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Combined genome-wide association study and epistasis analysis reveal multifaceted genetic architectures of plant height in Asian cultivated rice

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Abstract

Plant height (PH) in rice (*Oryza sativa*) is an important trait for its adaptation and agricultural performance. Discovery of the *semi-dwarf1* (*SD1*) mutation initiated the Green Revolution, boosting rice yield and fitness, but the underlying genetic regulation of PH in rice remains largely unknown. Here, we performed genome-wide association study (GWAS) and identified 12 non-repetitive QTL/genes regulating PH variation in 619 Asian cultivated rice accessions. One of these was an *SD1* structural variant, not normally detected in standard GWAS analyses. Given the strong effect of *SD1* on PH, we also divided 619 accessions into subgroups harbouring distinct *SD1* haplotypes, and found a further 85 QTL/genes for PH, revealing genetic heterogeneity that may be missed by analysing a broad, diverse population. Moreover, we uncovered two epistatic interaction networks of PH-associated QTL/genes in the *japonica* (*Geng*)-dominant $SD1^{NIP}$ subgroup. In one of them, the hub QTL/gene *qphSN1.4/GAMYB* interacted with *qphSN3.1/OsINO80*, *qphSN3.4/HD16/EL1*, *qphSN6.2/LOC_Os06g11130*, and *qphSN10.2/MADS56*. Sequence variations in *GAMYB* and *MADS56* were associated with their expression levels and PH variations, and MADS56 was shown to physically interact with MADS57 to coregulate expression of gibberellin (GA) metabolic genes *OsGA2ox3* and *Elongated Uppermost Internode1* (*EUI1*). Our study uncovered the multifaceted genetic architectures of rice PH, and provided novel and abundant genetic resources for breeding semi-dwarf rice and new candidates for further mechanistic studies on regulation of PH in rice.

KEYWORDS

epistatic interaction, GAMYB, genetic heterogeneity, MADS56/57, *Oryza sativa*, semi-dwarf1 (SD1)

Jun Hong and Su Su contributed equally to this work.

1 | INTRODUCTION

Plant height (PH) is primarily determined by internode elongation (Guo et al., 2020). Extensive genetic studies have revealed that the internode elongation rate changes during development; it is usually suppressed during early vegetative growth but significantly increases after the developmental transition from vegetative to reproductive growth. Rice, for example, elongates its uppermost internode dramatically at heading to prompt panicle exsertion from the flag leaf sheath (Iwamoto et al., 2010). Due to the importance of flowering/heading at the optimal time and height, elaborate regulatory mechanisms have evolved to coordinate the activities of related meristems during inflorescence development; however, the genetic networks governing this regulation remain elusive (McKim, 2020). PH is also affected by external environmental factors, such as temperature, photoperiod, and moisture and coordinates with other ecological traits to tailor plant architecture to survive in competitive environments (Guo et al., 2020).

In rice, gibberellins (GAs) promote internode elongation by activating cell division and elongation (Nagai et al., 2020). Mutational studies have revealed the roles of genes involving in GA homoeostasis (anabolism and catabolism) and signalling in rice PH regulation (Sakamoto et al., 2004; S. Wang & Wang, 2022). Defects in GA biosynthesis led to different dwarf phenotypes: mutants of *ent-copalyl diphosphate synthase1* (*OsCPS1*), *ent-kaurene synthase1* (*OsKS1*), *ent-kaurene oxidase2* (*OsKO2*) and *ent-kaurenoic acid oxidase* (*OsKAO*) are severe dwarfs without flower or seed development; mutants of *OsGA3ox2/D18* are severe dwarfs with seed development; while mutants of *OsGA20ox2/Semi-Dwarf1* (*SD1*) are semi-dwarf with seed development (Sakamoto et al., 2004). Mutations in GA degradation cause different PH phenotypes: mutants of *ELONGATED UPPERMOST INTERNODE1* (*EUI1*) are extremely tall (Zhu et al., 2006); mutants of *Shortened Basal Internodes* (*SBI*) are semi-dwarf due to reduced elongation of the basal internodes (C. Liu, Zheng, et al., 2018); while mutants of *OsGA2ox6* exhibit a range of PH effects (Lo et al., 2017). Mutations in GA reception and signalling also cause dwarfism: GA-INSENSITIVE DWARF1 (GID1) is a GA receptor in rice whose mutation results in severe dwarfs (Ueguchi-Tanaka et al., 2005); SLENDER RICE 1 (SLR1) is a DELLA protein that negatively regulate GA response by repressing GA-responsive genes such as *GAMYB* (Aya et al., 2009), and whose mutants are semi-dominant dwarfs (Asano et al., 2009; Z. Wu et al., 2018).

Many transcription factors (TF) and proteins regulate rice PH through transcriptional or posttranscriptional regulation of GA homoeostasis and signalling genes. The MADS-box TF OsMADS57 regulates expression of *EUI1* and *OsGA2ox3* (another GA catabolic gene) by directly binding to conserved CArG-box motifs in their promoter regions; *mads57* mutants are semi-dwarf (Chu et al., 2019). Early Flowering1 (EL1)/Heading Date16 (HD16), a casein kinase I, phosphorylates SLR1, and acts upstream of GAMYB (Aya et al., 2009); *el1* mutant plants are consistently taller, especially the uppermost internode (Dai & Xue, 2010).

Appropriate PH enhances yield, harvest uniformity and index, lodging resistance and crowding tolerance in rice (F. Liu, Wang, et al., 2018; Q. Liu et al., 2022). The discovery of the 'Green Revolution' gene *SD1* from dwarfing landrace Dee-geo-woo-gen and subsequent development of the 'miracle' variety IR8 significantly increased yield production and revolutionised rice breeding in the last century. Both Dee-geo-woo-gen and IR8 contain a 383 base-pair (bp) deletion across the first two exons of *SD1*, which creates a premature stop codon and causes semi-dwarf growth (Sasaki et al., 2002). Despite the successful characterisation of many GA homoeostasis and signalling genes in PH regulation, most also have detrimental effects on other agronomic traits, for example, small grain, excessive tillering and low seed setting rate (Z. Hong et al., 2005; Ji et al., 2019). As a result, only *SD1* has been successfully used in breeding programmes to generate semi-dwarf rice (Q. Liu et al., 2022; S. Wang & Wang, 2022). However, excessive utilisation of this one *sd1* allele may decrease genetic diversity and increase susceptibility to diseases and pests (Kadambari et al., 2018), so it is necessary to explore novel alleles or allelic combinations underlying PH variation to innovate and safeguard rice breeding.

Rice PH is a complex quantitative trait with a polytrophic nature (Su et al., 2021; Yano et al., 2019), so genome-wide association study (GWAS) struggles to dissect PH diversity due to spurious associations from strong population structures, high levels of linkage disequilibrium (LD) from self-pollination (Yano et al., 2016), and complex genetic architectures due to long selection for local adaptation (Lopez-Arboleda et al., 2021; Todaka et al., 2015). Although previous GWAS have reported several PH-associated genomic regions and single nucleotide polymorphisms (SNPs) (Kadambari et al., 2018; Ma et al., 2016; W. Yang et al., 2014), the genetic architecture of PH in rice remains largely unknown. Here, we use an elaborate GWAS design with an optimised population rich in geographical diversity, as well as smaller subgroups with fixed *SD1* alleles, to uncover the genetic architecture of PH in Asian cultivated rice.

2 | MATERIALS AND METHODS

2.1 | Plant materials and growth conditions

Population information for the 619 Asian cultivated rice (*Oryza sativa*) lines used in this study is shown in Supporting Information: Table S1. They were randomly selected from lines used in the 3000 Rice Genomes Project (3K RGP) (W. Wang et al., 2018).

All plants were grown in the experimental fields in Sanya (Hainan province, China; 31.03°N, 121.45°E) from January to April in 2015 and 2016 according to local cultivation practices. Plants were sown in a randomised complete block design (including two rows of each accession and eight plants in each row) (J. Hong et al., 2022). PH was measured using a ruler as the distance from ground to the top of the panicle at seed maturity (Yano et al., 2016). Four to six plants in the middle of each row were chosen for PH measurement, and the average across these eight–12 replicates was used for the further GWAS analysis.

2.2 | Data analysis

Two-way analysis of variance (ANOVA) was used to test significant differences between environments (two different seasons) and genotypes for PH variation across the 619 accessions (Supporting Information: Table S3). Pearson's correlations were used to examine the correlations between traits in 2015 and 2016. These statistical analyses were performed in the R software with script programming. Student's *t*-test was carried out using Microsoft Excel software.

2.3 | Genome-wide association study and two-locus epistasis interaction analysis

Two SNP genotype datasets – the 404k CoreSNP Data set and the 6.5 M filtSNP Data set (v0.4) – were downloaded from the Rice SNP-Seek database (https://snp-seek.irri.org/) (Alexandrov et al., 2015) and used for GWAS (J. Hong et al., 2022). In brief, the 3K RGP 6.5M filtSNP Dataset (v0.4) was used as association markers. After filtering out high missing rate sites (≥20%) and low minor allele frequency sites (≤0.05), the remaining SNPs were used for further analysis. Linear mixed model (LMM) was performed using Fast-LMM software (Lippert et al., 2011). Genetic relatedness was modelled as a random effect in LMM using the kinship matrix calculated from the 404k CoreSNP Dataset. The genome-wide significance threshold of the GWAS were determined using a modified Bonferroni correction, in which the total number of SNPs for threshold calculation was replaced by the effective number of independent SNPs in GWAS (M. X. Li et al., 2012). Graphical visualisation of *SD1* genotypes was done as previously described. (Yano et al., 2016).

Analysis of two-locus interactions was carried out between 10 selected significant lead SNPs identified by LMM. These 10 significant SNPs could be detected in both seasons and so were regarded as stable PH QTL. The two-locus epistatic interaction test was implemented in PLINK (Purcell et al., 2007).

2.4 | Linkage disequilibrium and haplotype analyses

Linkage disequilibrium (LD) was investigated based on standardised disequilibrium coefficients (D′), and squared allele-frequency correlations (r^2) for pairs of SNP loci or SNP – SV pairs. LD was determined and visualised using Haploview (Barrett et al., 2005).

For haplotype comparisons, haplotypes for a given gene were clustered based on all variants with a minor allele frequency (MAF) ≥ 0.05. Only haplotypes containing at least five natural accessions were selected for further comparative analyses. Duncan's multiple test was used to compare differences in the PH between haplotypes using the R software with script programming.

2.5 | Construction of transgenic lines

To generate the *MADS56* overexpression construct, a full-length cDNA corresponding to the coding sequence of *MADS56* was amplified from *O. sativa* var 9522 using cDNA from young leaf, and cloned into the binary pTCK303 vector under control of the maize *Ubiquitin* promoter to generate *pUbi::MADS56*. The construct was transformed into wild-type 9522 rice by the *Agrobacterium radiobacter* (EHA105) method as previously described (Su et al., 2021). All primers are listed in Supporting Information: Table S10.

2.6 | Expression analysis

For quantitative reverse-transcription PCR (qRT-PCR), total RNA from mature leaves, young (seedling) leaves, young leaf sheath, root and different panicle and elongation stem stages were extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions.

Total RNA (1 μg) from each sample was used to synthesise the first-strand cDNA using the PrimeScriptRT reagent kit with gDNA eraser (TaKaRa) according to the manufacturer's instructions. Quantification of transcript abundance was performed using SYBR Premix Ex Taq (TaKaRa) according to the manufacturer's instructions with the Bio-Rad Real-Time PCR System. The *OsActin* gene was used as an internal control to normalise the data using the comparative cycle threshold (ΔΔ*Ct*) method (J. Hong et al., 2022). Each experiment was repeated with three independent biological samples and three technical replicates. All primers are listed in Supporting Information: Table S10.

For in situ hybridisation, fresh elongation stem samples were collected and fixed in FAA solution (50% ethanol, 10% formaldehyde and 5% acetic acid), dehydrated with a graded ethanol solution, infiltrated, embedded in paraffin, and then sectioned into 8 μm sections using a Leica microtome (RM2235). Digoxygenin-labelled in situ hybridisation and probe detection were performed as previously described (Su et al., 2021). All primers are listed in Supporting Information: Table S10.

For histochemical analysis, a 3.5 kb DNA fragment upstream of the transcriptional start codon of *MADS56* was amplified and introduced into CAMBIA1301::GUS by In-Fusion (Clontech) according to the manufacturer's instructions. The *pMADS56::GUS* (β-glucuronidase) construct was transformed into 9522 rice callus using *A. tumefaciens*, as described above. GUS activity was determined by staining as described previously (Jefferson et al., 1987).

2.7 | Yeast two-hybrid assay

Full-length *MADS57* cDNA from 9522 was amplified and cloned into *Eco*RI and *Bam*HI restriction sites of pGADT7 (TaKaRa), which encodes the GAL4 activation domain. The coding sequences of full-length and truncated *MADS56* were amplified and cloned into *Eco*RI and *Bam*HI restriction sites of pGBKT7 (TaKaRa), which encodes the GAL4 binding domain. To detect protein-protein interactions, recombinant *pGBKT7-MADS56* and *pGADT7-MADS57* plasmids were co-transformed into

Saccharomyces cerevisiae strain AH109, according to the manufacturer's instructions (TaKaRa). Transformants were selected on SD medium lacking SD/-Trp/-Leu and SD/-Trp/-Leu/-His/-Ade.

2.8 | Bimolecular fluorescence complementation (BiFC) assay

Full-length *MADS57* and *MADS56* coding sequences were amplified and cloned into pXY104-cYFP and pXY106-nYFP plasmids, respectively. Constructs and controls were introduced into *A. tumefaciens* GV3101, which were grown in LB medium with 50 μg/mL kanamycin and 25 μg/mL rifampicin, resuspended in infection solution (10 mM MES and 200 μM acetosyringone), and co-infiltrated into 3-week-old *Nicotiana benthamiana* leaves. The BiFC assay was performed as previously described (Su et al., 2021). After 48 h incubation, fluorescent eYFP signals were detected at excitation 514 nm and emission 522–555 nm using a Leica SP8 confocal microscope.

2.9 | Luciferase assay

For split-luciferase assays, the *MADS57* coding sequence was cloned into the pCAMBIA 1300-nLuc, which encodes the N-terminal luciferase domain. The *MADS56* coding sequence was cloned into pCAMBIA 1300-cLuc, which encodes the C-terminal luciferase domain. Constructs and controls were introduced into *N. benthamiana* leaves as described above. After 36 h incubation in the dark, leaves were collected, and pictures were captured by a cooling CCD imaging apparatus (Tanon 5200).

For dual-luciferase reporter assays, effectors and control plasmids were generated by inserting the *MADS56*, *MADS57*, or *GFP* coding sequence into pGREEN000 plasmid under control of the 35 S promoter (p35S). Reporters were generated by inserting the 2.6 and 3.0 kb promoter sequence of *OsGA2ox3* and *EUI1* upstream of the luciferase (LUC) reporter gene in pGREENII0800 vector that constitutively expresses *p35S::Renilla* (REN). These two pGREEN vectors were kindly provided by Dr. Hao Yu, National University of Singapore, Singapore.

Different combinations of constructs were introduced in *N. benthamiana* leaves via *Agrobacterium*-mediated transformation, as described above. After 36 h incubation in the dark, leaves were ground in liquid nitrogen, and luciferase and Renilla activities were measured with a dual-luciferase reporter assay kit (Promega), according to the manufacturer's instructions.

3 | RESULTS

3.1 | There is significant PH variation in Asian cultivated rice

To explore the genetic basis of natural PH variation in rice, we examined the PH of randomly selected 619 Asian cultivated rice accessions (Supporting Information: Table S1) from the 3000 Rice Genomes Project (3K RGP) (W. Wang et al., 2018). Plants were grown in two consecutive seasons in 2015 and 2016. Natural variations in PH were significant, ranging from 67 to 179 cm and 55 to 183 cm in the 2 years (Figure 1a; Supporting Information: Table S2). The correlation coefficient of PH between the two seasons was remarkably high ($r^2 = 0.86$, $p = 2.2 \times 10^{-16}$), indicating a strong genetic influence on rice PH. Subsequent two-way ANOVA confirmed that genetic factors account for 89.3% of the total PH variation across the 619 accessions (Supporting Information: Table S3).

3.2 | At least 12 QTL/genes underlie PH variations in Asian cultivated rice

To identify underlying PH-associated QTL/genes in rice, we performed GWAS across the 619 accessions using the linear mixture model (LMM) with 6.5 M SNPs from the Rice SNP-Seek Database (Alexandrov et al., 2015), and identified 16 QTL exceeding the significant threshold (Figure 1b; Supporting Information: Figure S1; Table S4). Four QTL (*qPh1.1*, *qPh1.2*, *qPh1.3* and *qPh12.1*) could be detected in both seasons (Figure 1b; Supporting Information: Table S4), suggesting their independence from environmental cues, leaving 12 unique QTLs. Among them, *qPh1.3* has previously been characterised as *SD1* (Sasaki et al., 2002), the key PH regulator underlying the semi-dwarf phenotype. Candidate genes for *qPh1.1*, *qPh1.2* and *qPh12.1* could be assigned based on known functions of underlying or homologous genes. *OsWAK11*, the homologue of *qPh1.1*/*OsWAK10d* (Yue et al., 2022), is a known negative regulator of PH in rice; while *OsVQ4*, which underlies *qPh1.2* is a positive regulator of seed size and heading date in rice (Chan et al., 2021), and is a good candidate for the observed PH effect. Similarly, *DWARF53*, the homologue of *qPh12.1*/*D53L*, is a known positive regulator of rice PH (L. Jiang et al., 2013; F. Zhou et al., 2013). Thus, our GWAS results have led to successful identification of both known and unknown PH-associated QTL/genes in geographically diverse rice accessions.

3.3 | A structural variant in *SD1* underlies *qPh1.3*

To dissect the genetic bases underlying these PH-associated loci, we first focused on the peak on chromosome 1 with the highest signal, *qPh1.3* (Chr1:38538838; Figure 1b). Since the lead SNP (the most significant association) was close to (~157 kb downstream of) *LOC_Os01g66100/SD1*, we assumed that *SD1* is likely the causative gene for *qPh1.3* (Figure 2a). To identify the variants associated with PH in *SD1* gene region, we used the all 43 SNPs with minor allele frequency (MAF) > 0.05 in the *SD1* region (2 kb upstream of the *SD1* transcriptional start site to 1 kb downstream of the *SD1* stop codon) to perform a gene-based association study. To our surprise, associations detected for all these SNPs were much less significant ($p \geq 8.14 \times 10^{-6}$) than that of the lead SNP (Chr1:38538838),

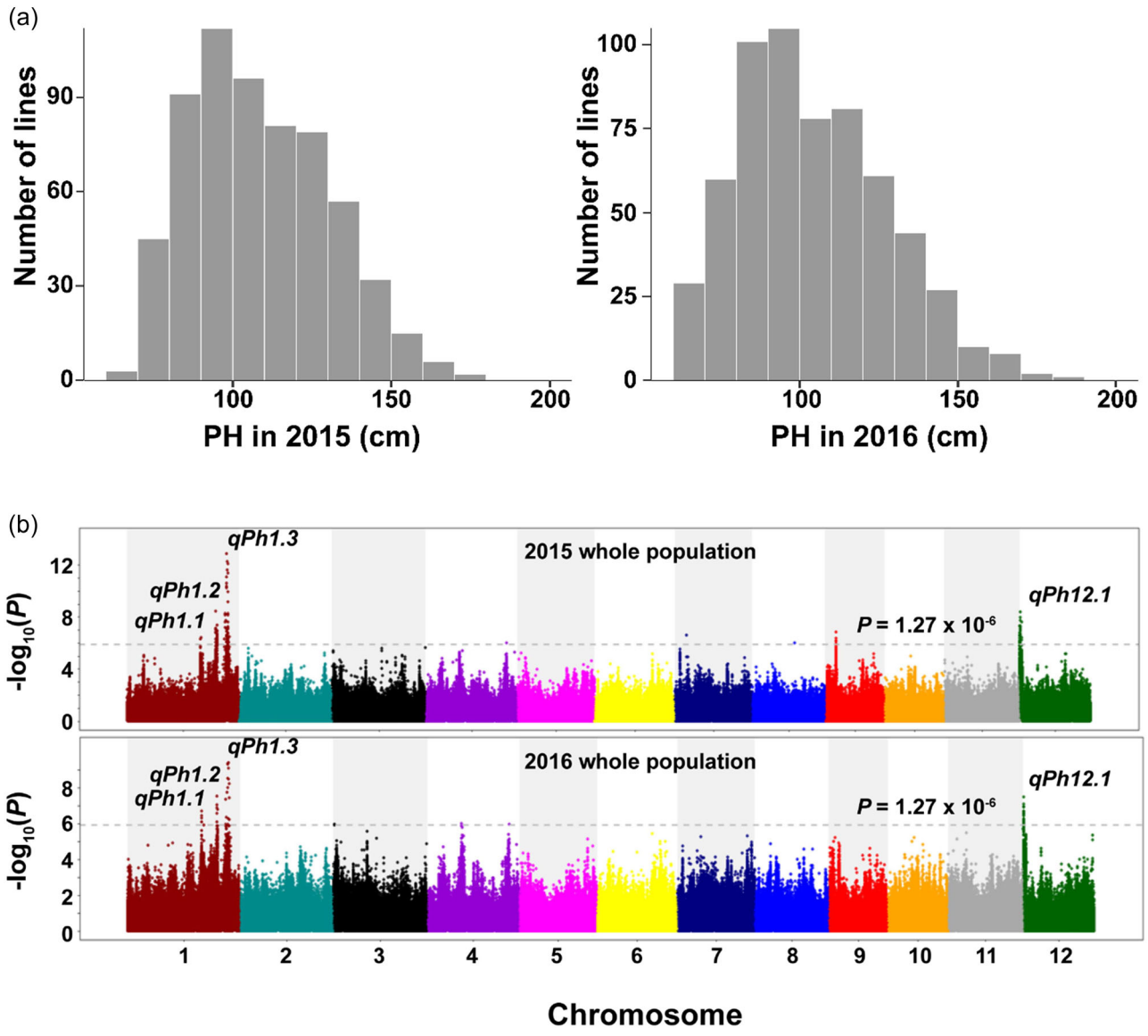

FIGURE 1 Genome-wide association study (GWAS) for plant height (PH) in 619 accessions in 2015 and 2016. (a) Distributions of PH for the whole panel. (b) Manhattan plots of GWAS for PH, indicating positions of 4 QTL of interest (3 on chromosome 1, 1 on chromosome 12).

suggesting that these SNPs cannot explain the GWAS signal in *qPh1.3* (Figure 2b).

Considering that a 383-bp deletion structural variant in *SD1* present in some accessions including IR8 (Sasaki et al., 2002) was not included in original SNP-based GWAS (Figure 1b; Supporting Information: Figure S1), to test its impact, we used a manually constructed binary structural variant based on all 15 accessible non-synonymous SNPs in this 383 bp genomic region (Figure 2c) to repeat the gene-based association study, and observed a significant association ($p = 8.48 \times 10^{-12}$) for this structural variant (Figure 2d,e). Interestingly, this structural variant exhibited a high LD ($r^2 = 0.82$) with the distant lead SNP in *qPh1.3* (Chr1:38538838) but low LD ($0.24 \le r^2 \le 0.40$) with other nearby SNPs in the *SD1* genomic region (Figure 2f). The LD analysis indicated that the 383 bp deletion in *SD1* is the causative variant for *qPh1.3*.

3.4 | Fixing the *qPh1.3/SD1* haplotype allows discovery of genetic heterogeneity of rice PH across subgroups

qPh1.3/SD1 exhibited a significantly lower *p*-value than any other QTL (Figure 1b), suggesting its strong effect on PH variation in Asian cultivated rice. As strong QTL can hinder identification of other less strong QTL in plant GWAS (Fang et al., 2017), we divided the 619 accessions into four subgroups with distinct *SD1* haplotypes ($SD1^{IR8}$, $SD1^{Kas}$ and $SD1^{NIP}$) or with an uncertain haplotype (accessions with missing or heterozygous genotypes in the three functional SNP positions; Figure 2g; Supporting Information: Table S2). $SD1^{Kas}$ and $SD1^{NIP}$ haplotypes were each defined using two (Chr1: 38382764 and Chr1: 38385057) of 55 non-synonymous SNPs (Asano

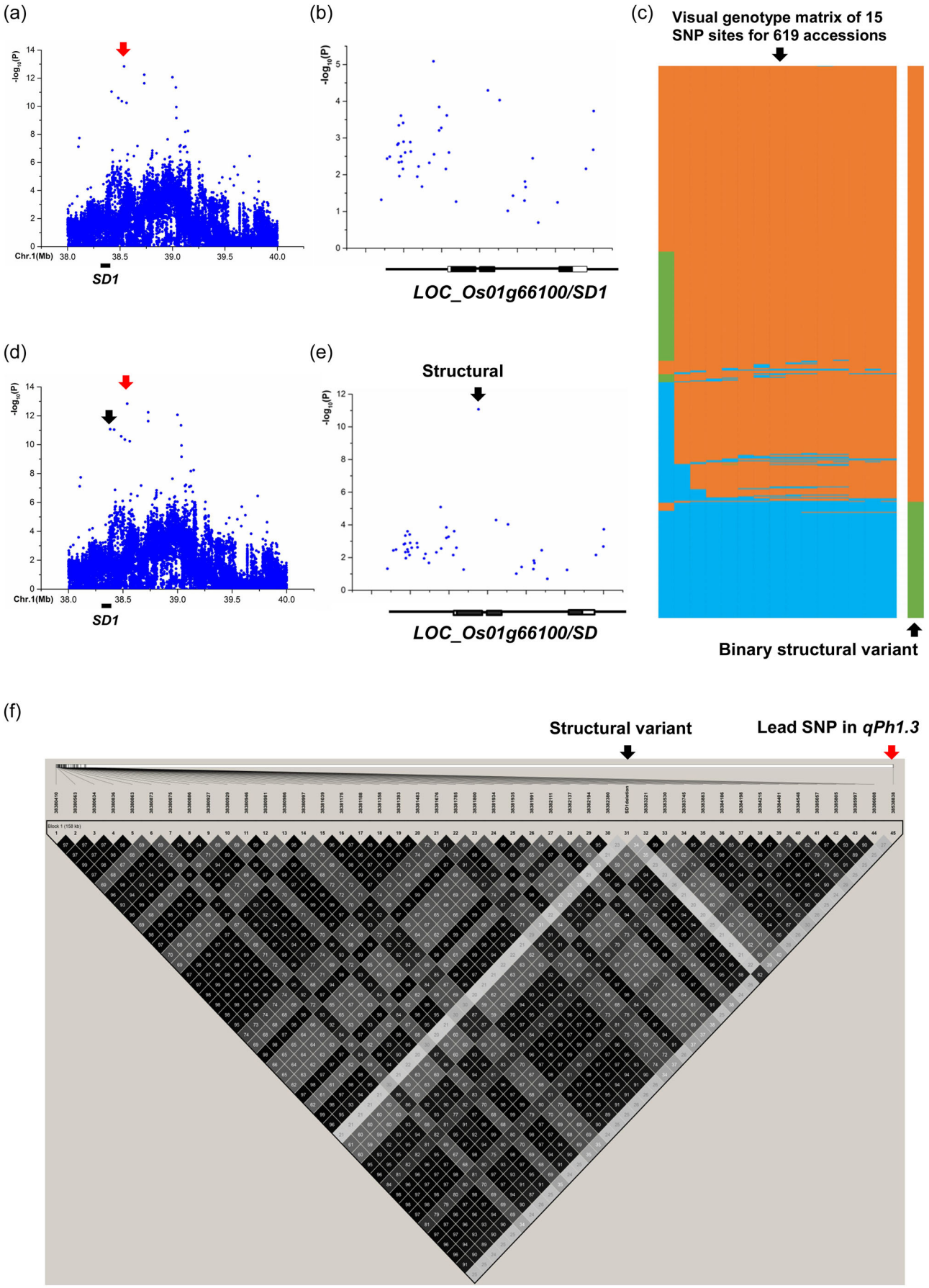

FIGURE 2 (See caption on next page)

et al., 2011) with distinctly higher MAFs across the 619 accessions (Supporting Information: Figure S2). The *SD1*^{IR8} haplotype contained the 383 bp deletion structural variant (Sasaki et al., 2002). By fixing the *SD1* allele, extensive phenotypic variations were observed within each of these three subgroups (Supporting Information: Figure S3), suggesting the existence of subgroup-specific genetic regulators not identified by our original GWAS.

GWAS within each subgroup identified 335, 995 and 242 significant SNP–trait associations in *SD1*^{IR8}, *SD1*^{Kas} and *SD1*^{NIP} subgroups, respectively (Supporting Information: Table S5; Figure S4), and uncovered 40, 13 and 50 QTL, respectively, based on the criteria for candidate gene selection (±200 kb, lowest *p*-value) (M. Yang et al., 2018). Of these QTLs, 17 were detectable in both seasons, leaving 86 unique QTL (Supporting Information: Table S6). Only four (*qphSK4.1/qPh4.1*, *qphSN4.3/qPh4.3*, *qphSN7.1/qPh7.1* and *qphSN8.2/qPh8.1*) had also be identified in the GWAS using all 619 accessions, albeit mostly in only one of the 2 years (Supporting Information: Table S4). Thus, analysis of distinct *SD1* subgroups revealed new regulators for PH whose effects were swamped by *SD1* in the broader population due likely to local adaptation (Lopez-Arboleda et al., 2021).

Two QTL in the *SD1*^{NIP} subgroup, *qphSN3.4* and *qphSN5.1*, were highly associated with known PH-related genes: *EL1/HD16* (Dai & Xue, 2010; Hori et al., 2013; Kwon et al., 2014) and *SBI/OsGA2ox4* (C. Liu, Zheng, et al., 2018), respectively (Supporting Information: Figure S4). Several significant SNPs in the *qphSN3.4* region closely colocated with the *EL1/HD16* gene (Supporting Information: Figure S5A), and three missense SNPs shaped four main haplotypes (Supporting Information: Figure S5B). Consistent with a previous report that allelic divergence between *HD16*^{Koshihikari} and *HD16*^{Nipponbare} affects culm length (Hori et al., 2013), we noticed that PH in accessions with HAP4/*HD16*^{Koshihikari} was significantly taller than for HAP1/*HD16*^{Nipponbare} accessions in both seasons (Supporting Information: Figure S5C); further, we found that PH of HAP3 accessions was also significantly higher than for HAP1 accessions (Supporting Information: Figure S5D). These results confirmed that different alleles of *EL1/HD16*, a flowering regulator, also affect PH, and that HAP3 is a newly identified allele of *EL1/HD16* associated with PH variation in rice.

Similar analysis on another QTL, *qphSN5.1*, revealed its co-location with the reported PH-related gene *SBI/OsGA2ox4* (Supporting Information: Figure S6A). We found that PH in *SBI/OsGA2ox4* HAP2 accessions, harbouring an amino acid substitution at position 255, was significantly taller than in *SBI/OsGA2ox4* HAP1 accessions in both seasons (Supporting Information: Figure S5c,d). Since a function for the plausible causative SNP (255) has not previously been reported, we propose that HAP2 is a novel functional allele of *SBI/OsGA2ox4* highly associated with PH variation.

3.5 | Epistatic interactions between identified QTL/genes contribute to PH variation in the *SD1*^{Nip} subgroup

A larger number of significant SNP–trait associations were observed in the *SD1*^{NIP} (995) than in the *SD1*^{IR8} and *SD1*^{Kas} subgroups (335 and 241, respectively; Supporting Information: Table S5). Almost all accessions (189/202) in the *SD1*^{NIP} subgroup are *japonica* (*Geng*; Supporting Information: Table S2), indicating a role for PH-related QTL in PH adaptation of *japonica* during rice domestication. Given that epistatic interactions between QTL across the genome have been well documented in plants subjected to selection and domestication (Misra et al., 2021; Soyk et al., 2020), we used a linear regression method implemented with PLINK (J. Zhang et al., 2015) to explore epistatic interactions between identified PH-related QTL in the *SD1*^{NIP} group.

We selected 10 QTLs detected in both seasons (Supporting Information: Figure S4c,d; Supporting Information: Table S6), and tested the resulting 45 potential pairs for epistatic interactions. Our analysis found six interaction pairs ($p < 0.05$) involving eight QTL, centred on two hub QTL (Figure 3a; Supporting Information: Table S7). The first hub *qphSN1.4* (chr 1: 34506482) interacted with four other QTL, *qphSN3.1*, *qphSN3.4/EL1/HD16*, *qphSN6.2* and *qphSN10.2*, while the second hub QTL *qphSN8.2* (chr 8: 16556183) interacted with *qphSN1.2* and *qphSN6.4*. These identified epistatic interactions between PH-associated QTL/genes in the *SD1*^{NIP} subgroup indicate a complex genetic network regulating rice PH.

3.6 | *GAMYB* is the causative gene for hub QTL *qphSN1.4*

In searching for the causative gene underlying the hub QTL *qphSN1.4* on chromosome 1, we noticed that one significantly associated SNP (chr 1:34506482; Supporting Information: Table S7) was located 2 kb upstream of *LOC_Os01g59660* (Figure 3b), which encodes a

FIGURE 2 A structural variant in the *semi-dwarf1 (SD1)* gene underlies the *qPh1.3* locus' significant contribution to PH variation across 619 accessions. (a) Regional genome-wide association study (GWAS) signal in 2 Mb region around *SD1* on chromosome 1. Red arrow shows the lead (most significant association) SNP at *qPh1.3*. (b) Regional GWAS signal in the *SD1* gene region. (c) Schematic representation of the structural variation of *SD1*, showing major (orange), minor (green), and missing (blue) genotype alleles at each polymorphic site. (d) Regional GWAS signal in 2 Mb region around *SD1* with SNP markers and the constructed structural variant. Red and black arrows show positions of *qPh1.3*and the constructed *SD1* structural variant, respectively. (e) Regional GWAS signal in the *SD1* gene region with SNP markers and constructed *SD1* structural variant (black arrow). (f) A representation of the pairwise r^2 values (linkage disequilibrium, LD between two SNPs) between all SNP markers (minor allele frequency > 0.05) located in the *SD1* gene region. [Color figure can be viewed at wileyonlinelibrary.com]

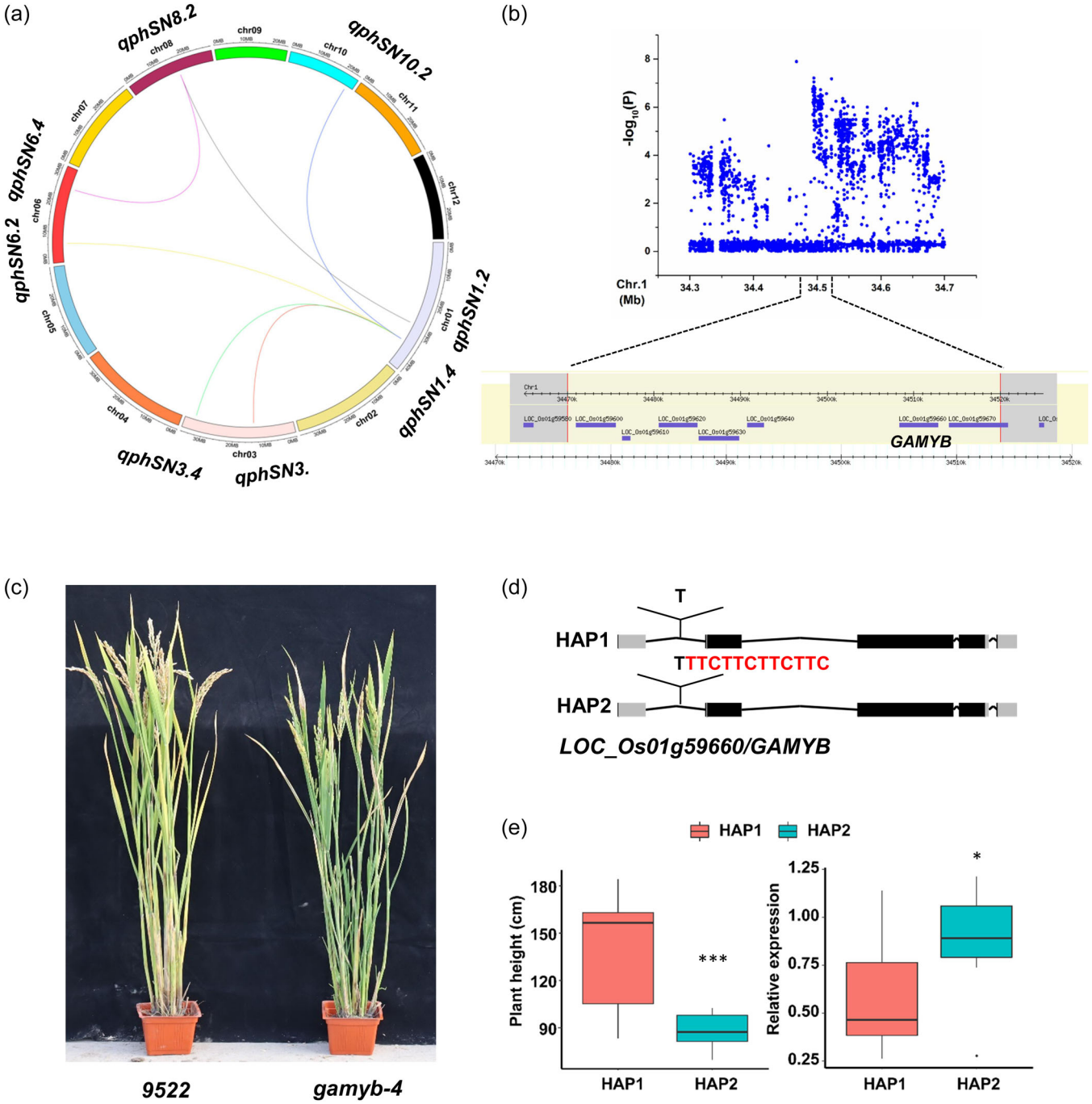

FIGURE 3 *qphSN1.4/GAMYB*, a hub QTL/gene, epistatically interacts with other four QTL/genes in the $SD1^{NIP}$ subgroup. (a) Circos plot showing the genome-wide distribution of the six most significant pairwise interactions detected in epistasis analysis. (b) Genome-wide association study (GWAS) revealed a significant peak for PH on chromosome 1 at about 34.5 Mb in the $SD1^{NIP}$ subgroup (top). Candidate genes within 50 kb of the lead SNP include *GAMYB* (bottom). (c) PH phenotypes of the wild type 9522 and *gamyb-4* mutant plants at maturity. (d) Sequence variations in *GAMYB* between two main haplotypes occur in the promoter. Grey boxes, untranslated regions; black boxes, exons; lines, introns. (e) PH and *GAMYB* expression between two main *GAMYB* haplotypes. Box horizontal lines represent the upper, middle and lower quantiles; whiskers and dots show maximum and minimum values. $^{*}p < 0.05$; $^{***}p < 0.001$ (Student's *t*-test). [Color figure can be viewed at wileyonlinelibrary.com]

MYB-family TF, GAMYB, involved in GA signalling and associated with internode elongation (Aya et al., 2009; Kaneko et al., 2004). We examined the effect of *GAMYB* on rice PH using a reported *gamyb-4* allele (Z. Liu et al., 2010), and found that *gamyb-4* plants were shorter than wild type (Figure 3c). These results suggest that *GAMYB* is a strong candidate gene for *qphSN1.4*.

To explore natural variants of *GAMYB* that lead to PH differences, we sequenced *GAMYB* genomic DNA across 24

accessions with contrasting PH values. A short tandem repeat (STR) variant in the *GAMYB* promoter region associated with PH variation, with high PH accessions lacking the tandem TTC repeat (Supporting Information: Figure S7). To further examine the correlation between this STR variant and the significant GWAS signal (chr 1:34506482), we queried this STR variant in the RiceVarMap2 database (http://ricevarmap.ncpgr.cn/) for $SD1^{NIP}$ accessions, and found that all accessions with A at the relevant SNP (chr 1:34506482) have lower TTC copy number and taller PH (Supporting Information: Table S8). Since STR variants are highly associated with gene expression levels and quantitative traits (Jakubosky et al., 2020; Si et al., 2016), we compared *GAMYB* expression in accessions of these two major *GAMYB* haplotypes (Figure 3d), and found that *GAMYB* expression positively correlated with TTC copy number, and negatively correlated with PH (Figure 3e). Based on these results, we concluded that *LOC_Os01g59660/GAMYB* is the causal gene underlying hub QTL *qphSN1.4*.

3.7 | *MADS56* is the causal gene underlying *qphSN10.2*

Subsequently, we investigated candidates for the second gene in the same network that underlies *qphSN10.2*. We found that only one gene, *LOC_Os10g39130*, is located in the interval of the sharp peak for *qphSN10.2* (Figure 4a). *LOC_Os10g39130* encodes a MADS-box gene *MADS56* that negatively regulates flowering in rice (Ryu et al., 2009). Similarly, we randomly sequenced and compared the *MADS56* genomic sequence of 16 GWAS accessions with contrasting PH values and found that five high PH accessions all have a 1008 bp deletion covering part of the 5′ untranslated region and the whole first exon of *MADS56* (Figure 4b). By manually constructing a binary structural variant in all $SD1^{NIP}$ subgroup accessions based on all 21 accessible SNPs in the 1008 bp genomic region, we found that PH in HAP2 accessions harbouring this deletion variant was significantly taller than that in HAP1 accessions in both seasons (Figure 4c). *MADS56* expression was very low in HAP2 accessions but significantly higher in HAP1 accessions (Figure 4d), indicating a negative association of *MADS56* expression with PH. To further confirm the function of *MADS56* function in PH, we analysed PH in three independent transgenic lines overexpressing *MADS56*. Plants in all three overexpression lines were significantly shorter than those in wild type at maturity (Figure 4e,f), supporting our conclusion that *MADS56* is a novel rice PH regulator that underlies *qphSN10.2*.

3.8 | MADS56 interacts with MADS57 to regulate expressions of GA metabolic genes

Based on qRT-PCR data, *MADS56* is ubiquitously expressed in vegetative and reproductive tissues throughout plant development (Supporting Information: Figure S8a). In situ hybridisation result revealed its expression in the vascular bundle of the stem (Supporting Information: Figure S8b) while histochemical analysis revealed its general expression in root, leaf sheath, stem node and panicle tissues (Supporting Information: Figure S8c) and, more specifically, in the vascular bundles of root and leaf (Supporting Information: Figure S8d).

Since *MADS56* (*qphSN10.2*) epistatic interacted with a GA signalling regulator *GAMYB* (*qphSN1.4*), we performed expression analysis of GA homoeostasis and signalling genes in stem tissues of wild type and *MADS56* overexpression lines, which showed that transcript levels of six GA biosynthetic genes (*OsCPS*, *OsKS*, *OsKO*, *OsKAO*, *GA20ox1*, *GA20ox2/SD1* and *GA3ox2*) were significantly reduced (Supporting Information: Figure S9) while expression of two GA deactivating genes (*EUI1 and GA2ox3*) was significantly increased by *MADS56* overexpression (Supporting Information: Figure S10). However, the expression of core GA signalling gene *SLR1* was not significantly changed by *MADS56* overexpression (Supporting Information: Figure S10). These results suggest that *MADS56* likely regulates rice PH by modulating GA homoeostasis.

Considering that MADS-domain proteins often function as hetero- and/or homo - dimers (Kaufmann et al., 2005), we performed a yeast two-hybrid (Y2H) assay to screen for MADS56 interactors. MADS56 could interact with MADS57 (Figure 5a), a known positive regulator of rice PH in the GA-mediated regulatory pathway (Chu et al., 2019). The MADS56–MADS57 interaction was further verified by both split-luciferase and bimolecular fluorescence complementation (BiFC) assays (Figure 5b,c). As the MADS56 protein is predicted to contain a MADS-box domain and a K-box domain (Supporting Information: Figure S11a; http://smart.embl-heidelberg.de/), we repeated the interaction experiments in yeast using truncated MADS56 constructs, and found that its K-box domain was required for interaction with MADS57 (Supporting Information: Figure S11b).

To examine the effect of the MADS56–MADS57 interaction on downstream genes, we further performed transcriptional activation assays in *N. benthamiana* leaves with various combinations of effectors and reporters (Figure 6a). We found that promoter activity of *EUI1* and *GA2ox3* was significantly more enhanced by co-expression of MAD56 and MADS57 than by expression of either MADS56 or MADS57 alone (Figure 6b,c). These results strongly indicate that MADS56 interacts with MADS57 to coregulate expression of GA catabolic genes *EUI1* and *GA2ox3* in rice.

3.9 | Putative candidate genes for the remaining QTL in the network

We finally attempted to identify the gene(s) underlying the last two QTL (*qphSN3.1* and *qphSN6.2*) in the GAMYB-centred QTL/gene interaction network (Figure 3a). The most significant association for *qphSN3.1* (chr 3: 13243402) was located in the genomic region of *LOC_Os03g22900*; this SNP caused a missense mutation and divided the GWAS population into two different haplotypes (Figure 7a,b). *LOC_Os03g22900* encodes a chromatin remodelling factor, OsINO80, that regulates GA biosynthesis by directly binding to the

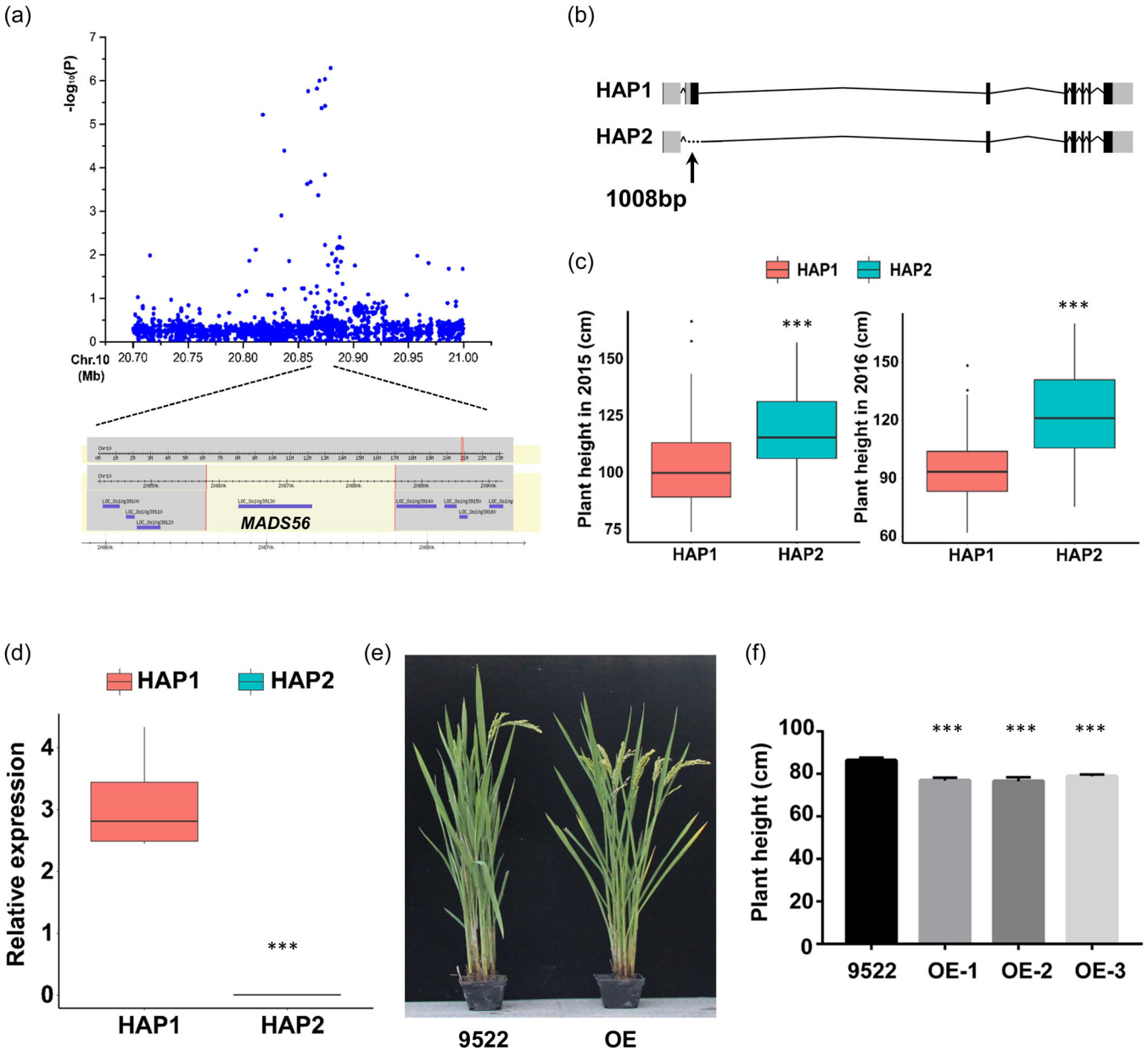

FIGURE 4 Identification and validation of the candidate gene *MADS56* underlying *qphSN10.2* in the $SD1^{NIP}$ subgroup. (a) Genome-wide association study (GWAS) revealed a significant peak for PH on chromosome 10 at 20.85–20.9 Mb in the $SD1^{NIP}$ subgroup (top). There is only one candidate gene (*MADS56*) within 28 kb of the lead SNP (bottom). (b) Sequence variations in *MADS56* between two main haplotypes reveal deletion of part of the 5' UTR and the first exon in HAP2. Grey boxes, untranslated regions; black boxes, exons; lines, introns. (c) PH between two main *MADS56* haplotypes in two successive seasons. (d) *MADS56* expression in two main *MADS56* haplotypes. Box horizontal lines represent the upper, middle, and lower quantiles; whiskers and dots show maximum and minimum values. ***$p < 0.001$ (Student's *t*-test). (e) PH phenotypes of wild type 9522 and *MADS56* overexpression plants at maturity. (f) PH of wild type 9522 and three independent *MADS56* overexpression lines at maturity. Mean ± SD of 8–12 plants; ***$p < 0.001$ (Student's *t*-test). [Color figure can be viewed at wileyonlinelibrary.com]

chromatin of *OsCPS1* and *GA3ox2* (C. Li et al., 2018). HAP2 accessions were significantly taller than HAP1 accessions in both seasons (Figure 7c), indicating that *OsINO80* is a good candidate gene for *qphSN3.1*.

Pairwise LD determination between the lead SNP (chr 6: 6150408) and other SNPs within 1000 kb for *qphSN6.2* identified 32 SNPs exhibiting high LD ($r^2 > 0.6$) with the lead SNP, corresponding to 26 putative candidate genes (Supporting Information: Table S9). Combined with expression data in RGAP, we selected 18 shoot-expressed candidate genes for *qphSN6.2* (Supporting Information: Table S9). Among them, *LOC_Os06g11130* encodes the putative gibberellin receptor GID1L2 family protein, which is involved in the GA pathway and regulates the elongation of rice pedicels and secondary branches (G. Jiang et al., 2014). We detected

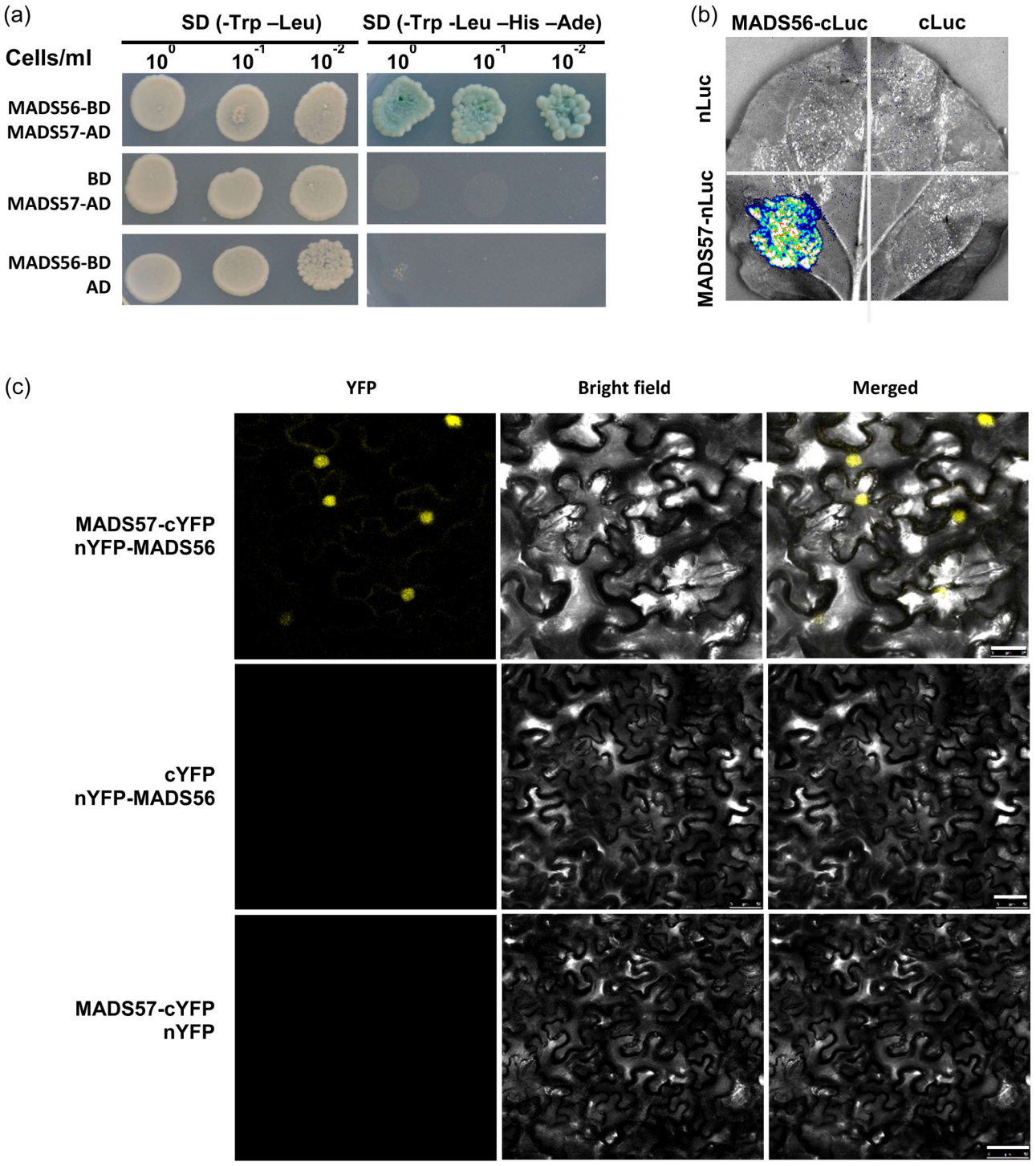

FIGURE 5 MADS56 interacts with MADS57. (a) Interaction between MADS56 and MADS57 in the yeast 2-hybrid assay. MADS56 was fused to the GAL4 binding domain (BD); MADS57 was fused to the GAL4 activation domain (AD). (b) Split-luciferase assays between MADS56 and MADS57 with controls in *Nicotiana benthamiana* leaves. cLuc, C-terminal luciferase; nLuc, N-terminal luciferase. (c) MADS56 interacts with MADS57 in a bimolecular fluorescence complementation (BiFC) assay in *N. benthamiana* leaves. MADS56 was fused to the N-terminal region of YFP (nYFP); MADS57 was fused to the C-terminal region of YFP (cYFP). [Color figure can be viewed at wileyonlinelibrary.com]

two missense mutations in the *LOC_Os06g11130* coding region, which divided the GWAS population into three distinct haplotypes (Figure 7d). HAP1 accessions were significantly shorter than HAP2 accession in both seasons (Figure 7e), suggesting that *LOC_Os06g11130* is a likely candidate gene for *qphSN6.2*.

We further employed PolyPhen-2 (Adzhubei et al., 2010) to quantitatively evaluate the effects of these non-synonymous SNP variants identified in *LOC_Os03g22900/CHR732* and *LOC_Os06g11130/GID1L2*, and found that PolyPhen-2 score of these tested three non-synonymous SNP variants are −1.793, 1.851 and

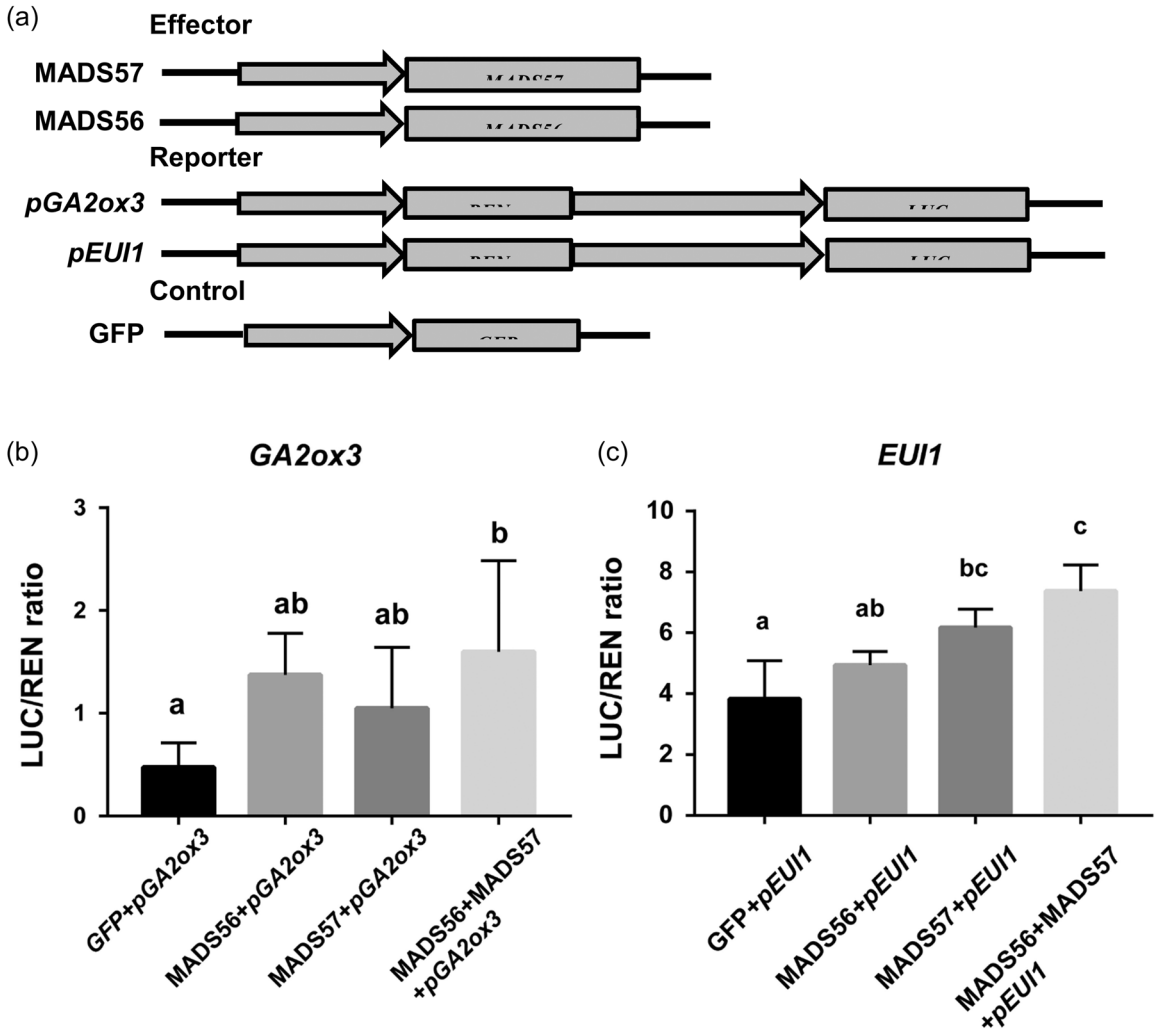

FIGURE 6 MADS56 and MADS57 jointly regulate expression of two downstream gibberellic acid (GA) catabolism genes. (a) Five constructs used for the *trans*activation assay. (b, c) Quantified Luciferase/Renilla (LUC/REN) activity with different combinations of constructs co-expressed in *Nicotiana benthamiana* leaves. Mean ± SE of 3–4 independent replicates. Different letters indicate significant differences at $p < 0.001$ (Duncan's multiple range test).

0.861, respectively. Since an absolute score over 1.5 means a deleterious mutation, we supposed that the non-synonymous SNP variant (chr 3: 13243402) in *LOC_Os03g22900/CHR732* and the non-synonymous SNP variant (chr 6: 5835826) in *LOC_Os06g11130/GID1L2* likely deleteriously affect the function of corresponding proteins.

4 | DISCUSSION

Appropriate PH has long been a major rice breeding target because semi-dwarf cultivars are positively associated with lodging resistance, harvest index and yield (Xin et al., 2022). To date, of the reported semi-dwarf QTL/genes in rice (Ji et al., 2019; Ma et al., 2016; S. Wang & Wang, 2022), only *SD1* alleles have been successfully used in breeding practice without penalty on other major agronomic traits (Q. Liu et al., 2022). To prevent over-use of fixed *SD1* alleles and the potential concomitant loss of genetic diversity, new PH-related QTL/genes suitable for breeding are required. However, while GWAS has been successfully used to dissect the genetic architecture of other quantitative traits in rice (Huang et al., 2010, 2012; W. Yang et al., 2014; K. Zhao et al., 2011), GWAS for PH in rice has had little success due to the strong population structure and larger LD (Yano et al., 2016). In this study, we use GWAS and two-locus epistatic interaction analyses on whole populations and subgroups with distinct *SD1* haplotypes to reveal different genetic architectures of PH, and provide valuable genetic resources that benefit both mechanistic studies and practical breeding programs for rice ideotypes.

Like SNPs, structural variants (SVs) are crucial elements in plant genome evolution and domestication, contributing significantly to phenotypes and their selection (Kou et al., 2020). Although thousands of SNP–trait associations can be identified by standard SNP-based GWAS in plants (Cortes et al., 2021), including rice

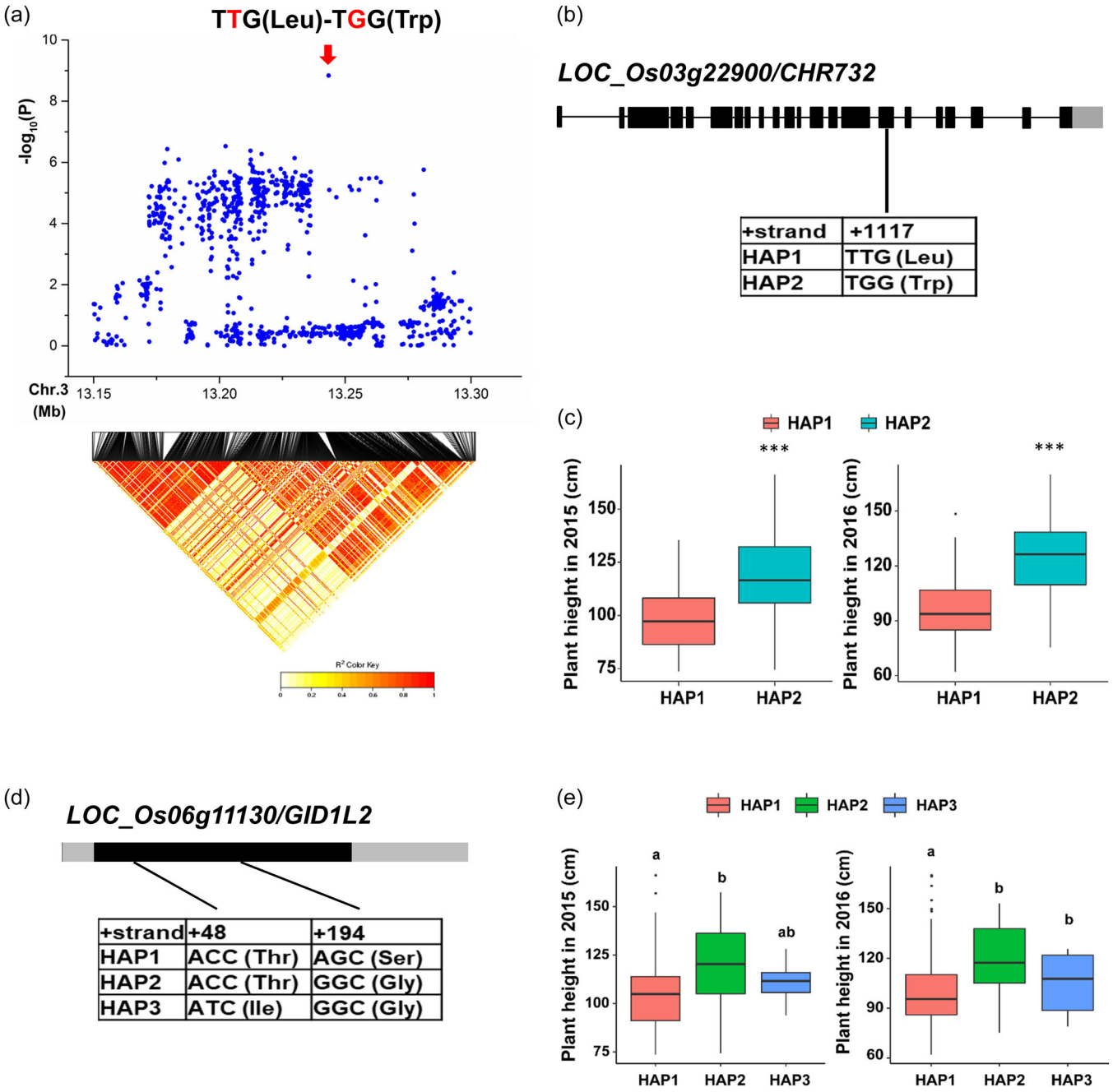

FIGURE 7 Candidate gene analysis for *qphSN3.1* and *qphSN6.2*. (a) Genome-wide association study (GWAS) revealed a significant peak (red arrow) for PH on chromosome 3 at 13.25 Mb in the $SD1^{NIP}$ (top). The underlying missense SNP variation is shown, as is the LD heatmap surrounding the peak (bottom). (b) Gene structure of *LOC_Os03g22900/OsINO80*, showing the position of the SNP defining HAP1 and HAP2. Grey boxes, untranslated regions; black boxes, exons; lines, introns. (c) PH between two main *LOC_Os03g22900/OsINO80* haplotypes in two successive seasons. Box horizontal lines represent the upper, middle and lower quantiles; whiskers and dots show maximum and minimum values. ***$p < 0.001$ (Student's *t*-test). (d) Gene structure of *LOC_Os06g11130/GID1L2*, showing positions of two SNPs defining three haplotypes. Grey boxes, untranslated regions; black boxes, exons; lines, introns. (e) PH between three main *LOC_Os06g11130/GID1L2* haplotypes in two successive seasons. Different letters indicate significant differences at $p < 0.001$ (Duncan's multiple range test). [Color figure can be viewed at wileyonlinelibrary.com]

(X. Zhou & Huang, 2019), most of them are difficult to be interpreted or translated back to causal genes/alleles (Lopez-Arboleda et al., 2021; Miao et al., 2019; Yano et al., 2016). In these scenarios, even though beneficial QTLs can still be used in molecular breeding, lack of information on underlying causative genes can seriously impede mechanistic studies or development of alternative, or more effective, varieties. Other association studies are needed to compensate for the limitations of SNP-based GWAS. In the case of *qPh1.3* identified in this study, the unexpected low LD of the leading SNP in *qPh1.3* with any other nearby SNPs (Figure 2f) required us to look for alternative

variants – in this case, a 383 bp deletion structural variant with high LD with the leading SNP. This result highlights the case where, if the causal variant is an SV, SNP-based GWAS could fail to detect it even when high-density SNP markers are used (Figure 2b). We firmly believe that, as rice pangenome studies advance (Q. Zhao et al., 2018; Qin et al., 2021; Shang et al., 2022; W. Wang et al., 2018), non-SNP-based GWAS, such as SV-based GWAS, will greatly facilitate identification of genetic factors, especially those such as PH with complex genetic regulatory networks and/or that have been heavily selected during rice local adaptation and domestication.

Since the release of the 3000-variety rice pangenome (W. Wang et al., 2018), these Asian cultivated accessions, rich in phenotypic, geographical and genetic diversity, have been widely and effectively used to unravel genetic architectures underlying complex traits, such as yield, via GWAS (J. Hong et al., 2022; Wei et al., 2021; Yuan et al., 2020; G. Zhang et al., 2021). Here, this same set of accessions again proved extremely useful for dissecting PH diversity. GWAS across both the whole 619 accessions and smaller subgroups with distinct *SD1* haplotypes had sufficient power to identify general QTL (Supporting Information: Table S4) and subgroup-specific QTL (Supporting Information: Table S6) associated with PH. GWAS on the 619 accessions identified fewer QTLs than in the small subgroups, and 82/86 of these identified QTL could only be detected in subgroups. These data support the idea that large populations from broad geographic regions may decrease GWAS power due to increased genetic heterogeneity and population structure (Lopez-Arboleda et al., 2021), so some, or even most, QTL can only be detected after the fixation of the major-effect QTL as observed here. Considering that a big (global) population may weaken the power to detect locally important allelic variants, while a small (local) population may not harbour globally important variants (Lopez-Arboleda et al., 2021), it is necessary to optimise GWAS populations when dissecting complex traits in rice.

Beneficial alleles of QTL/genes associated with major adaptative or agronomic important traits in plants have been strongly selected during local adaptation (especially within specific subspecies). Thus, fixation of haplotype in the major QTL/genes in plant GWAS may reduce negative effects of population structure and avoid effects of interactions of these major QTL/genes with other minor-effect ones (Fang et al., 2017; Lopez-Arboleda et al., 2021). Here, we found a much larger number of PH QTL/genes identified in subgroups after the fixation of the *SD1* haplotypes than in the broader population (Supporting Information: Tables S4 and S7). Therefore, it may be worthwhile to re-analyse published plant GWAS datasets using this strategy to identify novel causal genes, particularly in those experiments where GWAS on a large population exhibits a single sharp and significant peak in the Manhattan plot, to explore and unravel local adaptation and evolution of key traits.

Known and putative PH regulatory genes identified in the 619 accessions (Supporting Information: Table S4) were associated with hormone pathways, such as GA homoeostasis (*SD1*, Sasaki et al., 2002), brassinosteroid (BR) signalling (*WAK10d*, *WAK11* homologue, Yue et al., 2022; *OsVQ4*, *OsVQ25* homologue, Hao et al., 2022), strigolactone (SL) signalling (D53L, L. Jiang et al., 2013), and crosstalk between GA and BR (*NAL1*, Subudhi et al., 2020). This result agreed with existing knowledge that rice PH is regulated mainly by GA, BR and SL (F. Liu, Wang, et al., 2018), and reflected a general global architecture likely associated with rice domestication. Known PH QTL/genes identified in subgroups with distinct *SD1* haplotypes (Supporting Information: Table S7) were involved in a broader range of pathways. In the $SD1^{IR8}$ subgroup, *OsCIGR* (Kong et al., 2022) and *CYP94C2b* (Kurotani et al., 2015) are involved in GA signalling and jasmonic acid (JA) biosynthesis, respectively. In the $SD1^{NIP}$ subgroup, three genes, *GNP1/OsGA20ox1* (Y. Wu et al., 2016), *EL1/HD16* (Dai & Xue, 2010), and *SBI/OsGA2ox4* (C. Liu, Zheng, et al., 2018), are associated with GA homoeostasis, and one, *OsXTH8* (Jan et al., 2004), with GA signalling; further genes were associated with BR signalling (*XIAO*, Y. Jiang et al., 2012), abscisic acid signalling (*OsRNS4*, Zheng et al., 2014), auxin homoeostasis (*OsSAUR45*, Xu et al., 2017), RNA binding (*LGD1*, Thangasamy et al., 2012), fatty acid metabolism (*OsFAD8*; Gopalakrishnan Nair et al., 2009), and transcriptional activation (*OsSHI1*, Duan et al., 2019). Subgroup specific genetic architecture has been reported in Arabidopsis for flowering, a local adaptative trait (Lopez-Arboleda et al., 2021), our findings in this study strongly suggest that rice PH has also been involved in local adaptation.

Epistatic interactions present in natural accessions are important for capturing additional genetic information and understanding their roles in domestication and evolution (Malmberg & Mauricio, 2005; Misra et al., 2021). In this study, we identified six QTL interaction pairs centred on *qphSN1.4/GAMYB* and *qphSN8.2*, respectively, only in the *japonica*-dominated $SD1^{NIP}$ subgroup (Figure 3a; Supporting Information: Table S7). Notably, all five QTL/genes in this *GAMYB*-mediated genetic network are associated with the GA pathway, and all of them exhibit polytropic features in affecting both PH and heading date in rice. *GAMYB*, identified here as a novel *GAMYB* allele associated with PH variation (Figure 3), is a positive regulator of GA signalling, and adversely affects reproductive development, male sterility, and heading date (Kaneko et al., 2004; Z. Liu et al., 2010). The exact mechanisms of sequence difference between *HAP1* and *HAP2* for *GYMYB* remain unknown, our bioinformatic analysis with 125 bp sequence bearing STR variants (HAP2) upstream of the translation initiation site of GAMYB against New PLACE database (https://www.dna.affrc.go.jp/PLACE/?action=newplace) found two motifs, CGAAT and TTTCT, a CCAAT binding complex motif and a pollen-specific motif, respectively, are located closely to the STR variant. We supposed that this STR variant might affect the binding of upstream TFs to these motifs and thus affect the expression of GAMYB in different accessions. *OsINO80* and *EL1/HD16*, here shown to influence PH (Figures 3 and 7; Supporting Information: Figure S5), are both known to affect heading date through the GA synthetic and signalling pathways, respectively (Hori et al., 2013; Kwon et al., 2014; C. Li et al., 2018). *MADS56*, a positive regulator of PH (Figure 4c,f) identified here, positively regulates GA catabolism and heading date (Zhan et al., 2022), while *LOC_Os06g11130* (*qphSN6.2*), associated with PH variation found in this study (Figure 7e), encodes a putative

GA receptor and positively regulates heading date (G. Jiang et al., 2014).

In Arabidopsis, flowering time genes are pleiotropic with other functions that respond to environmental cues (Auge et al., 2019). During the spread of *japonica* cultivars to higher latitudes, we hypothesise that these heading date-related genes were collectively selected and maintained in an interactive network to facilitate local adaptation for heading date. These genes appear to operate in GA-centred pathways, now known to also affect PH (Figures 5–7). Harnessing epistasis effects hidden in natural accessions can help explain the molecular mechanisms underlying the biological pathways associated with the QTL/genes. Our future investigations will focus on the other genetic network identified here, centred on *qphSN8.2*, to see whether new mechanisms are involved.

In practice, SNPs detected in GWAS analyses can be used directly in marker-assisted selection without isolation of causal genes (X. Zhou & Huang, 2019). This study has provided >1500 associated SNPs for such purposes. However, the highest potential for breeding application relies on fine-tuning expression of causal genes, so identification of underlying causal genes can vastly improve results. Priority should therefore be given to exploring the agronomic effects of our newly identified candidate genes, especially the hub QTL identified in subgroups with distinct *SD1* haplotypes. Assessment of their utility in breeding programmes should focus on haplotype assessment and combination and fine-tuning gene expression via genome editing to target *cis*- or *trans*-regulatory elements in their promoters, to facilitate PH ideotype improvement while reducing undesirable effects (Hendelman et al., 2021; Lopez-Arboleda et al., 2021).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All our available data have been placed in supplementary materials. The authors are responsible for full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis presented in this article in accordance with the policy described in the Instructions for Authors (https://onlinelibrary.wiley.com/page/journal/13653040/homepage/forauthors.html?5) are: Dabing Zhang (zhangdb@sjtu.edu.cn), and Jianxin Shi (jianxin.shi@sjtu.edu.cn).

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REFERENCES

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P. et al. (2010) A method and server for predicting damaging missense mutations. *Nature Methods*, 7, 248–249.

Alexandrov, N., Tai, S., Wang, W., Mansueto, L., Palis, K., Fuentes, R.R. et al. (2015) SNP-seek database of SNPs derived from 3000 rice genomes. *Nucleic Acids Research*, 43, D1023–D1027.

Asano, K., Hirano, K., Ueguchi-Tanaka, M., Angeles-Shim, R.B., Komura, T., Satoh, H. et al. (2009) Isolation and characterization of dominant dwarf mutants, *Slr1-d*, in rice. *Molecular Genetics and Genomics*, 281, 223–231.

Asano, K., Yamasaki, M., Takuno, S., Miura, K., Katagiri, S., Ito, T. et al. (2011) Artificial selection for a green revolution gene during japonica rice domestication. *Proceedings of the National Academy of Sciences*, 108, 11034–11039.

Auge, G.A., Penfield, S. & Donohue, K. (2019) Pleiotropy in developmental regulation by flowering-pathway genes: is it an evolutionary constraint? *New Phytologist*, 224, 55–70.

Aya, K., Ueguchi-Tanaka, M., Kondo, M., Hamada, K., Yano, K., Nishimura, M. et al. (2009) Gibberellin modulates anther development in rice via the transcriptional regulation of GAMYB. *The Plant Cell*, 21, 1453–1472.

Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*, 21, 263–265.

Chan, A.N., Wang, L.L., Zhu, Y.J., Fan, Y.Y., Zhuang, J.Y. & Zhang, Z.H. (2021) Identification through fine mapping and verification using CRISPR/Cas9-targeted mutagenesis for a minor QTL controlling grain weight in rice. *Theoretical and Applied Genetics*, 134, 327–337.

Chu, Y., Xu, N., Wu, Q., Yu, B., Li, X., Chen, R. et al. (2019) Rice transcription factor OsMADS57 regulates plant height by modulating gibberellin catabolism. *Rice*, 12, 38.

Cortes, L.T., Zhang, Z. & Yu, J. (2021) Status and prospects of genome-wide association studies in plants. *Plant Genome*, 14, e20077.

Dai, C. & Xue, H.W. (2010) Rice early flowering1, a CKI, phosphorylates DELLA protein SLR1 to negatively regulate gibberellin signalling. *The EMBO Journal*, 29, 1916–1927.

Duan, E., Wang, Y., Li, X., Lin, Q., Zhang, T., Wang, Y. et al. (2019) OsSHI1 regulates plant architecture through modulating the transcriptional activity of IPA1 in rice. *The Plant Cell*, 31, 1026–1042.

Fang, C., Ma, Y., Wu, S., Liu, Z., Wang, Z., Yang, R. et al. (2017) Genome-wide association studies dissect the genetic networks underlying agronomical traits in soybean. *Genome Biology*, 18, 161.

Gopalakrishnan Nair, P.M.M.G, Kang, I.S., Moon, B.Y. & Lee, C.H. (2009) Effects of low temperature stress on rice (*Oryza sativa* L.) plastid ω-3 desaturase gene, OsFAD8 and its functional analysis using T-DNA mutants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 98, 87–96.

Guo, W., Chen, L., Herrera-Estrella, L., Cao, D. & Tran, L.S.P. (2020) Altering plant architecture to improve performance and resistance. *Trends in Plant Science*, 25, 1154–1170.

Hao, Z., Tian, J., Fang, H., Fang, L., Xu, X., He, F. et al. (2022) A VQ-motif-containing protein fine-tunes rice immunity and growth by a hierarchical regulatory mechanism. *Cell Reports*, 40, 111235.

Hendelman, A., Zebell, S., Rodriguez-Leal, D., Dukler, N., Robitaille, G., Wu, X. et al. (2021) Conserved pleiotropy of an ancient plant homeobox gene uncovered by cis-regulatory dissection. *Cell*, 184, 1724–1739.

Hong, J., Rosental, L., Xu, Y., Xu, D., Orf, I., Wang, W. et al. (2022) Genetic architecture of seed glycerolipids in Asian cultivated rice. Plant, Cell & Environment. https://doi.org/10.1111/pce.14378

Hong, Z., Ueguchi-Tanaka, M., Fujioka, S., Takatsuto, S., Yoshida, S., Hasegawa, Y. et al. (2005) The rice brassinosteroid-deficient dwarf2 mutant, defective in the rice homolog of Arabidopsis DIMINUTO/DWARF1, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, Dolichosterone. *The Plant Cell*, 17, 2243–2254.

Hori, K., Ogiso-Tanaka, E., Matsubara, K., Yamanouchi, U., Ebana, K. & Yano, M. (2013) Hd16, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. *The Plant Journal*, 76, 36–46.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y. et al. (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*, 42, 961–967.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q. et al. (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature Genetics*, 44, 32–39.

Iwamoto, M., Baba-Kasai, A., Kiyota, S., Hara, N. & Takano, M. (2010) ACO1, a gene for aminocyclopropane-1-carboxylate oxidase: effects on internode elongation at the heading stage in rice. *Plant, Cell and Environment*, 33, 805–815.

Jakubosky, D., D'Antonio, M., Bonder, M.J., Smail, C., Donovan, M.K.R., Young Greenwald, W.W. et al. (2020) Properties of structural variants and short tandem repeats associated with gene expression and complex traits. *Nature Communications*, 11, 2927.

Jan, A., Yang, G., Nakamura, H., Ichikawa, H., Kitano, H., Matsuoka, M. et al. (2004) Characterization of a xyloglucan endotransglucosylase gene that is up-regulated by gibberellin in rice. *Plant Physiology*, 136, 3670–3681.

Jefferson, R.A., Kavanagh, T.A. & Bevan, M.W. (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *The EMBO Journal*, 6, 3901–3907.

Ji, H., Han, C., Lee, G.S., Jung, K.H., Kang, D.Y., Oh, J. et al. (2019) Mutations in the microRNA172 binding site of SUPERNUMERARY BRACT (SNB) suppress internode elongation in rice. *Rice*, 12, 62.

Jiang, G., Xiang, Y., Zhao, J., Yin, D., Zhao, X., Zhu, L. et al. (2014) Regulation of inflorescence branch development in rice through a novel pathway involving the pentatricopeptide repeat protein sped1-D. *Genetics*, 197, 1395–1407.

Jiang, L., Liu, X., Xiong, G., Liu, H., Chen, F., Wang, L. et al. (2013) DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature*, 504, 401–405.

Jiang, Y., Bao, L., Jeong, S.Y., Kim, S.K., Xu, C., Li, X. et al. (2012) XIAO is involved in the control of organ size by contributing to the regulation of signaling and homeostasis of brassinosteroids and cell cycling in rice. *The Plant Journal*, 70, 398–408.

Kadambari, G., Vemireddy, L.R., Srividhya, A., Nagireddy, R., Jena, S.S., Gandikota, M. et al. (2018)) QTL-Seq-based genetic analysis identifies a major genomic region governing dwarfness in rice (*Oryza sativa* L.). *Plant Cell Reports*, 37, 677–687.

Kaneko, M., Inukai, Y., Ueguchi-Tanaka, M., Itoh, H., Izawa, T., Kobayashi, Y. et al. (2004) Loss-of-function mutations of the rice *GAMYB* gene impair α-amylase expression in aleurone and flower development. *The Plant Cell*, 16, 33–44.

Kaufmann, K., Melzer, R. & Theißen, G. (2005) MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. *Gene*, 347, 183–198.

Kong, W., Deng, X., Yang, J., Zhang, C., Sun, T., Ji, W. et al. (2022) High-resolution bin-based linkage mapping uncovers the genetic architecture and heterosis-related loci of plant height in indica–japonica derived populations. *The Plant Journal*, 110, 814–827. https://doi.org/10.1111/tpj.15705

Kou, Y., Liao, Y., Toivainen, T., Lv, Y., Tian, X., Emerson, J.J. et al. (2020) Evolutionary genomics of structural variation in Asian rice (*Oryza sativa*) domestication. *Molecular Biology and Evolution*, 37, 3507–3524.

Kurotani, K.I., Hattori, T. & Takeda, S. (2015) Overexpression of a CYP94 family gene *CYP94C2b* increases internode length and plant height in rice. *Plant Signaling & Behavior*, 10, e1046667.

Kwon, C.T., Yoo, S.C., Koo, B.H., Cho, S.H., Park, J.W., Zhang, Z. et al. (2014) Natural variation in *Early flowering1* contributes to early flowering in japonica rice under long days. *Plant, Cell & Environment*, 37, 101–112.

Li, C., Liu, Y., Shen, W.-H., Yu, Y. & Dong, A. (2018) Chromatin-remodeling factor OsINO80 is involved in regulation of gibberellin biosynthesis and is crucial for rice plant growth and development. *Journal of Integrative Plant Biology*, 60, 144–159.

Li, M.X., Yeung, J.M.Y., Cherny, S.S. & Sham, P.C. (2012) Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Human Genetics*, 131, 747–756.

Lippert, C., Listgarten, J., Liu, Y., Kadie, C.M., Davidson, R.I. & Heckerman, D. (2011) FaST linear mixed models for genome-wide association studies. *Nature Methods*, 8, 833–835.

Liu, C., Zheng, S., Gui, J., Fu, C., Yu, H., Song, D. et al. (2018a) *Shortened Basal Internodes* encodes a gibberellin 2-oxidase and contributes to lodging resistance in rice. *Molecular Plant*, 11, 288–299.

Liu, F., Wang, P., Zhang, X., Li, X., Yan, X., Fu, D. et al. (2018b) The genetic and molecular basis of crop height based on a rice model. *Planta*, 247, 1–26.

Liu, Q., Wu, K., Wu, Y., Song, W., Wang, S. & Fu, X. (2022) Beyond the green revolution: improving crop productivity and sustainability by modulating plant growth-metabolic coordination. *Molecular Plant*, 15, 573–576.

Liu, Z., Bao, W., Liang, W., Yin, J. & Zhang, D. (2010) Identification of *gamyb-4* and analysis of the regulatory role of GAMYB in rice anther development. *Journal of Integrative Plant Biology*, 52, 670–678.

Lo, S.F., Ho, T.H.D., Liu, Y.L., Jiang, M.J., Hsieh, K.T., Chen, K.T. et al. (2017) Ectopic expression of specific GA2 oxidase mutants promotes yield and stress tolerance in rice. *Plant Biotechnology Journal*, 15, 850–864.

Lopez-Arboleda, W.A., Reinert, S., Nordborg, M. & Korte, A. (2021) Global genetic heterogeneity in adaptive traits. *Molecular Biology and Evolution*, 38, 4822–4831.

Ma, X., Feng, F., Wei, H., Mei, H., Xu, K., Chen, S. et al. (2016) Genome-wide association study for plant height and grain yield in rice under contrasting moisture regimes. *Frontiers in Plant Science*, 7, 1801.

Malmberg, R.L. & Mauricio, R. (2005) QTL-based evidence for the role of epistasis in evolution. *Genetical Research*, 86, 89–95.

McKim, S.M. (2020) Moving on up-controlling internode growth. *New Phytologist*, 226, 672–678.

Miao, C., Yang, J. & Schnable, J.C. (2019) Optimising the identification of causal variants across varying genetic architectures in crops. *Plant Biotechnology Journal*, 17, 893–905.

Misra, G., Badoni, S., Parween, S., Singh, R.K., Leung, H., Ladejobi, O. et al. (2021) Genome-wide association coupled gene to gene interaction studies unveil novel epistatic targets among major effect loci impacting rice grain chalkiness. *Plant Biotechnology Journal*, 19, 910–925.

Nagai, K., Mori, Y., Ishikawa, S., Furuta, T., Gamuyao, R., Niimi, Y. et al. (2020) Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature*, 584, 109–114.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D. et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, 81, 559–575.

Qin, P., Lu, H., Du, H., Wang, H., Chen, W., Chen, Z. et al. (2021) Pan-genome analysis of 33 genetically diverse rice accessions reveals hidden genomic variations. *Cell*, 184, 3542–3558.

Ryu, C.H., Lee, S., Cho, L.H., Kim, S.L., Lee, Y.S., Choi, S.C. et al. (2009) OsMADS50 and OsMADS56 function antagonistically in regulating

long day (LD)-dependent flowering in rice. *Plant, Cell & Environment*, 32, 1412–1427.
Sakamoto, T., Miura, K., Itoh, H., Tatsumi, T., Ueguchi-Tanaka, M., Ishiyama, K. et al. (2004) An overview of gibberellin metabolism enzyme genes and their related mutants in rice. *Plant Physiology*, 134, 1642–1653.
Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D. et al. (2002) A mutant gibberellin-synthesis gene in rice. *Nature*, 416, 701–702.
Shang, L., Li, X., He, H., Yuan, Q., Song, Y., Wei, Z. et al. (2022) A super pan-genomic landscape of rice. *Cell Research*, 32, 878–896. https://doi.org/10.1038/s41422-022-00685-z
Si, L., Chen, J., Huang, X., Gong, H., Luo, J., Hou, Q. et al. (2016) OsSPL13 controls grain size in cultivated rice. *Nature Genetics*, 48, 447–456.
Soyk, S., Benoit, M. & Lippman, Z.B. (2020) New horizons for dissecting epistasis in crop quantitative trait variation. *Annual Review of Genetics*, 54, 287–307.
Su, S., Hong, J., Chen, X., Zhang, C., Chen, M., Luo, Z. et al. (2021) Gibberellins orchestrate panicle architecture mediated by DELLA–KNOX signalling in rice. *Plant Biotechnology Journal*, 19, 2304–2318.
Subudhi, P.K., Garcia, R.S., Coronejo, S. & De Leon, T.B. (2020) A novel mutation of the *NARROW LEAF1* gene adversely affects plant architecture in rice (*Oryza sativa* L.). *International Journal of Molecular Sciences*, 21, 8106.
Thangasamy, S., Chen, P.W., Lai, M.H., Chen, J. & Jauh, G.Y. (2012) Rice LGD1 containing RNA binding activity affects growth and development through alternative promoters. *The Plant Journal*, 71, 288–302.
Todaka, D., Shinozaki, K. & Yamaguchi-Shinozaki, K. (2015) Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Frontiers in Plant Science*, 6, 84.
Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M. et al. (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature*, 437, 693–698.
Wang, S. & Wang, Y. (2022) Harnessing hormone gibberellin knowledge for plant height regulation. *Plant Cell Reports*, 41, 1945–1953. https://doi.org/10.1007/s00299-022-02904-8
Wang, W., Mauleon, R., Hu, Z., Chebotarov, D., Tai, S., Wu, Z. et al. (2018) Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature*, 557, 43–49.
Wei, Z., Yuan, Q., Lin, H., Li, X., Zhang, C., Gao, H. et al. (2021) Linkage analysis, GWAS, transcriptome analysis to identify candidate genes for rice seedlings in response to high temperature stress. *BMC Plant Biology*, 21, 85.
Wu, Y., Wang, Y., Mi, X.F., Shan, J.X., Li, X.M., Xu, J.L. et al. (2016) The QTL GNP1 encodes GA20ox1, which increases grain number and yield by increasing cytokinin activity in rice panicle meristems. *PLoS Genetics*, 12, e1006386.
Wu, Z., Tang, D., Liu, K., Miao, C., Zhuo, X., Li, Y. et al. (2018) Characterization of a new semi-dominant dwarf allele of SLR1 and its potential application in hybrid rice breeding. *Journal of Experimental Botany*, 69, 4703–4713.
Xin, W., Liu, H., Yang, L., Ma, T., Wang, J., Zheng, H. et al. (2022) BSA-seq and fine linkage mapping for the identification of a novel locus (qPH9) for mature plant height in rice (*Oryza sativa*). *Rice*, 15, 26.
Xu, Y.X., Xiao, M.Z., Liu, Y., Fu, J.L., He, Y. & Jiang, D.A. (2017) The small auxin-up RNA OsSAUR45 affects auxin synthesis and transport in rice. *Plant Molecular Biology*, 94, 97–107.
Yang, M., Lu, K., Zhao, F.J., Xie, W., Ramakrishna, P., Wang, G. et al. (2018) Genome-wide association studies reveal the genetic basis of ionomic variation in rice. *The Plant Cell*, 30, 2720–2740.
Yang, W., Guo, Z., Huang, C., Duan, L., Chen, G., Jiang, N. et al. (2014) Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. *Nature Communications*, 5, 5087.
Yano, K., Morinaka, Y., Wang, F., Huang, P., Takehara, S., Hirai, T. et al. (2019) GWAS with principal component analysis identifies a gene comprehensively controlling rice architecture. *Proceedings of the National Academy of Sciences*, 116, 21262–21267.
Yano, K., Yamamoto, E., Aya, K., Takeuchi, H., Lo, P., Hu, L. et al. (2016) Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. *Nature Genetics*, 48, 927–934.
Yuan, J., Wang, X., Zhao, Y., Khan, N.U., Zhao, Z., Zhang, Y. et al. (2020) Genetic basis and identification of candidate genes for salt tolerance in rice by GWAS. *Scientific Reports*, 10, 9958.
Yue, Z.L., Liu, N., Deng, Z.P., Zhang, Y., Wu, Z.M., Zhao, J.L. et al. (2022) The receptor kinase OsWAK11 monitors cell wall pectin changes to fine-tune brassinosteroid signaling and regulate cell elongation in rice. *Current Biology*, 32, 2454–2466.
Zhan, P., Ma, S., Xiao, Z., Li, F., Wei, X., Lin, S. et al. (2022) Natural variations in grain length 10 (GL10) regulate rice grain size. *Journal of Genetics and Genomics*, 49, 405–413.
Zhang, G., Wang, R., Ma, J., Gao, H., Deng, L., Wang, N. et al. (2021) Genome-wide association studies of yield-related traits in high-latitude japonica rice. *BMC Genomic Data*, 22, 39.
Zhang, J., Singh, A., Mueller, D.S. & Singh, A.K. (2015) Genome-wide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. *The Plant Journal*, 84, 1124–1136.
Zhao, K., Tung, C.W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H. et al. (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications*, 2, 467.
Zhao, Q., Feng, Q., Lu, H., Li, Y., Wang, A., Tian, Q. et al. (2018) Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice. *Nature Genetics*, 50, 278–284.
Zheng, J., Wang, Y., He, Y., Zhou, J., Li, Y., Liu, Q. et al. (2014) Overexpression of an S-like ribonuclease gene, OsRNS4, confers enhanced tolerance to high salinity and hyposensitivity to phytochrome-mediated light signals in rice. *Plant Science*, 214, 99–105.
Zhou, F., Lin, Q., Zhu, L., Ren, Y., Zhou, K., Shabek, N. et al. (2013) D14–SCF^{D3}-dependent degradation of D53 regulates strigolactone signalling. *Nature*, 504, 406–410.
Zhou, X. & Huang, X. (2019) Genome-wide association studies in rice: how to solve the low power problems? *Molecular Plant*, 12, 10–12.
Zhu, Y., Nomura, T., Xu, Y., Zhang, Y., Peng, Y., Mao, B. et al. (2006) *ELONGATED UPPERMOST INTERNODE* encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. *The Plant Cell*, 18, 442–456.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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