

Molecular mapping of a novel non-canonical gibberellin-sensitive dwarfing gene Rht29 in wheat (*Triticum aestivum*)

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Research Article

Keywords: *Triticum aestivum*, Plant height, Rht genes, Gibberellin, GA3oxidase

Posted Date: February 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8634203/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Theoretical and Applied Genetics on June 17th, 2026. See the published version at <https://doi.org/10.1007/s00122-026-05299-2>.

Abstract

Breeding semi-dwarf cultivars has long been a major objective for wheat improvement due to the inseparable association between plant height (PH) and grain yield. Although the utilization of *Rht-B1b* and *Rht-D1b* genes successfully achieved semi-dwarfism in the 1960s, these genes were associated with undesirable traits. The current wheat breeding urgently requires continuously exploring PH-controlling genes and their regulatory mechanisms, which will expand the genetic diversity of the PH gene pool to achieve precise PH regulation while maintaining or even increasing the grain yield potential. In this study, we identified a gibberellin (GA) - sensitive dwarf mutant, designated *wph3* (*wheat plant height 3*). It showed GA biosynthesis deficiency and had pleiotropic effects on PH, spike length, grain weight, and grain number per spike. Using Exome Capture Sequencing for Bulk Segregant Analysis and molecular marker mapping, a novel recessive nuclear dwarfing gene was identified and localized into a ~ 3.9 Mb physical interval on chromosome 2B, designated *Rht29* (*Reduced height 29*). Transcriptome analysis and candidate gene mining indicated that *Rht29* may not encode a canonical key enzyme for GA biosynthesis, but participate in the GA biosynthesis by regulating the expression level of *GA3ox*. This study enriches the genetic resources available for wheat dwarfing breeding and establishes a foundation for further molecular characterization of phenotypic regulation by *Rht29*.

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most vital staple crops in the world, sustaining over one-third of the global population. Its yield and quality are thus directly linked to global food security. Plant height (PH) is a core but complex agronomic trait governed by both multiple quantitative trait loci (QTLs) and major genes. It critically determines lodging resistance and is intimately associated with harvest index, biomass partitioning, and yield potential (Song et al. 2023). Dwarfing alleles such as *Rht-B1b* and *Rht-D1b* successfully reduced wheat plant height, significantly improved lodging resistance and harvest index, thereby revolutionized wheat productivity during the Green Revolution in the 1960s (Mohan et al. 2021, Chai et al. 2022).

The primary dwarfing genes discovered in wheat were categorized into gibberellin (GA) signal transduction pathway genes (GA-insensitive type) and GA biosynthesis or deactivation pathway genes (GA-sensitive type). *Rht-B1b* and *Rht-D1b* belong to GA-insensitive type and encode N-terminally truncated DELLA proteins that conferred resistance to GA-dependent degradation, thereby suppressed stem elongation persistently. Conversely, there are some genes belonging to the GA-sensitive type. For example, *Rht12* (Sun et al. 2019) and *Rht18* (Grant et al. 2018) encode GA2-oxidase (GA2ox) A13 and GA2oxA9, respectively. The up-regulated expression of *Rht12* and *Rht18* significantly reduced endogenous GA levels, thereby reducing plant height (Ford et al. 2018, Buss et al. 2020).

Apart from genes directly participating in the GA biosynthesis and signaling pathways, dwarfing genes indirectly influencing these pathways have also been identified. The GA-sensitive gene *Rht8* encoding a ribonuclease (RNase) H-like protein modulated bioactive GA ratios through regulating the expressions of

GA biosynthetic enzyme genes. The *Rht8* mutations effectively reduced plant height (Chai et al. 2022, Xiong et al. 2022). *Rht-13* is a *nucleotide-binding site/leucine-rich repeat* (*NB-LRR*) gene and is GA-sensitive. A point mutation in the semi-dwarfing *Rht-B13b* allele resulted in autoactivation of *NB-LRR* genes and up-regulation of pathogenesis-related genes, thereby enhancing cell wall cross-linking and ultimately inhibiting cell elongation (Borrill et al. 2022). *Rht22* reduced plant height by disrupting cell proliferation and decreasing the number of internode cells, thereby shortening internodes (Wang et al. 2022). A recent study has revealed that GA-sensitive dwarfing gene *Rht25* encodes a PLATZ transcription factor regulating wheat plant height through directly interacting with DELLA. Consequently, loss of function mutations in *PLATZ-A1* reduced plant height (Zhang et al. 2023).

Despite the remarkable success of these dwarfing genes, their widespread deployment has also revealed limitations and potential drawbacks, including reduced coleoptile length, diminished early growth vigor, decreased grain size, and potentially increased susceptibility to certain diseases (Guo et al. 2022). Furthermore, modern breeding's heavy reliance on this limited set of major loci has narrowed genetic diversity. However, less attention has been paid to plant height of wheat in recent years due to the widespread perception that the lodging seemed solved by the Green Revolution. In the face of intensifying global climate change, increased frequency of extreme weather events, and escalating threats from pests and diseases, as well as the unknown weakness of the existing repertoire of dwarfing genes, exploring new genes regulating plant height is still necessary.

In this study, a plant height-reduced mutant was identified from an EMS-mutagenized population of an elite wheat variety Kechengmai 1 (K1), designated as *wheat plant height 3* (*wph3*). Phenotypical and genetic characterization of *wph3* were performed. The Exome Capture Sequencing based Bulk Segregant Analysis (BSE) and subsequent molecular mapping were conducted to localize causal gene for *wph3* in an approximately 3.9 Mb interval at the end of the long arm of chromosome 2B, representing a genetically novel dwarfing gene, designed as *Rht29*. We further analyzed its response to exogenous GA₃ application and endogenous GA content. Candidate gene and its potential mechanism in controlling plant height were also inferred and discussed by combining variant and transcriptome data. This research provided a novel dwarfing gene with breeding potential and laid the foundation for future cloning and functional characterization of *Rht29*.

Materials and methods

Plant Materials

Two elite wheat varieties, K1 and Kechengmai 6 (K6), were both developed and conserved by our lab. The dwarf mutant *wph3* was generated by EMS mutagenesis of K1 (Zhang et al. 2022). For genetic analysis, reciprocal crosses were performed between *wph3* and K1, as well as between *wph3* and K6. The hybrid seeds were collected and sown for summer propagation in Maerkang, Sichuan, China (31°54'25.99"N, 102°10'34.72"E, alt. 3025 m). Seeds of all F₁ of *wph3* × K6 and K6 × *wph3* were harvested

and planted in Shifang, Sichuan, China (31°06'37.07"N, 104°09'09.70"E, alt. 521 m) to generate the F₂ genetic population RK6-F₂ for inheritance investigation and molecular mapping.

Measurements of agronomic traits

At the jointing stage, ten randomly selected plants of K1, K6, and *wph3*, and all individuals of the population were phenotyped in the field for plant height (PH), spike length (SL), spikelet number per spike (SNS), and tiller number (TN). After seed harvest, thousand grain weight (TGW), grain length (GL), grain width (GW), and grain number per spike (GNS) were measured by using SC-G software (WSeen, Hangzhou, Zhejiang, China). All data were analyzed by Student's *t*-test ($P < 0.05$) or ANOVA.

Exogenous GA treatment and endogenous GA content measurement

GA₃ (MeilunBio[®]) was initially dissolved in a small amount of ethanol to formulate the 100 mM stock solution and then stored at 4°C. Thirty plants each of *wph3* and K1 were grown in the greenhouse under a 16-h light/8-h dark photoperiod, with the temperature maintained at 24 °C. At the initiation of the jointing stage, GA₃ stock solution was diluted 1000-fold then evenly sprayed onto the leaves and stems of both *wph3* and K1. The control group was sprayed with the solution containing 0.1% ethanol. Both treatments were performed in three replicates, with each replicate consisting of five plants.

For endogenous GA content measurement, thirty plants each of *wph3* and K1 were planted in the greenhouse. The peduncle tissues of *wph3* and K1 were collected during the jointing stage when the peduncles were about 2-3cm. Three biological replicates were performed, with each replicate consisting of samples from at least five uniformly growing plants. Fresh plant samples were immediately frozen in liquid nitrogen, ground into powder, and stored at -80 °C until needed. 50 mg sample from each replicate was weighed and transferred into a 2 mL microcentrifuge tube. GAs were extracted with 1 mL methanol/H₂O/formic acid (15:4:1, v/v/v) and quantified by MetWare (Wuhan, Hubei, China) based on the AB Sciex QTRAP[®]6500 + LC-MS/MS platform.

Exome capture sequencing based bulked segregant analysis (BSE)

The BSE was employed to identify key loci responsible for reduced plant height in *wph3*. At the flowering stage, leaves of the 30 individuals in RK6-F₂ population with extreme plant height were collected in equal amounts to construct two extreme phenotype bulks: a wild-type (WT) bulk and a dwarf bulk. Simultaneously, two parental pools were constructed using equal quantities leaf tissue from 30 individuals each of *wph3* and K6.

Library preparation and exome capture sequencing were performed by Tcuni Technology Co., Ltd. (Chengdu, Sichuan, China). The SpeedSeq pipeline was utilized for reads mapping and variant calling

(Chiang et al. 2015). Bulk segregant analysis was conducted using the Gene Mapping module of the Tcuni platform (<https://www.wheatgmap.org>).

Molecular marker development and gene mapping

Single nucleotide polymorphisms (SNPs) within the target region were selected to design molecular markers, including kompetitive allele-specific PCR (KASP) markers and Sanger sequencing markers for genotyping. Marker design and application followed the procedures described previously (Pu et al. 2021). KASP markers were subsequently used to genotype a subset of the F₂ population to construct a genetic linkage map. Once a primary genetic interval was determined, the fine mapping was performed by designing more markers within this interval to genotype more individuals in the mapping population. The key recombinants were validated by performing genotyping and phenotyping on their progeny.

RNA-seq analysis

The peduncle tissues of K1 and *wph3* were sampled when they elongated to 2–3 cm. The whole peduncles dissected from the plant was immediately flash-frozen in liquid nitrogen and subsequently stored at -80°C until further processing. To minimize experimental error, all samples were collected at the same time point. Three biological replicates were collected with each replicate consisting of samples from the main tiller of at least 10 individuals with similar growth status (Zhang et al. 2022).

RNA extraction, library preparation, RNA-seq and subsequent analysis were performed by Biomarker Technologies Co., Ltd. (Beijing, China). Paired-end sequencing was conducted on the Illumina NovaSeq platform. HISAT2 (Kim et al. 2015) was used to align the clean reads to the Chinese Spring reference genome (IWGSC RefSeq v2.1) (Zhu et al. 2021). Gene expression levels were quantified as Fragments Per Kilobase of transcript per Million mapped reads (FPKM). Differentially expressed genes (DEGs) between *wph3* and K1 were identified using DESeq2 (Love et al. 2014) with thresholds of false discovery rate (FDR) < 0.05 and absolute fold change ≥ 1.5. Finally, DEGs were functionally annotated via homology search against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2004) using DIAMOND (Buchfink et al. 2015).

Results

Phenotypic Characterization of *wph3*

A dwarf mutant, designated *wph3*, was derived from EMS-induced mutagenesis of K1. From the appearance, the plant height and spike length were significantly decreased in *wph3* compared to K1 (Fig. 1a, b). The length of all stem internodes of *wph3* was reduced and resulted in the 38.41% decrease in plant height (Fig. 1c, d). The uppermost internode (peduncle) contributed the most. Measurement of agronomic traits indicated that compared to the WT, the SL of the *wph3* decreased by 19.30% (Fig. 1e), while there was no significant change in the SNS, GL and GW ($P > 0.05$) (Fig. 1f-1h). Additionally, the

TGW, TN and GNS significantly decreased in *wph3* (Fig. 1i-1k). These results indicated that the mutation in *wph3* showed pleiotropic effects on agronomic traits.

Exogenous GA treatment and endogenous GA levels analysis

Given that most dwarfing genes participate in GA biosynthesis or signaling pathways, we exogenously applied GA₃ to *wph3* and the WT plants during the jointing stage. Our results indicated that both *wph3* and the WT were responsive to GA₃, and the dwarf phenotype of *wph3* could be completely rescued by GA₃ treatment (Fig. 2a, b), showing that *wph3* is a GA-sensitive dwarf mutant. This result suggested potential endogenous bioactive GA deficiency in *wph3*. Therefore, we further quantified the levels of endogenous bioactive GAs, along with precursors, intermediates and catabolites in the elongating peduncle. Compared to the WT, levels of bioactive GA₁ and GA₃, synthesized through the early-13-hydroxylation (13-OH) pathway, were dramatically reduced by 68.71% and 28.22% in the *wph3*, respectively. (Fig. 2c). Meanwhile, the intermediates of GA₁ and GA₃, including GA₂₀, GA₁₉, GA₄₄, and GA₅₃ in the 13-OH pathway were all decreased in *wph3*, while GA₅ showed a slight increase. Consistently, the level of GA₈, the catabolite of GA₁, was lower in *wph3* than in K1.

Furthermore, the levels of bioactive GA₄ and its intermediates GA₁₅ and GA₂₄ and catabolite GA₃₄ in the non-13-hydroxylation (13-H) pathway were not detectable or decreased in *wph3*, whereas the GA₉ slightly increased in *wph3*. GA₁₂, the precursor for bioactive GAs was not detectable in both *wph3* and K1. These results suggested significant suppression of GA biosynthesis in *wph3*.

*, **, and *** indicated $p < 0.05$, 0.01 , and 0.001 , respectively. n.s. indicated no significance. ND means not detectable.

Genetic Analysis and molecular mapping of reduced plant height phenotype in *wph3*

Reciprocal crosses were performed between *wph3* and each of two elite wheat varieties K1 and K6. All F₁ progeny from all crosses exhibited plant height comparable to that of the WT parents (K1 or K6) (Fig. 3a, b). Meanwhile, the PH in the RK6-F₂ population was continuously distributed within the range of 40 to 110 cm, with most values concentrated between 60 and 90 cm (Fig. 3c). Therefore, we hypothesize that multiple genetic loci regulating plant height may be present in the RK6-F₂ population.

To identify the mutant gene in *wph3*, we used the RK6-F₂ as the mapping population and performed BSE analysis with QTL-seq software (Takagi et al. 2013). A sharp peak of G' value was identified on chromosome 2B, indicating that a candidate chromosome region of approximately 750.0-817.0 Mb was responsible for the dwarf phenotype (Fig. 3d). We developed five KASP markers (*M1-M5*, Table S1) to genotype a total of 200 F₂ individuals (Table S2) from the population, and constructed a genetic linkage map (Fig. 3e). This map localized the target to an interval between markers *M3* and *M5*, corresponding to 799.5-807.3 Mb of chromosome 2B. Additional 562 F₂ individuals were further genotyped using markers

M3 and *M5*. As a result, 172 recombination events between the two markers were identified (Table S3). Five markers (*M6-M10*, Table S4) were further developed to genotype the recombinants and their F_{2:3} progeny, thereby further narrowing the candidate region. Finally, the candidate gene was mapped to a 3.9 Mb segment (799.5-803.4 Mb) on chromosome 2B, flanked by markers *M8* and *M10* (Fig. 3f). The plants carrying genotypes of the two markers derived from *wph3* exhibited a dwarf phenotype, indicating that the identified locus was derived from *wph3*. No GA-sensitive dwarf genes or their homologues had been reported in this region previously. Therefore, we proposed that it is a novel locus regulating plant height, designated *Reduced height 29 (Rht29)*.

Candidate gene mining

Based on the Chinese Spring reference genome annotation (IWGSC RefSeq Annotation v2.1) and BSE results, a total of 26 high-confidence genes carrying high or moderate variants were identified within the target region (Table S5). Given that *wph3* is an EMS-induced mutant of K1, the causal variant in *wph3* should be unique, and not present in Chinese Spring, K6 or K1. Therefore, whole-genome resequencing data of K1 was integrated with the reference genome (Li et al. 2021) to filter out the non-causal SNPs in *wph3*. Meanwhile, we focused on the typical EMS-induced guanine (G) → adenine (A) or cytosine (C) → thymine (T) transition. This integrated analysis resulted in only two candidate SNPs in two genes *TraesCS2B03G1539200* and *TraesCS2B03G1527800* (Table 1). *TraesCS2B03G1539200* encodes a peroxidase and *TraesCS2B03G1527800* encodes the SKP1-interacting partner 15 (SKIP15) (Table 1). The candidate SNPs on these two genes resulted in amino acid changes or splice region variants. Thus, they were predicted as the potential causal genes for *Rht29*.

Table 1
Candidate SNPs in the target interval.

Position	SNP	Type	Gene	Variant DNA	Variant Protein
800072182	C/T	missense_variant	TraesCS2B03G1527800	G1214A	Gly405Asp
803273626	C/T	missense_variant & splice_region_variant	TraesCS2B03G1539200	G215A	Gly72Asp

Transcriptome analysis

To further mine the genes and transcription network responsible for the dwarf phenotype of the *wph3*, we collected peduncle samples of *wph3* and K1 during the jointing stage for RNA sequencing (RNA-seq). Principal Component Analysis (PCA) revealed that the K1 and *wph3* groups showed a clear clustering pattern in the 3D PCA space. Sample points within each group were relatively tightly clustered, indicating good consistency in transcriptome expression patterns among biological replicates of the same group (Fig. 4a). Quantification of gene expression level using FPKM values was performed. From this dataset, we found that the candidate gene *TraesCS2B03G1539200* was not expressed in either K1 or *wph3*, while

TraesCS2B03G1527800 exhibited no significant expression difference between *wph3* and K1 (Fig. 4c). This result indicated that *TraesCS2B03G1539200* could be excluded from candidate genes.

Based on the threshold of $FDR < 0.05$ and fold change ≥ 1.5 , a total of 3479 DEGs, including 2228 up-regulated and 1251 down-regulated, were identified (Fig. 4b). Since exogenous GA application could restore the PH phenotype of *wph3* and *wph3* exhibited apparent deficiency in bioactive GAs, as well as their intermediates and catabolites, we focused on DEGs related to the GA biosynthesis pathway. Several key enzymes, such as GA 13-oxidase (GA13ox), GA 20-oxidase (GA20ox) and GA3-oxidase (GA3ox), participate in converting the common precursor GA₁₂ to several intermediates and the bioactive GAs through 13-OH pathway (GA₁ and GA₃) and the 13-H pathway (GA₄ and GA₇) (Shani et al. 2024). The bioactive GAs could also be further deactivated by GA2-oxidase (GA2ox). According to our transcriptome data, seven genes encoding key enzymes of GA biosynthesis were detected to express in the peduncle and exhibit differential expression levels (Fig. 4d). One DEG encoding GA2ox2 was up-regulated in *wph3*, and four GA2oxs were identified showing down-regulated expression in *wph3* compared to K1. Interestingly, two genes, annotated as GA3ox2-2 and GA3ox2-3, encoding GA3ox, which catalyzes the key step for bioactive GA biosynthesis, exhibited significantly down-regulated expression in *wph3* (Fig. 4d).

Effects of the *Rht29* on agronomic traits

To evaluate effects of *Rht29* on agronomic traits in different genetic background, we genotyped RK6-F_{2:3} population using a molecular marker *M4* tightly linked with *Rht29* and randomly selected 84 homozygous K6-type individuals and 73 mutant individuals for phenotyping. Compared to K6-type, *wph3*-type showed average 34.13% decrease in PH, 15.35% decrease in SL, but similar SNS and TN (Fig. 5a-d). For the grain-related traits, GL, GW, and TGW decreased by 5.99%, 6.58%, and 11.51% in *wph3*, respectively (Fig. 5e-g). Similar to that in the genetic background of K1, the 30.41% decrease of GNS was found in the *wph3*-type plants (Fig. 5h).

PH segregation in the RK6-F₂ population did not show monogenic inheritance, suggesting potential additional dwarf gene(s) besides *Rht29* between K6 and *wph3*. Based on our exome sequencing data, we found a premature termination variant in K6 and a missense variant in *wph3* of *Rht-B1*, corresponding to alleles *Rht-B1b* and *Rht-B1i*, respectively (Table S6). We therefore analyzed interaction between *Rht-B1* and *Rht29*. We used molecular marker *KASP_Rht-B1* linked with *Rht-B1* (Li et al. 2022) and those linked with *Rht29* to identify F₂ individuals homozygous at both loci. Under the background of *Rht-B1b* or *Rht-B1i*, mutant allele of *Rht-29* (*Rht-B29b*) caused approximately 23% PH decrease. A greater reduction (32.67%) in PH could be observed when comparing the combination of *Rht-B1b* and *Rht-B29b* to the combination of *Rht-B1i* and WT *Rht-29* (*Rht-B29a*) (Fig. 5i).

Discussion

Rht29 is a novel wheat dwarfing gene

In this study, a dwarfing gene was identified within the physical interval of 799.5-803.4 Mb on the long arm of chromosome 2B of wheat. Several *Rht* genes have been cloned or localized on the specific chromosomes. For example, *Rht1* and *Rht2* were located on chromosomes 4B and 4D, respectively (Peng et al. 1999); *Rht5* was on chromosome 3B (Cui et al. 2022); *Rht14*, *Rht18*, *Rht24*, and *Rht25* were all located on chromosome 6A (Haque et al. 2011, Ford et al. 2018, Tian et al. 2022, Zhang et al. 2023). Several *Rht* genes have been reported to be derived from the group 2 homoeologous chromosomes. *Rht8* was derived from chromosome 2D and its homologous locus *Rht8-2B* was located on the short arm of chromosome 2B (Chai et al. 2022). *Rht28* (Liu et al. 2025) was located at 558.90-562.08Mb on chromosome 2A, which was not collinear with the interval we defined for *Rht29*. The homologues of the predicted candidate genes of *Rht28* on chromosome 2B, *TraesCS2B03G0888600* (495,354,127–495,360,583) and *TraesCS2B03G0889100* (496,131,191–496,141,611), were far apart from *Rht29*. *Rht4* was the dwarfing gene closest to *Rht29* on chromosome 2B (770–773 Mb) (Qiao 2020). However, it still did not overlap with the *Rht29* interval. Its candidate gene, *TraesCS2B03G1429200*, exhibited no variant and expression in K6 and *wph3*. Additionally, other known genes associated with plant height on chromosome 2B, such as *TaAP1-3-2B*, *TaAP1-2-2B*, and *TaARF12-2B* (Li et al. 2019, Li et al. 2022), also fall outside the *Rht29* region. Therefore, *Rht29* is a novel dwarfing gene.

***Rht29* is potentially a non-canonical GA-sensitive dwarfing gene**

In this study, *Rht29* was characterized as a GA-sensitive dwarfing gene due to the two aspects: First, the PH of *wph3* is sensitive to exogenous GA₃ application, which can rescue the dwarf phenotype of *wph3*. Second, *wph3* showed apparent GA biosynthesis deficiency (Fig. 2). Quantitative analysis of GAs revealed that both the 13-OH and 13-H pathways were suppressed in the *wph3*. The intermediates of the 13-OH pathway (e.g. GA₅₃, GA₄₄, GA₁₉, GA₂₀) exhibited a more pronounced reduction compared to those of the 13-H pathway. This observation was consistent with previous study showing that GA biosynthesis in wheat mainly relies on the 13-OH pathway, with GA₁ as the major bioactive GA. In line with this, 13-hydroxylated GAs, such as GA₁, GA₂₀, and GA₁₉, predominantly accumulate in vegetative tissues of wheat, whereas GA₄ from the 13-H pathway is present at very low levels in these tissues (Appleford et al. 2006).

Typical GA-sensitive dwarfing genes usually encode key enzymes for bioactive GA synthesis or deactivation. For example, rice ‘green revolution’ gene *sd1* encodes a GA20ox (Sasaki et al. 2002). In wheat, *Rht12* (Sun et al. 2019) and *Rht18* (Grant et al. 2018) encode GA2oxA13 and GA2oxA9, respectively. However, some non-canonical GA-sensitive dwarfing genes have also been identified. *Rht8* encodes an RNase H-like protein which can modulate bioactive GA ratios by regulating the expressions of GA13ox and GA20ox (Xiong et al. 2022). GA-sensitive *Rht13* encodes an NB-LRR protein (Borrill et al. 2022) and *Rht25* encodes a PLATZ transcription factor, which regulated wheat PH via interacting with DELLA (Zhang et al. 2023).

Two high-confidence genes, *TraesCS2B03G1527800* and *TraesCS2B03G1539200*, carrying *wph3*-specific SNPs were identified within the *Rht29* interval. Our transcriptome data showed that

TraesCS2B03G1539200 was not expressed either in *wph3* or in K1 (Table S7). We also queried the spatiotemporal gene expression dataset of Chinese Spring (Consortium 2014) and found that it did not express in the stem. Thus, *TraesCS2B03G1527800* is considered to be the most possible candidate gene for *Rht29*. However, it did not encode the known key enzymes for GA synthesis or metabolism, suggesting that *Rht29* might be a non-canonical GA-sensitive dwarfing gene.

Potential functions of *Rht29* in GA-dependent PH modulation

Previous studies have shown that expression of *GA20ox* and *GA3ox* genes is under negative feedback control of GA, whereas that of *GA2ox* genes is under positive feedforward control (Hedden and Phillips 2000, Olszewski et al. 2002). Consistent with these reports, a *GA20ox* gene and four *GA2ox* genes of *wph3* showed up-regulated and down-regulated expression in response to GA deficiency, respectively (Fig. 4d). However, the expression of two detectable *GA3ox* genes in *wph3* was significantly suppressed, which contradicted the negative feedback regulation pattern of GA. Therefore, we speculated that *Rht29* may function in bioactive GA biosynthesis through regulating *GA3ox* expression.

The candidate gene *TraesCS2B03G1527800* is homologous to *BAF1* of *Arabidopsis*, which encodes an F-box family E3 ubiquitin ligase. *BAF1* interacts with *BRI1-EMS-SUPPRESSOR1* (*BES1*), the core transcription factor of the brassinosteroid signaling pathway, and mediates its ubiquitination and degradation (Wang et al. 2021). Notably, *BES1* plays a key role in the crosstalk between Brassinosteroids (BRs) and GA biosynthesis pathways. It has been reported that *BES1* was able to interact with the promoter of *GA3ox1D* and activate its expression to promote fiber cell elongation in cotton (Hou et al. 2025). Therefore, it is possible that the mutation in *BAF1* of *wph3* may enhance the degradation of *BES1*, thereby preventing the activation of *GA3ox* expression. This model proposed the functional relevance between *TraesCS2B03G1527800* and the dwarf phenotype of *wph3*.

Rht29 has pleiotropic effects on agronomic traits

By comparing with WT and analyzing its effects in RK6 population, *Rht29* was found to cause more than 30% PH and more than 15% SL decrease (Fig. 1 and Fig. 5). This combined effect was consistent with the GA-sensitive *Rht8* (Chai et al. 2022) and *Rht25* (Zhang et al. 2023), as well as GA-insensitive *Rht-B1* (Song et al. 2023), indicating that this is a common feature of GA-related dwarfing genes. *Rht29* showed relatively large degree of PH reduction under semi-dwarf genetic background and exhibited additive effects with different *Rht-B1* alleles. Such effect may originate from the significant suppression of *GA3ox* and the consequent extensive inhibition of synthesis of bioactive GA_1 , GA_3 , and GA_4 (Fig. 2). This also led to a significant decrease in biomass, which may contribute to the decrease of the grain weight. In contrast, *Rht8* functions in modulating the ratio of GA_3 and GA_4 by regulating the expression of *GA20ox* and *GA13ox*. Such a relatively mild change in GA synthesis generated ~ 20% PH decrease and had no obvious negative impact on grain yield (Xiong et al. 2022).

Additionally, GA is closely associated with TN and inflorescence development. For example, the wheat *ga3ox2* mutant showed approximately 3-fold lower concentration of the bioactive GA_1 compared to the

WT, and exhibited significantly more TN, fewer spikelets, and complete sterility (Phillips et al. 2025). In this study, *wph3* showed significantly down-regulated expression of *GA3ox* genes and a similar reduction of GA_1 , but exhibited different performance on tiller and inflorescence development compared to the *ga3ox2* mutant. We observed ~ 30% TN reduction in *wph3* compared with that in K1 (Fig. 1k). However, this negative impact was undetectable in RK6 population, indicating that the influence of *Rht29* on TN may be genetic background-dependent. Additionally, *wph3* showed no difference in SNS under different backgrounds (Fig. 1g and Fig. 5c) and significantly decreased GNS (Fig. 1k and Fig. 5h), suggesting that *Rht29* does not affect differentiation of spikelet. Although it remains unclear that whether *Rht29* affects GNS by regulating the floret number or floret fertility, our results indicated that the pleiotropy of *Rht29* on agronomic traits is not equivalent to that of the *GA3ox* mutant. Thus, further causal gene cloning, functional validation, and molecular mechanism dissection are worth conducting, which will facilitate gaining deeper insights into the mechanisms underlying the transcriptional regulation of *GA3ox2* genes and the maintenance of bioactive GA homeostasis, as well as their pleiotropic effects on agronomic traits. This will lay the foundation for breaking the linkage between *Rht29* and unfavorable traits and expanding wheat yield potential through fine tuning plant height in breeding.

Author contribution statement

GD and HL conceived and designed the study. YT developed mapping population, FD, YT, FH and GD undertook the field and greenhouse trials, sampling, phenotyping and genotyping, JZ, TL, JW, JL and HZ assisted in genetic mapping, FD, JZ, and HL analyzed data, drafted and revised the manuscript, and all authors reviewed the manuscript.

Declarations

Conflict of interest

All authors declare that they have no conflict of interest.

Funding

This work is supported National Natural Science Foundation of China (32272125, 32301790), the National Key R&D Program of China (2024YFD1201200), Sichuan Science and Technology Program, China (2022ZDZX0014), and Sichuan Provincial Agricultural Department Innovative Research Team (SCCXTD-2024-11).

Author Contribution

GD and HL conceived and designed the study. YT developed mapping population, FD, YT, FH and GD undertook the field and greenhouse trials, sampling, phenotyping and genotyping, JZ, TL, JW, JL and HZ assisted in genetic mapping, FD, JZ, and HL analyzed data, drafted and revised the manuscript, and all authors reviewed the manuscript.

Data Availability

The BSA data and transcriptome data generated and used in this study have been deposited in The Genome Sequence Archive (GSA) (Accession No. XXXXXXXXXX, under checked), Beijing Institute of Genomics, Chinese Academy of Sciences, and are publicly available.

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Figures

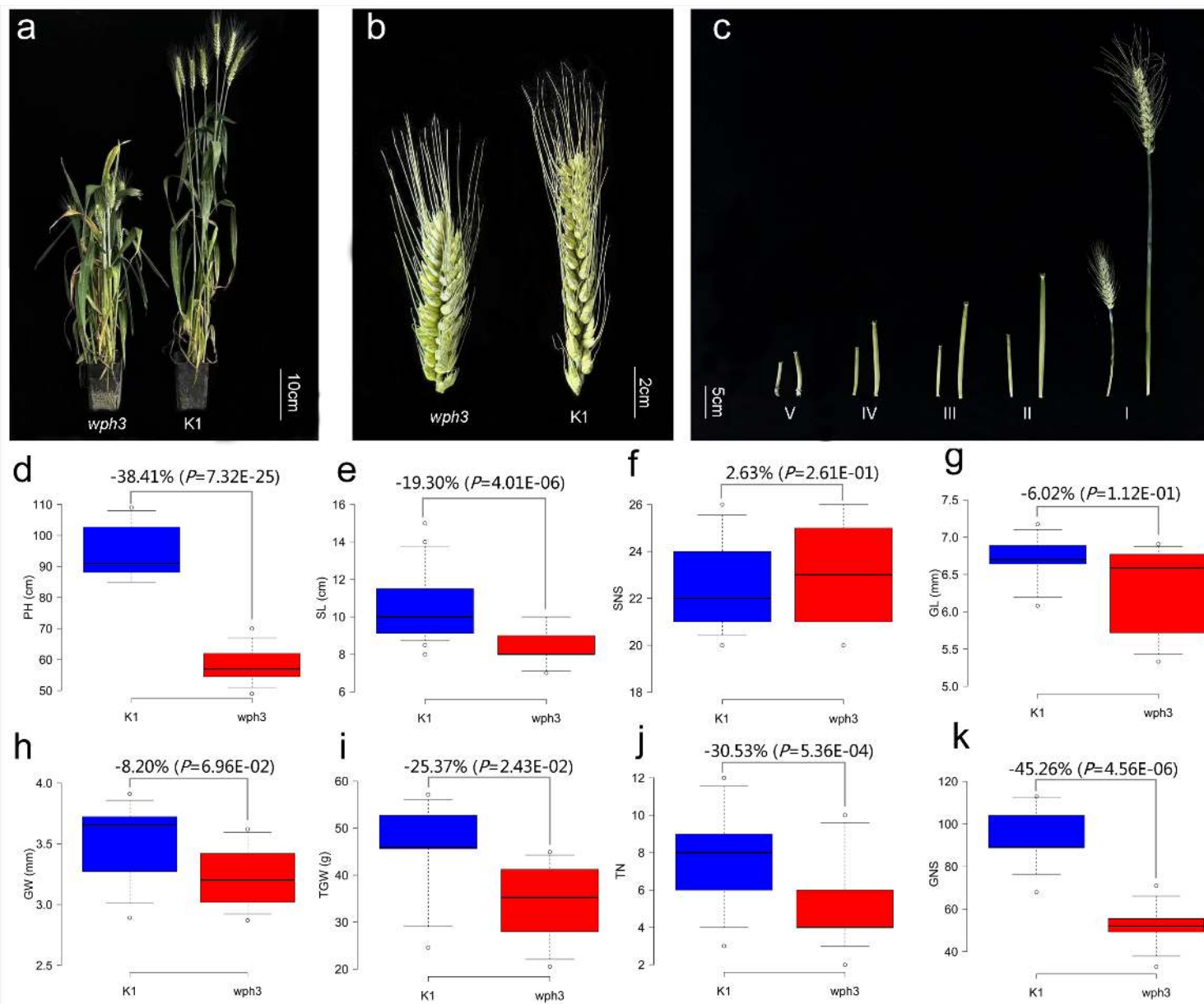


Figure 1

Phenotypic comparison of *wph3* and K1. **a-c**, indicated morphology of the whole plant, spike, and the internodes of *wph3* and K1, respectively. **d-k**, indicated comparison of PH, SL, SNS, GL, GW, TGW, TN and GNS between *wph3* and K1, respectively.

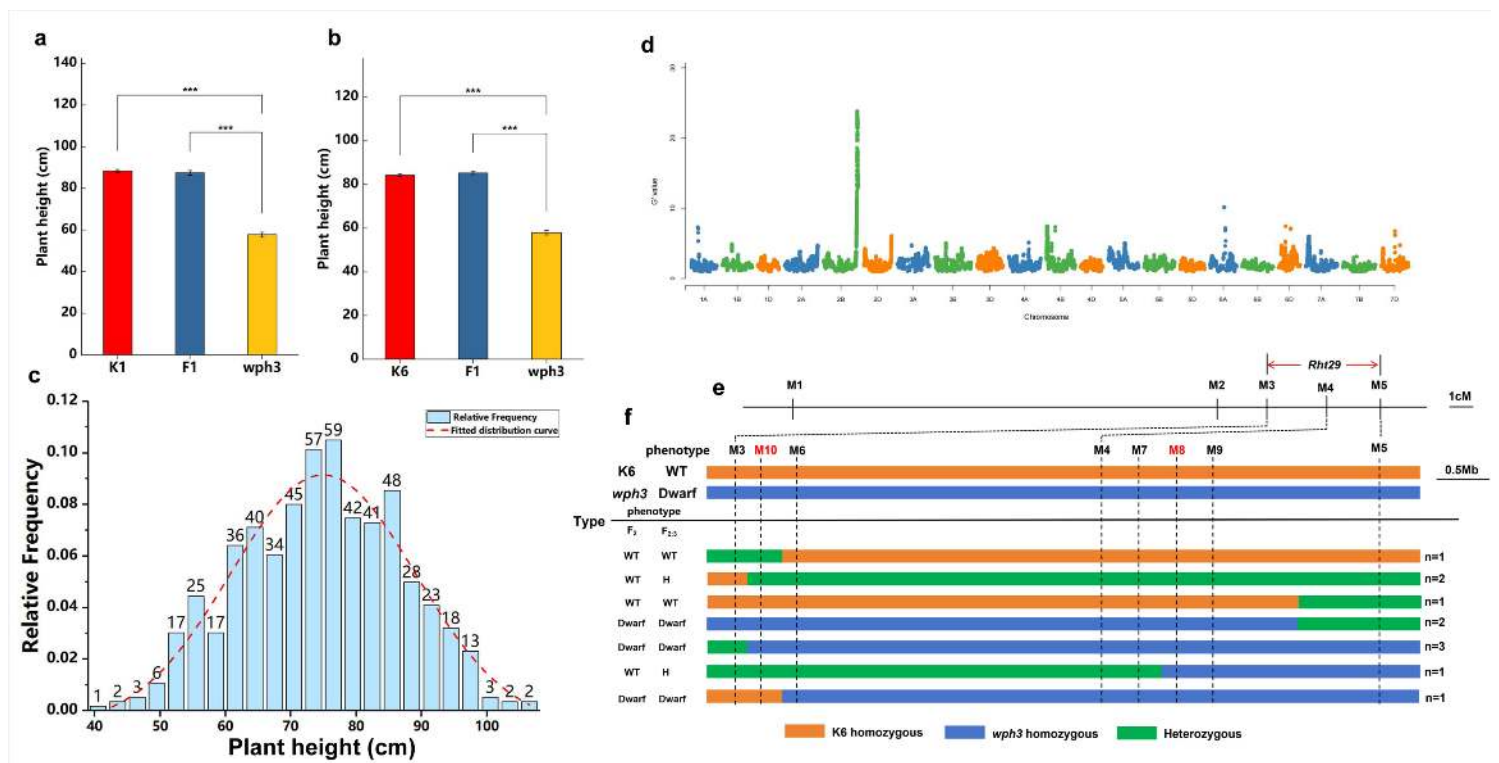


Figure 3

Genetic analysis and molecular mapping of *Rht29*. **a** and **b**, Phenotypic comparison between parental lines and F₁ hybrids. **c**, Plotting of frequency distribution of plant height in the RK6-F₂ population. **d**, BSE based on plotting of G' value on 21 chromosomes of wheat. **e**, Genetic map of *Rht29*. **f**, Fine mapping of *Rht29*. *** indicated $p < 0.001$.

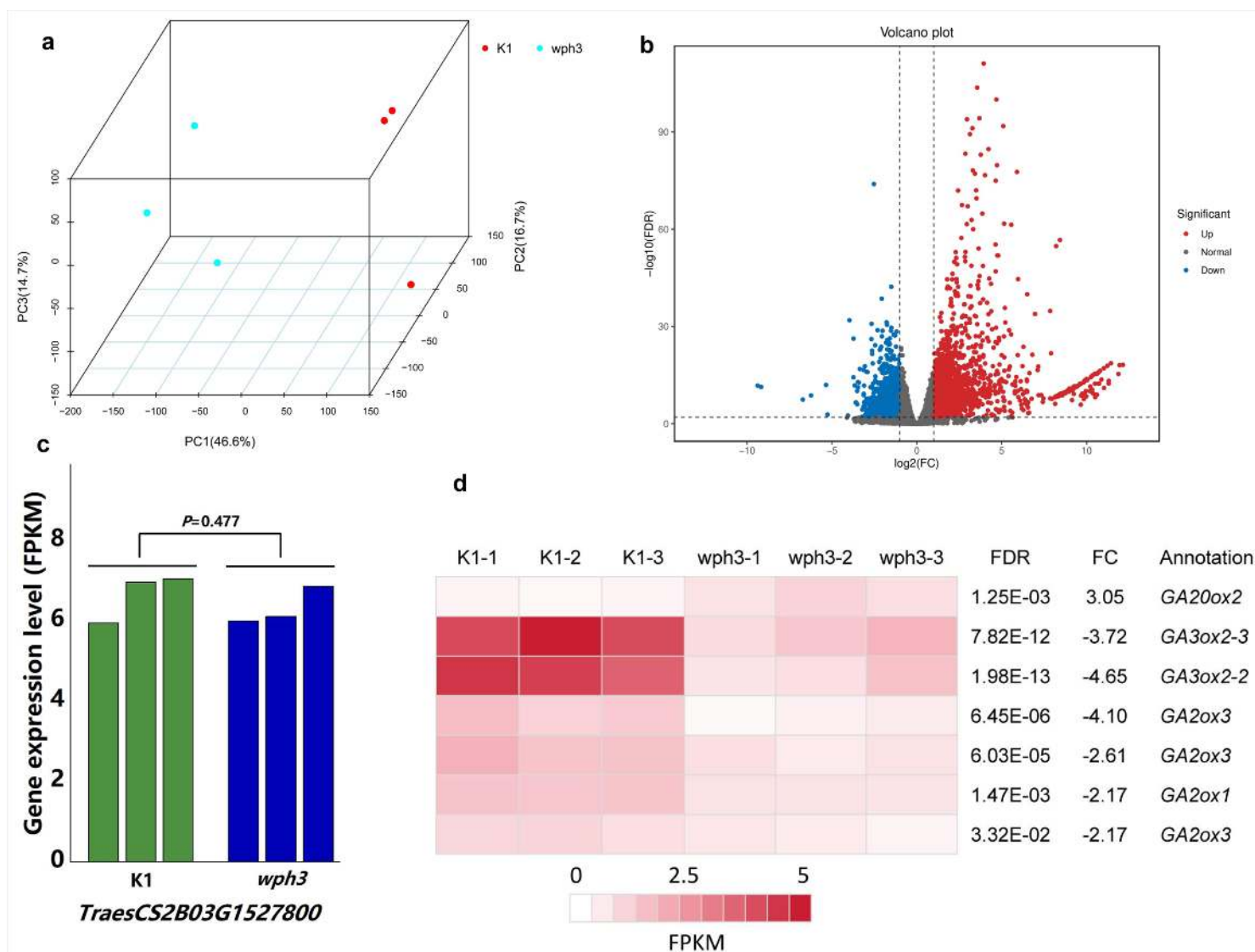


Figure 4

Transcriptome analysis of the elongating peduncle. **a**, Principal Component Analysis (PCA) based on DEGs. **b**, Volcano plotting of DEGs. **c**, The expression of candidate gene *TraesCS2B03G1527800* in K1 and *wph3*. **d**, Comparison of expression levels of DEGs encoding key enzymes catalyzing bioactive GA biosynthesis and metabolism. FDR, False discovery rate; FC, fold change.

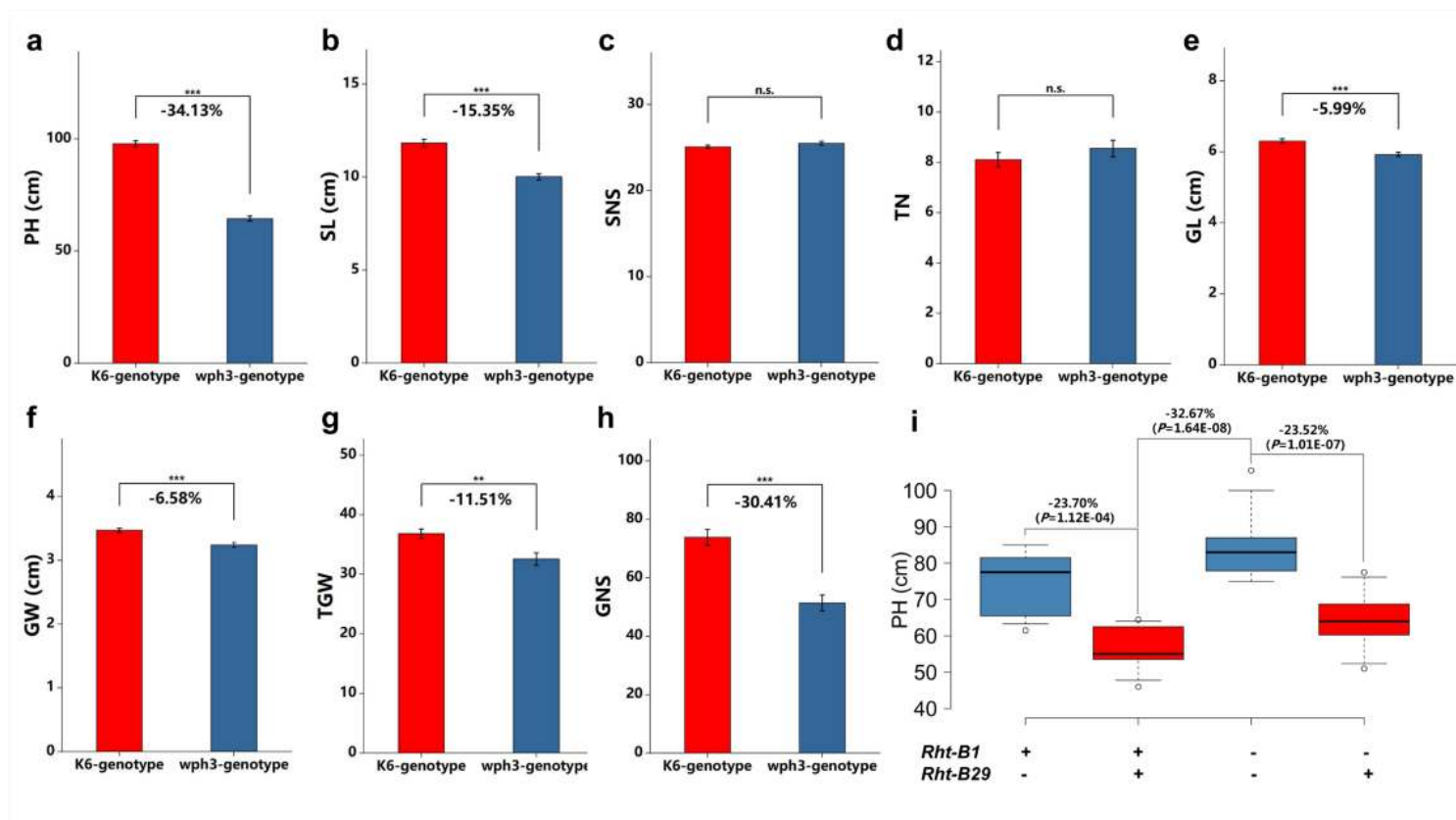


Figure 5

Analysis of the genetic effects of the mutant gene in the RK6-F_{2:3} population. **a-h**, corresponded to PH, SL, SNS, TN, GL, GW, TGW, and GNS, respectively. **i**, indicated the interaction between *Rht-B1* and *Rht-29* (*Rht-B29*). + or - for *Rht-B1* indicated *Rht-B1b* or *Rht-B1i*, and for *Rht-29* indicated WT (*Rht-B29a*) or mutant type (*Rht-B29b*), respectively. *** indicated $p \leq 0.001$, n.s. indicated no significant difference.

Supplementary Files

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