Phenotypic characterization and hormone-related gene expression analysis of the rice dwarf and small grain mutant *dsg2*

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Abstract

Plant height and grain shape are important agronomic traits in rice and are closely associated with plant architecture, lodging resistance, and yield. Genetically, they often behave as linked traits regulated by the same gene; therefore, studies of related mutants can provide important genetic resources for the improvement of plant height and grain shape. This study aimed to characterize the phenotypic features of a novel dwarf and small-grain rice mutant (dwarfandsmallgrain2, dsg2) and to examine the expression levels of key genes in plant hormone signaling pathways using RT-qPCR, in order to elucidate the regulatory mechanism of the DSG2 gene on multiple agronomic traits. The results showed that: (1) Compared with WT, the plant height of the dsg2 mutant was reduced by approximately 18.9%, indicating a semi-dwarf mutant, while grain length, grain width, grain thickness, and 1,000-grain weight decreased by 11.8%, 20.4%, 17.7%, and 34.2%, respectively. (2) In addition, the dsg2 mutant exhibited a significantly increased tiller number, delayed heading date, and markedly inhibited root growth. (3) The auxin biosynthesis gene OsGH3-8 and signaling pathway gene OsIAA3, the gibberellin biosynthesis genes sd1 and SDG2, and the brassinosteroid biosynthesis genes D2 and D4 were all significantly downregulated, whereas the brassinosteroid signaling genes DLT and BZR1 were significantly upregulated. In summary, the mutant gene DSG2 is pleiotropic and coordinately affects rice plant height, grain shape, heading date, and root development by regulating the homeostatic balance of multiple hormones, including auxin, gibberellin, and brassinosteroid, providing an important genetic resource for the coordinated improvement of rice plant architecture and grain shape.

Full Text

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Abstract: Plant height and grain shape are important agronomic traits in rice, closely related to plant architecture, lodging resistance, and yield. The two traits are often genetically linked and regulated by the same gene; therefore, studies of relevant mutants can provide important genetic resources for the improvement of plant height and grain shape. This study aimed to identify the phenotypic

characteristics of a novel rice dwarf and small grain mutant (*dwarf and small grain 2*, *dsg2*) and to measure, using RT-qPCR technology, the expression levels of key genes in plant hormone signaling pathways, so as to reveal the regulatory mechanism of the *DSG2* gene for multiple agronomic traits. The results showed that: (1) Compared with WT, the plant height of the mutant *dsg2* was reduced by approximately 18.9%, indicating that it was a semi-dwarf mutant, while grain length, grain width, grain thickness, and thousand-grain weight were reduced by 11.8%, 20.4%, 17.7%, and 34.2%, respectively. (2) In addition, the number of tillers of the mutant *dsg2* increased significantly, heading was delayed, and root growth was also markedly inhibited. (3) The growth hormone biosynthesis gene *OsGH3-8* and signaling-pathway gene *OsIAA3*, the strigolactone biosynthesis genes *sd1* and *SDC2*, and the brassinosteroid biosynthesis genes *D2* and *D4* were all significantly downregulated, whereas the brassinosteroid signaling genes *DLT* and *BZR1* were significantly upregulated. In summary, the mutant gene *DSG2* has pleiotropic effects; by regulating the homeostatic balance of multiple hormones, including auxin, strigolactones, and brassinosteroids, it coordinately affects rice plant height, grain shape, heading date, and root development, thereby providing important genetic resources for the coordinated improvement of rice plant architecture and grain shape.

Keywords: rice; dwarf; small grain; plant hormones; gene expression

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Phenotypic characterization and hormone Related gene expression analysis of the dwarf and small grain rice mutant

dsg2

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Abstract: Plant height and grain shape are important agronomic traits in rice, closely related to plant architecture, lodging resistance, and yield. These two traits are often genetically linked and regulated by the same gene; therefore, studying relevant mutants can provide valuable genetic resources for the improvement of plant height and grain shape. This study aimed to characterize

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the phenotypic features of a novel rice dwarf and small grain mutant (*Dwarf and small grain 2*, *dsg2*), and to examine the expression levels of key genes involved in major plant hormone signaling pathways using RT-qPCR, in order to elucidate the regulatory role of the *DSG2* gene in multiple agronomic traits. The results were as follows: (1) Compared with WT, the plant height of *dsg2* was reduced by approximately 18.9%, classifying it as a semi-dwarf mutant, while grain length, grain width, grain thickness, and 1 000-grain weight decreased by 11.8%, 20.4%, 17.7%, and 34.2%, respectively. (2) In addition, *dsg2* exhibited a significantly increased tiller number, delayed heading date, and markedly inhibited root growth. (3) The auxin biosynthesis gene *OsGH3-8*, the auxin signaling gene *OsIAA3*, the gibberellin biosynthesis genes *sd1* and *SDG2*, and the brassinosteroid biosynthesis genes *D2* and *D4* were all significantly downregulated, while the brassinosteroid signaling genes *DLT* and *BZR1* were significantly upregulated. In conclusion, the *DSG2* gene exhibits pleiotropic effects and coordinately regulates rice plant height, grain shape, heading date, and root development by modulating the homeostasis of multiple phytohormones, including auxin, gibberellin, and brassinosteroid, thereby providing important genetic resources for the synergistic improvement of plant architecture and grain shape in rice.

Key words: rice, dwarf, small grain, plant hormone, gene expression

Rice plant height and grain shape are key traits for shaping an ideal plant type and determining yield. They not only directly affect lodging resistance and harvest index, but also constitute an important basis for grain weight and yield per plant. Therefore, the coordinated improvement of the two has always been a core objective in high-yield rice breeding (Ouyang et al., 2022; Wang et al., 2024). However, plant height and grain shape are often regulated by the pleiotropic effects of the same gene, presenting genetic linkages such as "dwarf-small grain" or "tall-large grain," which severely hinder the aggregation of favorable traits (Wang Yan, 2022). Therefore, identifying genetic materials with coordinated variation in plant height and grain shape and analyzing their molecular regulatory mechanisms are of important theoretical significance and application value for breaking linkage drag and achieving coordinated improvement of both traits.

Phytohormones play central roles in the regulatory networks of plant height and grain shape. Signaling pathways such as auxin (IAA), gibberellic acid (GA), and brassinosteroid (BR) are all involved in the coordinated regulation of rice plant height and grain shape, and abnormalities in their biosynthesis or signaling lead to related phenotypic defects (Wang et al., 2018). In the auxin pathway, YUCCA-family flavin monooxygenases (such as *OsYUC1* and

OsYUC9) catalyze the rate-limiting steps of auxin biosynthesis, and their expression levels directly affect endogenous auxin content, thereby regulating cell elongation and organ size (Yamamoto et al., 2007; Abu-Zaitoon et al., 2012). In the gibberellin pathway, *sd1* encodes GA20 oxidase and catalyzes the synthesis of bioactive GA; loss of its function results in a deficiency of active GA in culms, suppression of internode cell elongation, and a semi-dwarf phenotype (Monna et al., 2002). The transcription factor *SGD2* positively regulates internode cell elongation and grain size by regulating the expression of GA biosynthesis genes (Chen et al., 2019). In the brassinosteroid pathway, the biosynthetic gene *D11* encodes a cytochrome P450 protein and participates in key catalytic steps of BR biosynthesis; its mutation causes BR deficiency, inhibited cell elongation, severe dwarfism, and shortened grain length (Tanabe et al., 2005). The signaling factors *DLT* and *BZR1*, as key transcription factors in BR signal transduction, regulate downstream cell

the expression of genes related to cell elongation and cell division, thereby coordinately regulating internode elongation and grain size (Bai et al., 2007; Tong et al., 2009). Through complex crosstalk, the above hormone pathways constitute a coordinated regulatory network that jointly determines plant height and grain shape in rice (Wang et al., 2018). In addition to hormone pathways, the spatial structure of microtubules is also an important link in plant architecture establishment: the formin protein FH5/RMD maintains the correct arrangement of microtubule filaments by regulating actin dynamics and plays a key role in morphogenesis (Yang et al., 2011); the plant-specific protein OsSCD2 is associated with reticulon proteins, participates in vesicle transport, and regulates cell expansion, and its mutation causes almost all organs of the plant to become smaller (Wang et al., 2022).

Dwarf and small-grain mutants are ideal genetic materials for dissecting the coordinated regulation of plant height and grain shape. With the aid of such materials, many important functional genes have been successfully cloned, and key mechanisms underlying trait coordination have been revealed. However, most currently reported dwarf and small-grain mutants mainly show changes in plant height, with grain-shape changes as a secondary effect, and their simultaneous regulatory patterns mainly depend on a single pathway (Yang et al., 2011; Chen et al., 2019; Niu et al., 2022; Wang et al., 2022). It is noteworthy that Lü Zhaokun et al. (2020) obtained, through EMS mutagenesis, a dwarf small-grain mutant *dsg1*, whose plant height was semi-dwarf and whose grain shape was significantly smaller; the mutant gene was mapped to a 52.28 kb interval on chromosome 4, suggesting the existence of a gene type in which grain-shape regulation is primary and plant-height regulation is secondary. However, such genetic materials remain relatively scarce, related research has progressed slowly, and more genes remain to be mined and analyzed. In this study, a new dwarf small-grain mutant *dsg2* and its wild type were used as the research objects. By systematically identifying the mutant phenotype of *dsg2* and analyzing its hormone regulatory pathways, we aimed to explore the following questions: (1) the patterns of changes in plant height and grain-shape traits of the mutant *dsg2*

and changes in its key agronomic traits; (2) the relationship between the mutant phenotype of *dsg2* and key plant hormone signaling pathways. The results of this study may lay a foundation for map-based cloning and functional analysis of the mutant gene *DSG2*, and also provide clues for revealing new mechanisms of coordinated regulation of plant height and grain shape.

1 Materials and Methods

1.1 Experimental Materials

The mutant *dsg2* is a mutant material discovered during callus tissue culture of the conventional japonica rice variety Nipponbare. After three generations of planting, it was confirmed that its dwarf and small-grain mutant traits could be stably inherited. In this study, in July 2023, WT and *dsg2* were planted in the Science and Technology Park of Jiangxi Agricultural University, managed under conventional water and fertilizer conditions, and investigated for yield-related agronomic traits.

1.2 Phenotypic Observation

At maturity, 10 WT and *dsg2* plants with relatively uniform growth vigor were selected to measure plant height and panicle number. After harvest and drying, 10 main panicles each from WT and *dsg2* were taken to examine yield-related traits, including panicle length, primary branch number, secondary branch number, and 1,000-grain weight. In addition, 30 fully filled grains each from WT and *dsg2* were taken, and a vernier caliper was used to measure grain length, grain width, and grain thickness. To investigate the difference in heading date between WT and *dsg2*, seeds of the two were sown in an artificial climate chamber (10 h light/14 h dark), and their heading date was examined. The heading date of a single rice plant was defined as the number of days from sowing to the first panicle emerging 1 cm from the flag leaf sheath; 20 plants each of WT and *dsg2* were counted.

1.3 Investigation of Root Development in the Mutant *dsg2*

Plump WT and *dsg2* seeds were selected, soaked to induce germination, and then sown in hydroponic boxes. When the seedlings grew to the one-leaf-one-heart stage, 20 plants each of WT and *dsg2* were taken, and traits such as seedling height, root length, and root number were examined.

1.4 RT-qPCR Analysis of Genes Related to Plant Hormone Pathways

Based on previous studies, plant hormones often regulate two linked traits—plant height and grain shape—through the same gene. However, differences exist in the panicle development process between *dsg2* and WT, making it difficult to obtain grain samples at identical developmental stages; by contrast,

stem development stages are easier to determine and unify. Therefore, in this study, stems of WT and *dsg2* at the booting stage were selected for expression analysis of hormone-related genes. Specifically, internodes II to III of WT and *dsg2* plants with relatively consistent booting stages in the field were collected, and the stems were ground into powder in liquid nitrogen. Total RNA extraction and reverse transcription were then performed using an RNA extraction kit (TaKaRa MiniBEST Universal RNA Extraction Kit, 9767) and a reverse transcription kit (PrimeScript™ RT reagent Kit with gDNA Eraser, RR037Q), respectively, following the instructions of the kits. An RT-qPCR reaction system was prepared using a fluorescence quantitative kit (Hieff UNICON® Universal Blue qPCR SYBR Master Mix, 11184ES03), and gene expression levels were detected with a BIO-RAD CFX96 real-time fluorescence quantitative PCR instrument. The rice *Ubiquitin* (LOC_Os03g13170) gene was used as the internal reference. Each sample included three biological replicates, and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of each gene in WT and *dsg2*. The primer sequences used in this experiment are shown in Table 1.

Table 1 List of primers for RT-qPCR

Primer name	Forward primer (5′(→)3′)	Reverse primer (5′(→)3′)
d18	GCCGACGAGTTGCTGA	GGCTTCTCGCCGATCCTCCTC
sd1	GTCGCTGGCGTTCTTC	ATGATGGTCGGCCCAGGTGAA
SGD2	GGAGAGCTTCCGCCTC	AAGCCGCTTCAGGTACTCGCA
D1	ACCAAGGAACTCTTTG	GCTGCAGTGCTTGCTGCTCTGG
EUI1	TTCCGGGACATGCAGT	GGCGTGGTGCATGGTGGACA
GID1	CATGTCGCAGCCGTTC	GTAGCCAGGATGTTGCCGCAGA
OsYUC1	AGGGCCCATCGAGCTC	GAACGTTGCTCCTGTAGCCT
OsYUC9	CGCCACCTCCATCGTC	TTGAGCAGCACCACCTTGTCG
OsYUC11	TATGCTGCGCAAAGGG	TGGTCAGAGGGCTGCGGATG
OsRGB1	CCATCAGGGCATACGG	ACTAGCCCGGCTTGCAATTCT
OsGH3-8	GATTGGGCCGCTCGAG	ACCGTGGCACCTTGTACTGG
OsARF12	AGGTGTACGCGCAGAT	GATCGTCCGCGGGAAGGTACG
OsIAA3	ACGACGACGAAGAGCA	GGCCTCCGTCCATGCTCACC
D11	TGAGGTTCCTCAGTCC	TAATCACCCTCCCATACCTGGAG
D2	TTCAACCCATGGAGGT	AGCATGGATGACCAAGTGGTGAAG
D4	TAAACGAGACGTTGCG	GATAGGCTTGGAATGTCATAACCC
BRI1	AGCTTCAGATGCACCA	TCAAGCAGCTCCAGCAACACAAC
DLT	GGCGATGAGGTATGGT	CGACTTGCTGTACTCCTCCT
BZR1	TCCCGTACCTGTCATG	GGTATCGTCAAAGCGATCATGCC

Figure 1

Figure 1: Figure 1

2 Results and Analysis

2.1 The mutant gene *DSG2* positively regulates plant height and grain shape

Compared with WT, the most significant characteristic of the mutant *dsg2* was reduced plant height (Fig. 1A), and both grain length and width became smaller (Fig. 1B, D). After removing the hull, it was found that the length and width of brown rice grains in the mutant *dsg2* were also smaller than those of WT (Fig. 1C, E). Statistical analysis showed that, compared with WT, the plant height of mutant *dsg2* decreased by approximately 18.9% at maturity, while grain length, grain width, grain thickness, and thousand-grain weight decreased by 11.8%, 20.4%, 17.7%, and 34.2%, respectively (Fig. 1F–J). These data indicate that the mutant gene *DSG2* positively regulates the establishment of rice plant height and grain shape. Further investigation of agronomic traits related to heading and other yield components in WT and *dsg2* revealed that the heading stage of mutant *dsg2* was delayed by approximately 6 d compared with WT (Fig. 2A, B), the number of tillers increased by approximately 64.7%, and panicle length was shortened by approximately 17.6% (Fig. 2C, D), whereas traits such as primary branches, secondary branches, and grains per panicle showed no significant changes (Fig. 2E–G).

A. Plant morphology of WT and mutant *dsg2* at heading stage, bar = 10 cm; **B–E.** rice grain length (**B**) and brown rice grain length (**C**) of WT and *dsg2* (bar = 1 cm), rice grain width (**D**) and brown rice grain width (**E**) (bar = 5 mm); **F–J.** statistical analysis of plant height, thousand-grain weight, grain length, grain width, and grain thickness of WT and mutant *dsg2*. Data are expressed as mean ± standard deviation; ** indicates an extremely significant level ($P < 0.01$; Student' s *t*-test), the same below.

A. Plant morphology of WT and mutant *dsg2* at heading stage, bar = 10 cm; **B–E.** Rice grain length (**B**) and brown rice grain length (**C**) of WT and mutant *dsg2* (bar = 1 cm), rice grain width (**D**) and brown rice grain width (**E**) of WT and mutant *dsg2* (bar = 5 mm); **F–J.** Statistical analysis of plant height, thousand-grain weight, grain length, grain width, and grain thickness between WT and *dsg2* mutant. Values are presented as means $\bar{x} \pm s$; ** indicates statistically significant difference is marked with asterisks ($P < 0.01$; Student' s t-test), the same below.

Fig. 1 Comparisons of plant height and grain shape between WT and mutant *dsg2*

A, B. In the short-day artificial-climate chamber, plant morphology (**A**) and heading-date statistics (**B**) of WT and the mutant *dsg2*; red arrows indicate the

panicles emerged from WT. **C–G.** Statistical analysis of tiller number, panicle length, primary branches, secondary branches, and grains per panicle in WT and the mutant *dsg2*.

A, B. Plant morphology (**A**) and heading date statistics (**B**) of WT and mutant *dsg2* in the short-day phytotron; red arrows indicate the emerged panicles of WT. **C–G.** Statistical analysis of primary branches, secondary branches, grains per panicle, panicle length, and tiller number between WT and mutant *dsg2*.

Fig. 2 Comparisons of heading date and yield traits between WT and *dsg2*

Fig. 2 Comparisons of heading date and yield traits between WT and mutant *dsg2*

2.2 The mutant gene *DSG2* regulates rice root growth

To investigate whether the mutant gene *DSG2* affects root development, seedlings of WT and *dsg2* were examined after germination followed by 7 d of hydroponic culture, with traits including seedling length, primary root length, and root number assessed. The results showed that, compared with WT, growth of the aboveground part, primary root system, and root hairs of the mutant *dsg2* was markedly inhibited (Fig. 3: A, D); statistical analysis showed that the seedling length, primary root length, and root number of the mutant *dsg2* were reduced by 31.7%, 46.2%, and 18.5%, respectively, compared with WT (Fig. 3: B, C, E), indicating that the mutant gene *DSG2* regulates root growth and development.

A. Seedling phenotype of WT and mutant *dsg2*, bar = 5 cm; **B**, **C**. Statistical analysis of seedling length and primary root length between WT and mutant *dsg2*; **D**, **E**. Root system phenotype and statistical analysis of root number between WT and mutant *dsg2* (**D**, bar = 5 cm).

Fig. 3 Seedling and root phenotypes of WT and mutant *dsg2*

2.3 The mutant gene *DSG2* affects signal transduction of multiple important plant hormones

Auxin, GA, and BR have been widely reported to participate in the regulation of plant height and grain shape. To explore the potential association between the mutant gene *DSG2* and these hormone pathways, this study examined the expression of key genes in the three signaling pathways in WT and mutant *dsg2*, including the auxin-pathway genes *OsYUC1*, *OsYUC9*, *OsRGB1*, *OsGH3-8*, *OsARF12*, and *OsIAA3*; the gibberellin-pathway genes *d18*, *sd1*, *SGD2*, *D1*, *EUI1*, and *GID1*; and the brassinosteroid-pathway genes *D11*, *D2*, *D4*, *BRI1*, *DLT*, and *BZR1*. The encoded products and functions of these genes are shown in Table 2. The results showed that, compared with WT, in mutant *dsg2* the expression levels of the auxin-biosynthesis-pathway flavin monooxygenase genes *YUC1* and

YUC9, the G protein β subunit gene *OsRGB1*, and the signaling response factor *OsARF12* did not change significantly; however, the expression levels of the auxin-degradation indole-3-acetic acid–amido synthetase gene *OsGH3-8* and the signaling repressor *OsIAA3* were significantly decreased (Fig. 4). In the GA signaling pathway, the expression levels of the GA oxidase gene *d18*, the key GA-degradation gene *EUI1*, the signaling-pathway regulatory gene *D1*, and the GA receptor *GID1* did not change significantly, whereas the expression levels of GA oxidase *sd1* and the HD-Zip transcription factor *SGD2* were significantly decreased (Fig. 5). In addition, in the brassinosteroid biosynthesis pathway, the expression levels of the cytochrome P450 genes *D2* and *D4* were significantly downregulated, whereas the expression level of the *D11* gene was significantly increased; meanwhile, the expression levels of the core transcription factors *BZR1* and *DLT* in the BR signal-transduction pathway were significantly increased, whereas its receptor ···

The expression of the receptor gene *BRI1* showed no significant change (Fig. 6). The above results indicate that, in the *dsg2* mutant, the expression of key genes in the growth hormone, gibberellin, and brassinosteroid signaling pathways all changed significantly, suggesting that loss of *DSG2* gene function is closely associated with an imbalance in the homeostasis of these three hormone pathways.

Table 2 Information of hormone pathway genes used for RT-qPCR analysis

Signaling pathway	Gene name	Locus No.	Coding product	Function and reference
Auxin pathway	*OsYUC1*	LOC_Os01g45760	Flavin monooxygenases	IAA biosynthesis (Yamamoto et al., 2007)
Auxin pathway	*OsYUC9*	LOC_Os01g16714	Flavin monooxygenases	IAA biosynthesis (Abu-Zaitoon et al., 2012)
Auxin pathway	*OsRGB1*	LOC_Os03g46650	G protein β subunit	IAA biosynthesis (Zhang et al., 2021)
Auxin pathway	*OsGH3-8*	LOC_Os07g40290	Indoleacetic acid-amido synthetase	IAA degradation (Ding et al., 2008)

Signaling pathway	Gene name	Locus No.	Coding product	Function and reference
Auxin pathway	*OsARF12*	LOC_Os04g57614	ARF transcription factor	IAA transcriptional activator (Qi et al., 2012)
Auxin pathway	*OsIAA3*	LOC_Os01g13030	Aux/IAA protein	IAA transcriptional repressor (Jain et al., 2006)
GA pathway	*d18*	LOC_Os01g08220	GA oxidase	GA biosynthesis (Itoh et al., 2001)
GA pathway	*sd1*	LOC_Os01g66100	GA oxidase	GA biosynthesis (Monna et al., 2002)
GA pathway	*SGD2*	LOC_Os01g45570	HD-Zip protein	GA biosynthesis (Chen et al., 2019)
GA pathway	*D1*	LOC_Osp5g26890	G protein α subunit	GA signaling (Seo et al., 1995)
GA pathway	*EUI1*	LOC_Os05g40384	Cytochrome P450	GA degradation (Zhu et al., 2006)
GA pathway	*GID1*	LOC_Os05g33730	GID protein	GA receptor (Ueguchi-Tanaka et al., 2005)
BR pathway	*D11*	LOC_Os04g39430	Cytochrome P45	BR biosynthesis (Tanabe et al., 2005)
BR pathway	*D2*	LOC_Os01g10040	Cytochrome P45	BR biosynthesis (Hong et al., 2003)

Signaling pathway	Gene name	Locus No.	Coding product	Function and reference
BR pathway	*D4*	LOC_Os03g12660	Cytochrome P45	BR biosynthesis (Sakamoto et al., 2006)
BR pathway	*BRI1*	LOC_Os01g52052	BR receptor kinase	BR receptor (Yamamuro et al., 2000)
BR pathway	*DLT*	LOC_Os06g03710	GRAS protein	BR signaling (Tong et al., 2009)
BR pathway	*BZR1*	LOC_Os07g39220	BZR protein	BR signaling (Bai et al., 2007)

A–F are the expression comparisons of *OsYUC1*, *OsYUC9*, *OsRGB1*, *OsGH3-8*, *OsARF12*, and *OsIAA3* in WT and mutant *dsg2* plants, respectively. * indicates the significance level ($P < 0.05$; Student' s *t*-test), the same below.

Fig. 4 Relative expression of IAA signaling pathway genes in WT and mutant *dsg2*

A–F are the comparisons of the expression levels of *d18*, *sd1*, *SGD2*, *D1*, *EUI1*, and *GID1* in WT and mutant *dsg2* plants, respectively.

Fig. 5 Relative expression of GA signaling pathway genes in WT and mutant *dsg2*

A–F are the comparisons of the expression levels of *D11*, *D2*, *D4*, *BRI1*, *DLT*, and *BZR1* in WT and mutant *dsg2* plants, respectively; $n = 3$.

Fig. 6 Relative expression of BR signaling pathway genes in WT and mutant *dsg2*

3 Discussion and Conclusion

This study identified a new rice dwarf small-grain mutant, *dsg2*, whose phenotype shows pleiotropic effects: in addition to reduced plant height and smaller grains, it is also accompanied by delayed heading, increased tiller number, and inhibited root growth. The decrease in plant height of *dsg2* was only 18.9%, indicating that it is a semi-dwarf mutant; meanwhile, grain length, grain width, and grain thickness were all significantly reduced. Compared with most reported dwarf small-grain mutants (Tanabe et al., 2005; Bai et al., 2007; Yang et al., 2011; Chen et al., 2019; Niu et al., 2022; Wang et al., 2022), *dsg2* has distinct characteristics dominated by changes in plant height and grain shape. Notably, compared with another dwarf small-grain mutant, *dsg1*, although the two are

relatively consistent in the direction of changes in plant height and grain shape, they show completely opposite trends in tiller and root phenotypes: the tiller number of *dsg1* is significantly reduced and root length shows no significant change, whereas *dsg2*

This indicates that the number of tillers increased significantly, whereas root length and root number both decreased significantly (Lü Zhaokun et al., 2020). These phenotypic differences suggest that, although *DSG2* and *DSG1* have similar regulatory effects on plant height and grain shape, they may not be allelic genes and may act in different developmental regulatory modules. This functional differentiation provides valuable genetic material for further dissecting node-specific features of the rice plant-type regulatory network.

To explore the hormonal regulatory basis underlying the *dsg2* mutant phenotype, this study analyzed changes in the expression of genes related to the Auxin, GA, and BR pathways. In the GA pathway, the expression of the biosynthetic genes *sd1* and *SGD2* was significantly downregulated in *dsg2*, which is highly consistent with the phenotypes of dwarfing and smaller grains (Monna et al., 2002; Chen et al., 2019). The simultaneous repression of two nonredundant GA biosynthetic genes in the *dsg2* mutant suggests that *DSG2* may be located at an upstream node in the regulation of GA biosynthesis, rather than representing a simple downstream feedback response. However, the degree of dwarfing in *dsg2* (18.9%) is far lower than that in the typical *sd1* mutant (40%–50%), indicating that inhibition of the GA pathway contributes only partially, and that the involvement of other hormone pathways—especially BR—may have alleviated the excessive reduction in plant height. In contrast, the BR pathway exhibited a more complex regulatory pattern in *dsg2*: biosynthesis was partially inhibited and key signaling factors were aberrantly activated. Specifically, the BR biosynthetic genes *D2* and *D4* were repressed, whereas *D11* expression was upregulated. We speculate that loss of *DSG2* function causes dysregulation of upstream regulatory nodes in the BR biosynthetic pathway, leading to differential expression of *D2*, *D4*, and *D11* and thereby disrupting feedback regulation of BR biosynthesis (Hong et al., 2003; Tanabe et al., 2005; Sakamoto et al., 2006). Meanwhile, the transcriptional upregulation of the key positive regulators of BR signal transduction, *DLT* and *BZR1*, does not necessarily indicate enhanced BR signaling; rather, it may represent a compensatory transcriptional feedback initiated when plants perceive insufficient BR biosynthesis or impaired signal output. However, this compensation cannot effectively restore BR signal output, because the roots of the *dsg2* mutant are significantly shortened, and root growth is a sensitive indicator of BR signaling. This precisely indicates that the functional output of BR signaling is inhibited (Bai et al., 2007; Tong et al., 2009). In the auxin pathway, expression of the IAA-amido synthetase gene *OsGH3-8* was significantly downregulated, which theoretically would reduce the inactivation of free IAA and increase the level of active auxin (Ding et al., 2008). However, the overall growth of *dsg2* is markedly inhibited, indicating that the growth-promoting effects potentially produced by auxin may be masked by the severe defects in the GA and BR pathways, or suggesting the existence of high-

level feedback regulation that prevents effective auxin signal output. Taken together, *DSG2* may integrate multiple hormonal signaling pathways, including Auxin, GA, and BR, and coordinately regulate plant height and grain shape in rice by maintaining the dynamic balance among multiple hormones. This multi-hormone signaling regulatory model has been validated in our previous study of the transcription factor *SHI1*, which precisely shapes rice plant type by coordinating multiple signaling pathways including ABA, BR, and IAA (Duan et al., 2023).

Nevertheless, it should be noted that although this study detected significant changes in the expression of genes from multiple hormonal pathways in the *dsg2* mutant by RT-qPCR, these changes may partly arise from secondary feedback effects caused by macroscopic phenotypes such as plant dwarfing and smaller grains, rather than being direct regulatory consequences of the *DSG2* gene. Therefore, we infer that loss of *DSG2* function is closely associated with disruption of the homeostasis of these hormonal signals; whether it is a direct "integrator" of these hormonal signals still requires more direct biochemical evidence. Subsequent map-based cloning and functional dissection of *DSG2* will be conducted to further clarify its precise site of action in the hormonal regulatory network, providing theoretical support and genetic resources for the coordinated improvement of plant type and grain shape in rice.

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Note: Figure translations are in progress. See original paper for figures.