Genomics Data Center (NGDC) of China under BioProject accession number PRJCA056159.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2026.02.008

References

Hou L, Zhu L, Hao M, Liang Y, Xiao G. 2025. Brassinosteroids enhance gibberellic acid biosynthesis to promote cotton fibre cell elongation. *Plant Biotechnology Journal*, **23**, 1213–1215.

Joo S H, Jang M S, Kim M K, Lee J E, Kim S K. 2015. Biosynthetic relationship between C28-brassinosteroids and C29-brassinosteroids in rice (*Oryza sativa*) seedlings. *Phytochemistry*, **111**, 84–90.

Li X, Li J, Zabed H M, Li J, Xiong M, Shi H, Li J. 2025. Manipulating brassinosteroid signaling pathway to genetically improve horticultural plants. *aBIOTECH*, **6**, 328–345.

Manghwar H, Hussain A, Ali Q, Liu F. 2022. Brassinosteroids (BRs) role in plant development and coping with different stresses. *International Journal of Molecular Sciences*, **23**, 1012.

Nakamura A, Fujioka S, Sunohara H, Kamiya N, Hong Z, Inukai Y, Miura K, Takatsuto S, Yoshida S, Ueguchi-Tanaka M, Hasegawa Y, Kitano H, Matsuoka M. 2006. The role of *OsBRI1* and its homologous genes, *OsBRL1* and *OsBRL3*, in rice. *Plant Physiology*, **140**, 580–590.

Nam K H, Li J. 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell*, **110**, 203–212.

Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S, Ashikari M, Kitano H, Matsuoka M. 2000. Loss of function of a rice *brassinosteroid insensitive1* homolog prevents internode elongation and bending of the lamina joint. *The Plant Cell*, **12**, 1591–1605.

Yu Y, He R R, Yang L, Feng Y Z, Xue J, Liu Q, Zhou Y F, Lei M Q, Zhang Y C, Lian J P, Chen Y Q. 2024. A transthyretin-like protein acts downstream of miR397 and LACCASE to regulate grain yield in rice. *The Plant Cell*, **36**, 2893–2907.

Yuan H, Xu Z, Chen W, Deng C, Liu Y, Yuan M, Gao P, Shi H, Tu B, Li T, Kang L, Ma B, Wang Y, Wang J, Chen X, Li S, Qin P. 2022. OsBSK2, a putative brassinosteroid-signalling kinase, positively controls grain size in rice. *Journal of Experimental Botany*, **73**, 5529–5542.

Zhang C, Bai M Y, Chong K. 2014. Brassinosteroid-mediated regulation of agronomic traits in rice. *Plant Cell Reports*, **33**, 683–696.

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