GW10, a Member of P450 Subfamily Regulates Grain Size and Grain Number in Rice

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GW10, a member of P450 Subfamily regulates grain size and grain number in rice

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Abstract

Grain size and grain number play extremely important roles in rice grain yield. Here, we identify *GW10*, which encodes a P450 subfamily protein and controlls grain size and grain number by using Lemont (*tropical japonica*) as donor parent and HJX74 (*indica*) as recipient parent. The *GW10* locus was mapped into a 20.1 kb region on the long arm of Chromosome 10. Lower expression of the *gw10* in panicle is contributed to the shorter and narrower rice grain, and the increased number of grains per panicle. Furthermore, the higher expression levels of some of the brassinosteroid (BR) biosynthesis and response genes are associated with the NIL-*GW10*, which strongly suggests that the *GW10* is a key node in the brassinosteroid-mediated regulation of rice grain shape and grain number.

Key words

Rice, *GW10*, SSSL, Grain size, Grain number, BR

Abbreviations

cM Centimorgan QTL Quantitative trait loci SSR Simple sequence repeats SSSL Single segment substitution line

Declarations

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Author contribution statement

SW and GZ designed and supervised works. PZ performed most of the experiments, analyzed experimental data and prepared the draft of manuscript. XW, XW, ZX,SM, SL, and FL conducted a part of experiments. SB, ZL, HZ and GL developed the materials. SW wrote the paper. All authors read and approved the final manuscript.

Key message

A quantitative trait locus *GW10* is located on Chromosome 10 by map-based cloning, which encodes a P450 Subfamily protein. The *GW10* regulates grain size and grain number in rice involved in the BR pathway.

Introduction

Rice (*Oryza sativa* L.) is one of the most crucial cereal foods in the world, which provides over 21% calorie for the world population and over 76% calorie for the Asians, and it has been deeply ingrained in their daily lives (Fitzgerald et al. 2009). Grain yield controlling factors include grain weight, grain number per panicle, and panicle number per plant (Zuo et al. 2014). Grain size plays a key role in grain weight, and it is one of the most frequently selected traits during domestication and breeding (Meyer et al. 2013).

Grain weight and grain size are controlled mainly by the grain length, grain width and grain thickness. A number of major quantitative trait loci (QTLs) controlling grain size have been successfully cloned and described. Grain size could be controlled by transcriptional regulators including *GW8* (Wang et al. 2012), *GLW7* (Si et al. 2016), *GS2* (Hu et al. 2015), *GL4* (Wu et al. 2017), *MADS1* (Yu et al. 2018), *GS9* (Zhao et al. 2018) and *GW6a* (Song et al. 2015; Li et al. 2019). Previously, some major QTLs such as *TGW3* (Hu et al. 2018), *GS5* (Li et al. 2011), *GW5*（Liu et al. 2017） *TGW6* (Ishimaru et al., 2013),

GW6 (Shi et al., 2020) and *GS9* (Zhao et al. 2018) were proved to change grain size by phytohormone signals. In the auxin signaling pathway, *TGW6* encodes an indole-3-acetic acid (IAA)-glucose hydrolase activity protein and negatively regulates grain size. TGW3 protein interacts with OsARF4 (AUXIN RESPONSE FACTOR 4) repressing downstream auxin response genes' expression (Ishimaru et al. 2013; Hu et al. 2018). *GW6* encodes a GA-regulated GAST family protein and positively regulates grain width (Shi et al. 2020). The other three major QTLs of grain size, *GS3*, *GW2* and *GL3.1*, are involved in G-protein signaling pathway, ubiquitin-proteasome pathway and cell cycle regulation, respectively (Song et al. 2007; Mao et al. 2010; Qi et al. 2012). In the BR signaling pathway, GS5 binds to OsBAK1−7 affecting BR signaling and grain size (Li et al. 2011). GW5 protein represses GSK2 activity resulting in the altered expression of BR-response genes (Liu et al. 2017). GSK2 influences BR signaling pathway by the phosphorylates OsBZR1 and DLT (Tong et al. 2012). GS9 interacts with OsOFP14 and OsOFP8, whereas OsGSK2 interacts and phosphorylates OsOFP8 affecting the BR signaling pathway (Zhao et al. 2018).

The cytochrome P450 family is one of the biggest protein families in plants. In several sequenced angiosperms, the CYP genes constitute up to 1% of the total gene annotations of each plant species (Nelson et al. 2004). Several P450 genes have been studied to participate in biosynthesis and catabolism of phytohormones (Mizutani 2010). *ELL1* encodes a cytochrome P450 monooxygenase, and influences chlorophyll contents and ROS accumulation (Cui et al. 2021). CYP701A and CYP88A regulates Gibberellins (GAs) biosynthesis to influence seed germination and shoot growth in *Arabidopsis* (Helliwell et al. 1998; Helliwell et al. 2001). Some P450 genes regulates Brassinosteroids (BRs) biosynthesis to influence plant growth development and regulates Strigolactones (SLs) biosynthesis to influence seed germination of root parasitic plants (Mizutani 2012). In rice, *DWARF11* (*D11*)/ *CPB1* /*GNS4*, encoding cytochrome P450 proteins are involved in grain size control and brassinosteroid (BR) biosynthesis pathway (Tanabe et al. 2005; Wu et al. 2016; Zhou et al. 2017).

In this study, we identified a P450 subfamily gene, named as *GW10* (Grain Width gene on Chromosome 10), which controlled grain size and grain number. The near-isogenic line *gw10* (NIL-*gw10*) produces more grain number per panicle than that of the NIL-*GW10* line, while the grain size of the NIL-*gw10* is smaller than that of the NIL-*GW10*. The expression levels of the BR biosynthesis and response genes, such as *D2*, *DWARF*, *CPD*, *BZR1* and *DLT*, are higher in the NIL- *GW10* than those in the NIL-*gw10*, which suggests that *GW10* might be involved in the BR signaling pathway to regulate grain size

and grain number in rice. The *GW10* might be a potential target for rice breeding by design.

Materials and methods

Plant materials and growth conditions

We have developed a library with 2360 SSSLs by using seven AA genome *Oryza* species as donor parent and Huajingxian74 (HJX74), an elite *xian* (*indica*) cultivar from South China as recipient parent. Each of the SSSLs carries only one substitution segment from donor parents on the HJX74 genetic background (Zhang 2019). We generated a SSSL carrying $qgw10^{Lemont}$ (NIL-*gw10*) which was derived from the Lemont×HJX74 F_1 hybrid with an additional five times backcrossing to HJX74 in the SSSL library. The NIL-*gw10* carried an substitution segment from Lemont. The segment was determined between marker RM258-RM147 on chromosome 10. All of the plant materials were planted in paddy field in South China Agriculture University, Guangzhou, China (23°07′ N, 113°15′ E). Seedings were transplanted in paddy field after about 25 days, and each line was grown in a single row of ten plants (Fang et al., 2019).

Genetic analysis and fine mapping of the *GW10*

We developed F_2 population from the cross between NIL-*GW10* (HJX74) and NIL-*gw10*. We selected polymorphic simple sequence repeat (SSR) markers between these two NILs according to the rice linkage map (http://www.gramene.org). A series of Indel and SNP polymorphic markers which were designed according to the sequence variations between the *japonica* cultivar Nipponbare (https://rapdb.dna.affrc.go.jp) and HJX74 (http://192.168.87.153/) were used for *GW10* fine mapping (Li et al., 2021) (Table S1). The linkage analysis between the markers and the grain width locus for the F_2 population mapped the *GW10* gene to a chromosome 10 region flanked by polymorphic markers.

Genomic DNA extraction and PCR analysis

The fresh young leaves of individual rice were collected into 2 ml centrifuge tube and then were grounded in liquid nitrogen. The solution used in the experiment has been reported previously (Chen et al., 2015). The PCR program for the initial denaturing step was at 94℃ for 5 min, followed by 38 cycles for 30 s at 94℃, 30 s at 55℃, 45 s at 72℃, with a final extension at 72℃ for 5 min. The 6% nondenaturing polyacrylamide gel was used for separating PCR products and the genomic DNA polumorphic analysis was carried out by the silver staining method.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from young panicles of NIL-*GW10* and NIL-*gw10* by using TRIZOL

reagent (Invitrogen) following the manufacturer's instruction. First-strand cDNA was reverse transcribed from 5× All-In-One RT MasterMix (with AccuRT Genimic DNA Removal Kit), which was implemented for detected gene semi-quantitative/quantitative analysis with specific primers. Gene transcript levels were measured by qRT-PCR using the ABI 7500 real-time PCR system while actin gene was used as internal control. Each qRT-PCR program was performed in total volume of 20 μl schema containing 1×SYBR Green Master Mix. Each experiment was repeated three times, and the relative quantitative method was used to evaluate quantitative variation. The qRT-PCR reaction program was directed at 94℃ for 10 min, followed by 40 cycles at 94℃ for 30 s, 60℃ for 1min. Primers for experiment were referred to the Table S2.

Gene cloning and sequence analysis

Candidate genes were cloned by the gene specific primers, and the primers were produced by using HJX74 genome sequence as reference. The KOD FX DNA polymerase (TOYOBO, Japan) was employed in the PCR assay. The information of ORFs (Open Reading Frames) and its corresponding predicted protein sequence was obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The multiple DNA and protein sequences of candidate genes in NIL-*GW10* and NIL-*gw10* were aligned by software DNAman. The phylogenetic analysis was presented by MAGA6 based on the Neighbor-Joining (NJ) model and the bootstrap values were estimated with 1000 replicates.

Vector construction and rice transformation

To generate the Cas9 vector for *GW10*, two targets were designed in the ORF of candidate gene. The intermediate SK-gRNA vector was constructed using the isocaudamer ligation method. The SK-gRNA-target1 and SK-gRNA-target2 were digested with *Kpn*I/*Nhe*I and *Xba*I/*Bgl*II, respectively. The digested products were gel purified and ligated into pC1300-Cas9 binary vector (digested with *Kpn*I/*Bam*HI). The vector was transformed into *Agrobacterium tumefaciens* EHA105 cells, and resulting strains were implemented to transform the callus of HJX74 (Hu et al., 2018).

Agronomic traits evaluation and statistical analysis

Agronomic traits were evaluated in the different period of plant growth. Plant height and number of effective tillers were measured at the maturity stage. Heading date was calculated at the time when the first panicle sprouting. Meanwhile, panicle length, primary branch, secondary branch, 1000-grain weight, grain length and grain weight were investigated after the rice harvest at the maturity stage. Grain length and grain weight were measured by Microtek ScanWizard EZ scanner V-2.140 and Wan Shen grain

analyzer software. Each set of groups was recorded about ten plants. All the data was analyzed using IBM SPASS statistic 20. The significance was accepted at $P < 0.05$ and $P < 0.01$.

Results

Comparison of grain and panicle traits of NIL-*GW10* and NIL-*gw10*

Grain size and grain number per panicle play extremely important roles in rice grain yield (Li et al. 2019). We constructed a set of SSSLs by crossing Lemont (donor parent) and HJX74 (recipient parent). Each of the SSSLs carries only one substitution segment from the Lemont on the genetics background of HJX74. Then QTL analysis showed that the presene of a minor grain size locus *qGW10* on the long arm of chromosome 10. The near-isogenic line *gw10* which was drived from Lemont produced significantly shorter grain length than that of NIL-*GW10* (namely HJX74) (Fig. 1c and d) and the plant of NIL-*gw10* is slightly higher than that of the NIL-*GW10* (Fig. 1a). The grain width of NIL-*gw10* was also markedly narrower than that of NIL-*GW10* and the 1000-grain weight decreased accordingly in NIL-*gw10* (Fig. 1c,e and f). In addition, plant height (Fig. 2b) and panicle length (Fig. 2d) of NIL-*gw10* were markedly higher and longer than that of NIL-*GW10*. There were no difference between the two NILs in the heading date (Fig. 2a) and number of tillers (Fig. 2c). Moreover, the secondary branch per panicle of NIL-*gw10* were much more than that of NIL-*GW10*, which resulted in an increased number of grains per plant (Fig. 2e and f). These results indicated that the introgressed substitution segment from Lemont in HJX74 contributed to the decrease in grain length and grian width, meanwhile, to the increase in the grain number per panicle.

Genetic analysis and mapping of *GW10*

To study the genetic factor for *qGW10,* we crossed NIL-*GW10* with NIL-*gw10* to generate F_2 population. The grain width was used as a target trait. The genotype and phenotype of F_2 population conformed to a segregation ratio 1:2:1 ($\chi^2 = 0.11 < \chi^2_{0.01,2} = 9.21$). The inheritance patterns of the F_2 plants indicated that a semidominant *qGW10* allele from HJX74 controlled grain size (Fig. 1b).

A 3200 F_2 segregants was bred from the cross between the NIL-*gw10* and HJX74. A subsequent high resolution map was constructed on the basis of the F_2 population. The region of *qGW10* was narrowed down to a 20.1 kb flanked by marker Z4 and Z5 on chromosome 10 (Fig.3a and b). The segment contains only 1 predicted open reading frames (*ORF1*) and is very close to the start codon of *ORF2* according to the HJX74 genome(Fig. 3c). The *ORF1* and *ORF2* correspond to *Os10g0515400* and *Os10g0515900* in Nipponbare respectively. The expression profiles of *ORF1* in various tissues of NIL-

GW10 and NIL-*gw10* were tested by RT-PCR analysis. No expression of *ORF2* was detected at all the tissues (Fig. S1b), while the qPCR analysis indicated that *ORF1* was expressed in root, stem, leaf and developing panicles (Fig. S1a). The expression was significantly reduced in the young panicle of the NIL-*gw10* compared to that of the NIL-*GW10* especially in the panicles of 6 cm in length (Fig. 3d). It is strongly suggested *ORF1* is the candidate gene for *GW10*. The *ORF1*(*Os10g0515400*) encodes a P450 subfamily protein CYP89A2 (Fig. S2). The sequence comparison of *GW10* in Lemont and HJX74 revealed four polymorphisms in the promoter region and two polymorphisms in the coding sequence. There is one synonymous polymorphism (C 792 G) and an in frame 3 bp Indel in the coding sequence. A 3326 bp Indel in the uptream of the coding region of *Os10g0515400* gene locus was probablely associated with the down regulation of the gene in NIL-*gw10* (Fig. 3c and Table S3). The role of *Os10g0515400* in rice development is not clear. Phylogenetic analysis of GW10 protein indicates that it is ubiquitous in the poaceae (Fig. S3), suggests that GW10 might play a vital role in the poaceae development.

_GW10_ controls grain size and grain number

We generated the *GW10* knockout transgenic plants by the CRISP/cas9 genome editing system in HJX74 and obtained homozygous transgenic T_2 plants (Fig. S4 and S5). The grain length and grain width were examined in the NIL-*GW10*, NIL-*gw10* and KO-*GW10* lines at the mature stage. The NIL-*GW10* line produced grains with length of 8.40 mm and width of 2.82 mm, while the NIL-*gw10* line produced grains with length of 7.93 mm and width of 2.72 mm. The KO-*GW10* line had a grain length of 7.71 mm and a grain width of 2.71 mm (Fig.4a, c and d). These results indicate that the grain size of KO-*GW10* line was significantly smaller than that of NIL-*GW10* and NIL-*gw10*. The 1000-grain weight of KO-*GW10* line decreased 5.5% and 14.6% compared to that of NIL-*gw10* and NIL-*GW10*, respectively (Fig. 4e). Interestingly, the number of grains per plant was obviously different in the three lines. The KO-*GW10* line had much more grain number compared to the NIL-*GW10* and NIL-*gw10* line (Fig.4b and f). The heading date, the number of tillers and the panicle length of KO-*GW10* were no difference compared to those of NIL-*GW10* (Fig. S5a, b, d and e). The plant height of KO-*GW10* was higher than that of NIL-*GW10* with $P = 0.03$ (Fig. S5c). These results indicate that the GW10 protein regulates grain size and grain numbers in rice.

_GW10_ involved in the BR biosynthesis pathway

The *GW10* encodes a cytochrome P450 subfamily 89A2 homology protein. In the previous reports,

a new allele of *DWARF2* (*D2*) , *smg11*, encoding a cytochrome P450 protein which controls grain size and grain number by involving in brassinosteroid (BR) biosynthesis pathway (Fang et al. 2016). Our meterials have similar phenotypes to those of the *smg11*. Therefore, the *GW10* might participate in the BR biosynthesis pathway. We examined the expression levels of BR biosynthetic and BR-signaling genes in the 0.2 cm young panicle. The expression levels of *D2*, *DWARF*, *CPD*, *BZR1* and *DLT* of NIL-*GW10* were markedly higher than those of NIL-*gw10*, while the expression levels of *BU1* and *GSK2* were significantly lower in NIL-*GW10* than those in NIL-*gw10*. The *BIR1* expression level was not altered in the NIL-*gw10* and NIL-*GW10* lines (Fig. 5a). The expression levels of *GS2* and *GS9* were no significant difference in the two NILs, but the *GW5* and *DEP2* expression were significantly different with $P < 0.01$ and $P < 0.05$ levels, respectively (Fig. 5b). These results indicated that the GW10 protein might regulate BR-signaling genes by influencing *GW5* expression.

Discussion

It was difficult to determine the precise location of QTLs in rice genome by using the F_2 plants, Recombinant Inbred Lines (RILs), Backcross Inbred Lines (BILs) and Doubled Haploid Lines (DHLs) (Ashikari et al. 2006). Consequently, the development of Nearly Isogenic Lines (NILs), Chromosome Fragment Substitution Line (CSSL) and Single Segment Substitution Line (SSSL) were necessary for QTL fine mapping and gene cloning (Ashikari et al. 2006; Guo et al. 2016; Zhou et al.2017; Wang et al. 2018; Yang et al. 2018; Luan et al. 2019; Tan et al. 2020; Tan et al. 2021), especially for the minor genes. The additive effect value of was *GW10* lower than some major genes for grain size, such as *gs3*, *GW5* and *GW7*. The SSSLs were excellent material for the cloning and functional analysis of the *GW10*. Many QTLs for grain size have been identified and distributed across nearly all the 12 chromosomes in rice. However, only a few QTLs for grain size were reported on chromosome 10 (Li et al. 2019). The *GW10* was located on the long arm of chromosome 10 and was incomplete dominant inheritance (Fig. 1b). The qPCR was performed to examine the expression level of *GW10* in various rice tissues, including roots, stems, leave and panicles in different growth stages. The *GW10* is constitutively transcribed in all tested tissues (Fig 3d and Fig S1a) The grain size, plant height, number of secondary branch and grain number per panicle were difference between the line of NIL-*GW10* and NIL-*gw10* (Fig. 1 and 2), which illustrated that the *GW10* was a pleiotropic gene in rice.

The *GW10* encodes a P450 Subfamily 89A2 homology protein. The cytochrome P450 family is one of the biggest protein families in plants and several P450 proteins were found that invoved in the

controlling of rice grain size. The *CPB1* which encoding a cytochrome P450 superfamily protein effected grain size and other agronomic traits in rice. The OE-*CPB1* transgenic plants showed increasing grain length while the RNAi-*CPB1* transgenic plants showed smaller grain size and semidwarf in stature (Wu et al. 2016; Shi et al. 2018). A small grain (*smg11*) mutant in rice exhibited more secondary panicle branches, small grains and increased number of grains per panicle (Fang et al. 2016). The *NBG4*, also reported as *Small grain 4* (Shi et al. 2015)/ *Dwarf 11* (Tanabe et al. 2005)/ *Clustered spokelets4* (Guo et al. 2014)/ *GNS4* (Zhou et al. 2017) encodes a cytochrome P450 (CYP724B1). A 10-bp deletion in the CDS resulted in the lower expression of *nbg4* than that of the NBG4 in ZH11 and the deletion was contributed to the grain shape (Tong et al. 2018). In this study, the lower expression level of *gw10* in young panicle was associated with the smaller grain size and the more grain number in NIL-*gw10* (Fig. 1 and Fig. 2f). Additionally, the grain size of the KO-*GW10* line was smaller than that of the NIL-*gw10* and of the NIL-*GW10* (Fig. 4a) while the grain number of the KO-*GW10* line was obviously increased comparing with NIL-*gw10* and NIL-*GW10* (Fig. 4b). It implied that the four variations in the promoter (Fig. 3c, Table S3) would be the key potential *cis*-regulators for the smaller grain size in NIL-*gw10*.

It was reported that the CYP724B1 could control grain size by affectting BR-related genes (Shi et al. 2015; Zhou et al. 2017). BRs regulate various aspects of plant development, including root development, anther and pollen development, stem elongation and cellulose biosynthesis in plants (Yang et al. 2011). Our study demonstrated that *GW10* is a key factor in the BRs mediated regulation of rice grain size (Fig 5a). *GSK2* is one of the orthologs of *BIN2* which plays crucial roles in the BR signaling pathway, and *GSK2* could inhibit the activities of *OsBZR1* and *DLT* by phosphorylating the two proteins (Tong et al. 2012). *BU1* protein acts as a positive regulator of the BR pathway and participates in BR signaling pathways through *OsBRI1* and *RGA1* (Tanaka et al. 2009). *GW5* could physically interact with *GSK2* and inhibit its kinase activities toward *BZR1* and *DLT* (Liu et al. 2017). In NIL-*gw10*, the expression of *GW5* was significantly higher than that of NIL-*GW10*. Beside, a series of *GSK2* downstream genes including of *BZR1*, *DLT*, *D2*, *Dwarf* and *CPD* expression was significantly down regulated (Fig. 5a), which implies *GW10* induces the decline of *GSK2* expression by influencing *GW5*. *DEP2* mainly affects the rapid elongation of rachis and primary and secondary branches (Li et al. 2010) . The expression level of *DEP2* is higher in NIL-*gw10* than in NIL-*GW10* , which suggests that *GW10* should involve in the regulatory of *DEP2* for grain size and secondary branch (Fig. 5b). The rice grain size is regulated by a complicated network. Although recent studies have identified some key grain size

genes and molecular pathways, the complete regulatory network for grain size is poorly understood (Li et al. 2019). Our work provides a piece of this complex puzzle and make a contribution to the enrichment of breeding germplasm resources bases.

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Figure legends

Fig. 1 Morphological characteristics of NIL-*GW10* and NIL-*gw10*. **a.** The morphology of the NIL-*GW10* and NIL-*gw10* plants. Scale bar, 15 cm. **b.** Frequency distribution of grain width in F_2 population. **c.** Grain shape of NIL-*GW10* and NIL-*gw10*. Scale bar, 5 mm. **d.** Grain length ($n \geq 100$). **e.** Grain width ($n \geq 100$). **f.** 1000-grain weight, 500 grains was weighted by electronic balance and the data was converted into 1000-grain weight, 3 repeats. Data in **d-f** are given as means ± SD. Student's *t*-test were employed for the evaluation of *P* value. $*P \leq 0.05$,$**P \leq 0.01$.

Fig. 2 The agronomic traits of NIL-*GW10* and NIL-*gw10*. **a.** Heading date. **b.** Plant height. **c.** Number of effective tiller. **d.** Panicle length. **e.** Secondary branches per panicle. **f.** Number of grains per plant. 2019ES, the early season in 2019; 2019LS, the late season in 2019. Values are means ± SD. $*P \leq 0.05$ $**P \leq 0.01$.

Fig. 3 Fine-mapping of *GW10*. **a.** The substitution segment on chromosome 10. **b.** *qGW10* was mapped to 20.1 kb region between marker Z4 and Z5 using 3,200 BC_5F_3 plants. The number above the line indicate the number of recombinants between each of the two adjacent markers. Black filled and open bars represent substitution segments homozygous for the Lemont and HJX74 alleles, respectively. Grain width is shown for recombinant plants (I-IV) and NILs. Data are shown as means ± SD (n≥ 100). **c.** Allelic variation in the region of candicate gene *ORF1* (*Os10g0515400*) between Lemont and HJX74. **d.** Expression of *ORF1* in NIL-*GW10* and NIL-*gw10*. 0.2 cm -8 cm, young panicles and the number showed the length of the panicle (cm).

Fig. 4 The effects of *GW10* on grain size and grain number. **a**. The grains from NIL-*GW10*, NIL-*gw10* and target-gene edited NIL-*GW10* (KO-*GW10*). Scale bar, 5 mm. **b** The grains from NIL-*GW10*, NIL-*gw10* and KO-*GW10*. Scale bar, 5 cm. **c**. Grain length (n≥ 100). **d**. Grain width (n≥ 100). **e**. 1000-grain weight, 3 repeats. **f**. number of per plants (n≥ 20). Values shown in **c-f** are means ± SD. a, b, c $P \leq 0.05$.

Fig. 5 Transcript levels of BR responsive and BR-related grain size genes in young panicle. **a** BR responsive genes. **b.** BR-related grain size genes. Expression is shown relative to that of NIL-*GW10* plants, which was set to 1. Data are shown as means ± SE. *P* values from the *t*-test were indicated, $*P \leq 0.05$, $**P \leq 0.01$.

Figures

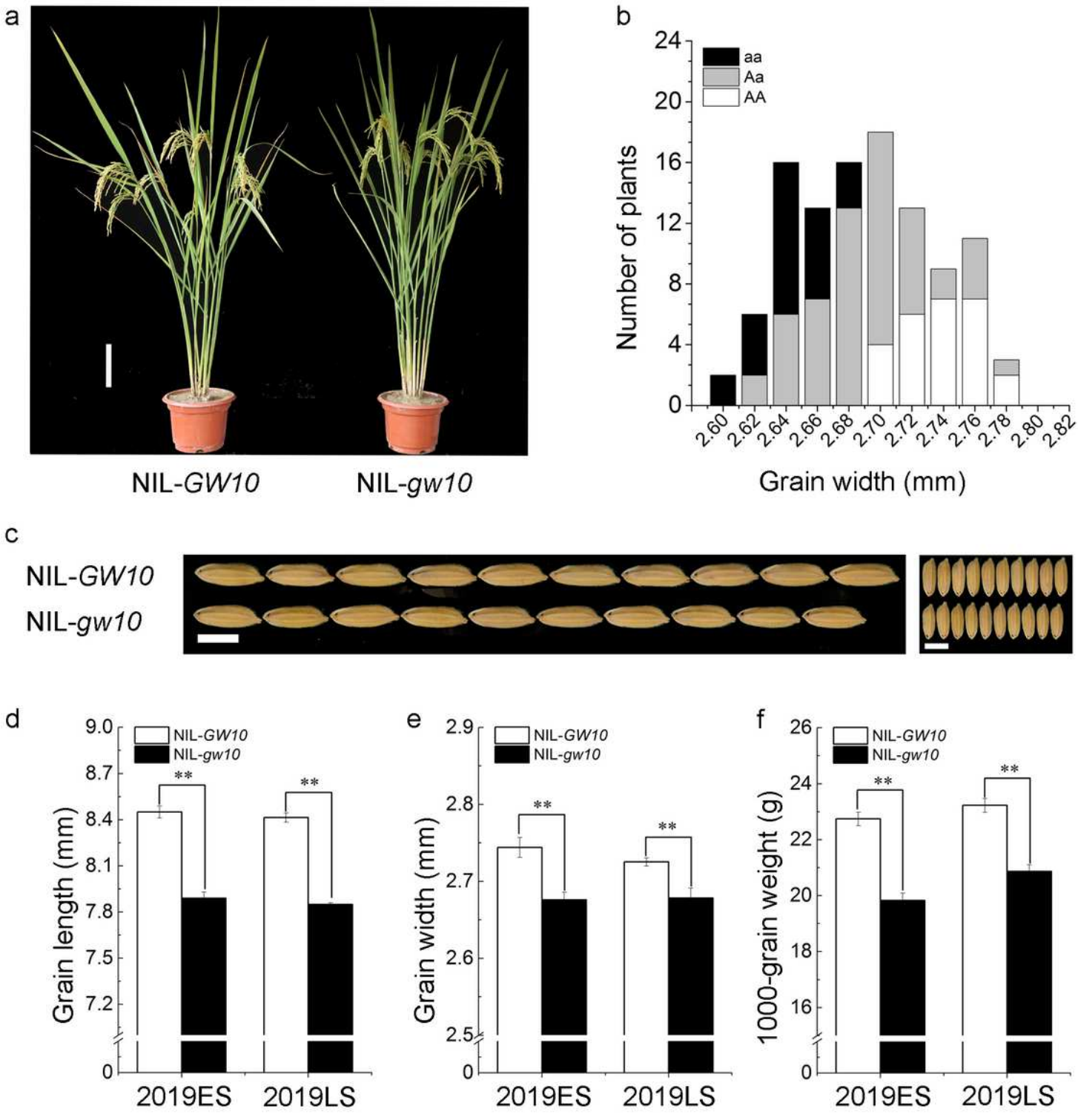

Figure 1

Morphological characteristics of NIL-GW10 and NIL-gw10. a. The morphology of the NIL-GW10 and NIL-gw10 plants. Scale bar, 15 cm. b. Frequency distribution of grain width in F2 population. c. Grain shape of NIL-GW10 and NIL-gw10. Scale bar, 5 mm. d. Grain length (n ≥ 100). e. Grain width (n ≥ 100). f. 1000-

grain weight, 500 grains was weighted by electronic balance and the data was converted into 1000-grain weight, 3 repeats. Data in d-f are given as means ± SD. Student's t-test were employed for the evaluation of P value. *P ≤ 0.05 ,**P ≤ 0.01.

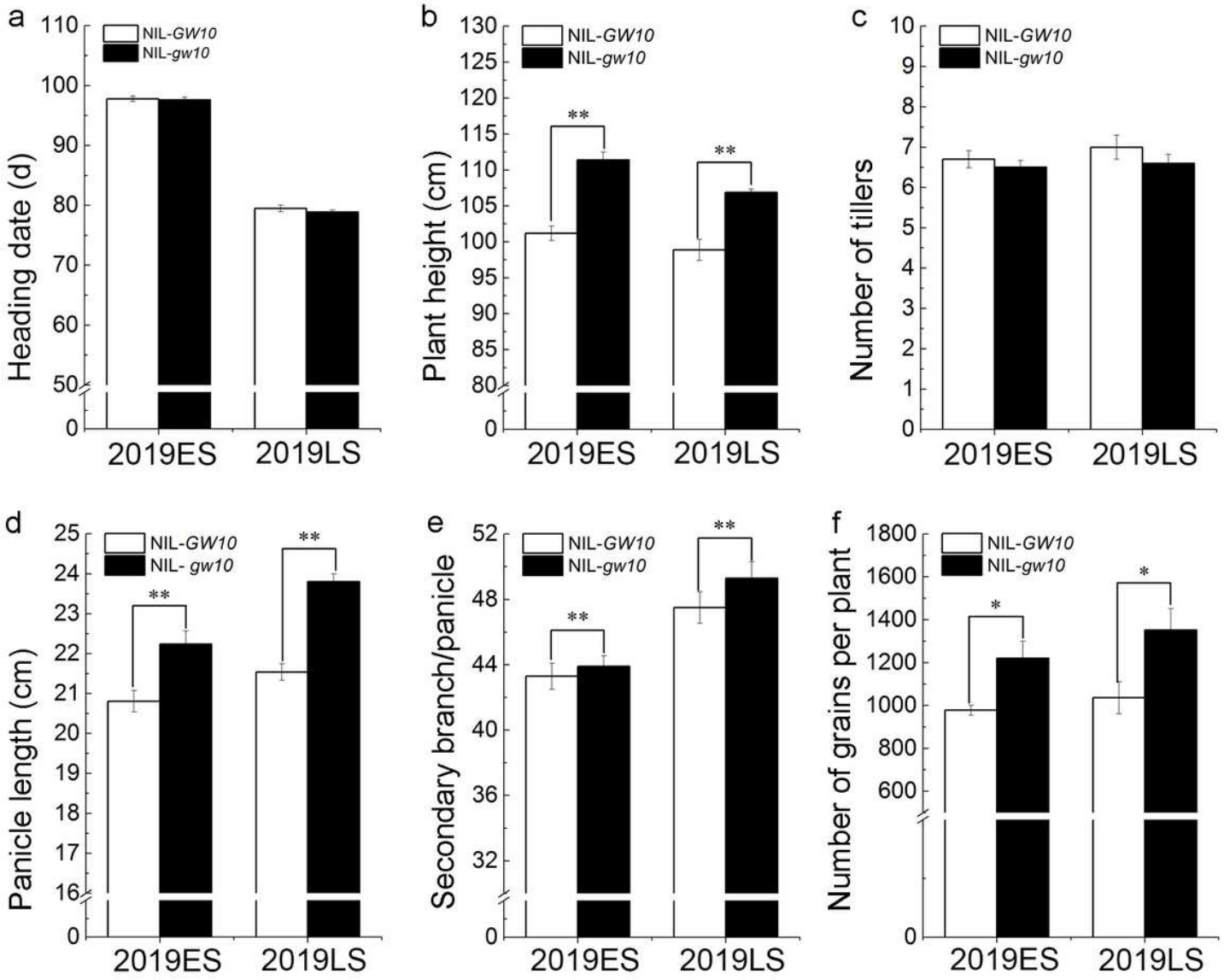

Figure 2

The agronomic traits of NIL-GW10 and NIL-gw10. a. Heading date. b. Plant height. c. Number of effective tiller. d. Panicle length. e. Secondary branches per panicle. f. Number of grains per plant. 2019ES, the early season in 2019; 2019LS, the late season in 2019. Values are means ± SD. *P ≤ 0.05 **P ≤ 0.01.

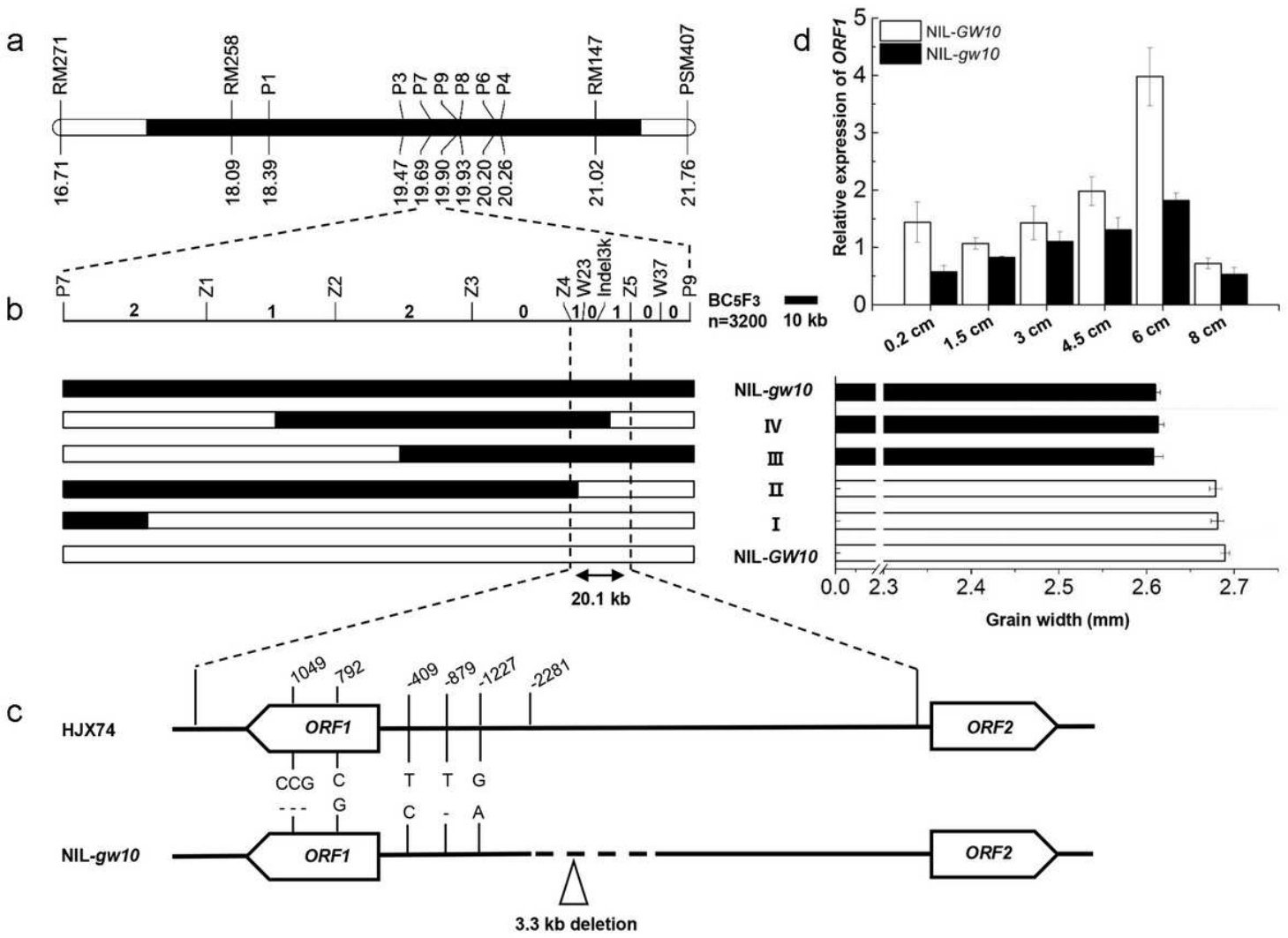

Figure 3

Fine-mapping of GW10. a. The substitution segment on chromosome 10. b. qGW10 was mapped to 20.1 kb region between marker Z4 and Z5 using 3,200 BC5F3 plants. The number above the line indicate the number of recombinants between each of the two adjacent markers. Black filled and open bars represent substitution segments homozygous for the Lemont and HJX74 alleles, respectively. Grain width is shown for recombinant plants (I-IV) and NILs. Data are shown as means ± SD (n≥ 100). c. Allelic variation in the region of candicate gene ORF1 (Os10g0515400) between Lemont and HJX74. d. Expression of ORF1 in NIL-GW10 and NIL-gw10. 0.2 cm -8 cm, young panicles and the number showed the length of the panicle (cm).

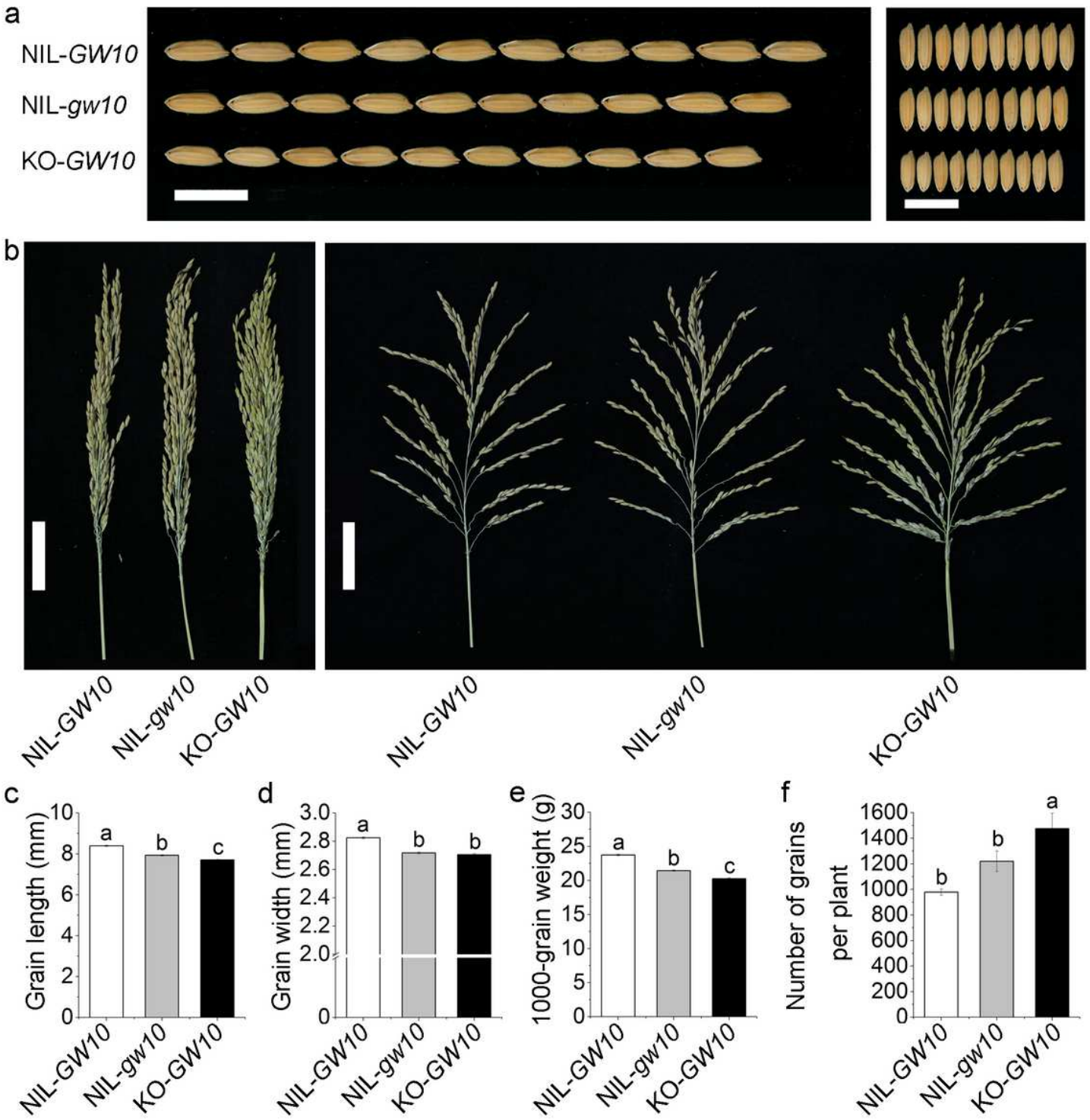

Figure 4

The effects of GW10 on grain size and grain number. a. The grains from NIL-GW10, NIL-gw10 and target-gene edited NIL-GW10 (KO-GW10). Scale bar, 5 mm. b The grains from NIL-GW10, NIL-gw10 and KO-GW10. Scale bar, 5 cm. c. Grain length (n≥ 100). d. Grain width (n≥ 100). e. 1000-grain weight, 3 repeats. f. number of per plants (n≥ 20). Values shown in c-f are means ± SD. a, b, c $P \leq 0.05$.

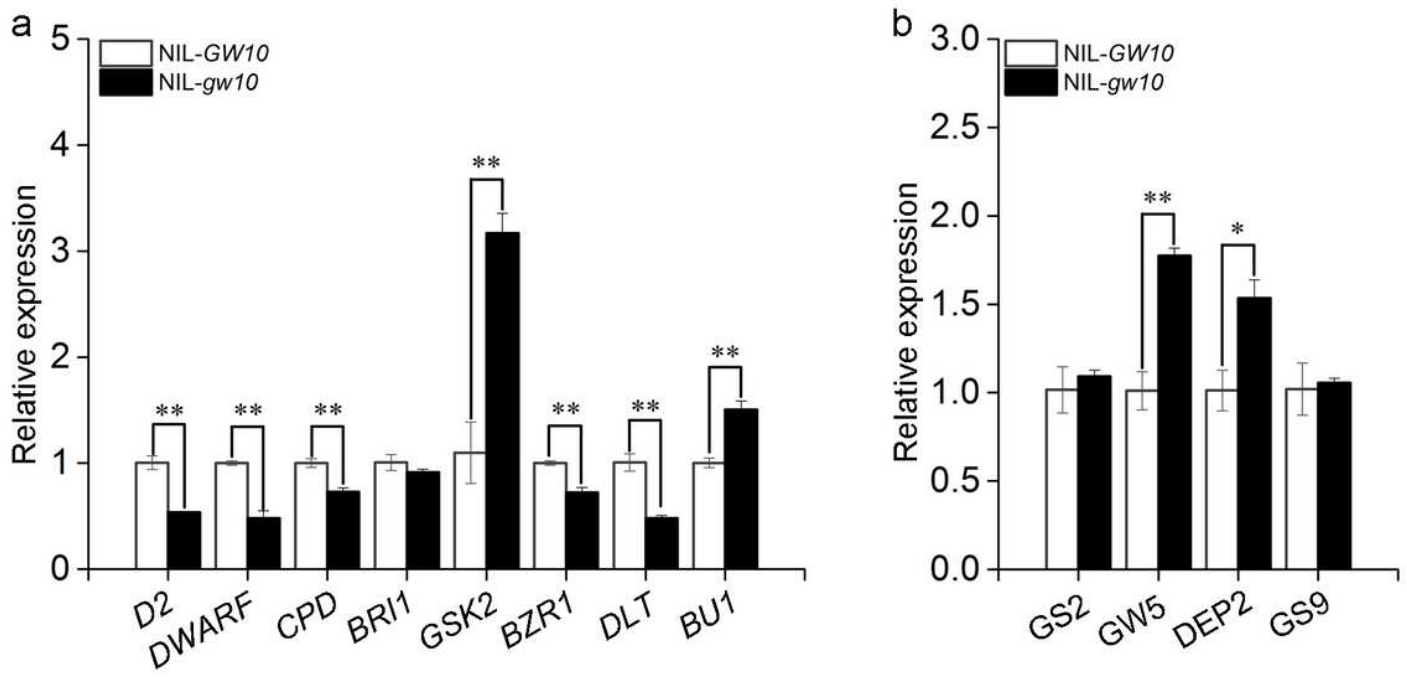

Figure 5

Transcript levels of BR responsive and BR-related grain size genes in young panicle. a BR responsive genes. b. BR-related grain size genes. Expression is shown relative to that of NIL-GW10 plants, which was set to 1. Data are shown as means ± SE. P values from the t-test were indicated, *P ≤ 0.05, **P ≤ 0.01

Supplementary Files

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- Supplementarydata1.pdf