Fine Mapping and Cloning of the Novel Gene Qph-IAA30, which Simultaneously Affects the Plant Height, Panicle Length, Spikelet Number and Yield in Rice (Oryza Sativa L.)

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Research Article

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Abstract

Background: The plant height is one of the most important agronomic traits in rice (Oryza sativa L.), and the introduction of semidwarf rice led to record yield increases throughout Asia in the 1960s. Near-isogenic lines (NILs) are the most powerful tools for the detection and precise mapping of quantitative trait loci (QTLs).

Results: In this study, 176 NILs were produced from the crossing and back-crossing of two rice cultivars. Specifically, Jiafuzhan, an indica rice cultivar, served as the recipient, and Hui1586, a restorer japonica cultivar, served as the donor. Using the 176 NILs, we identified a novel QTL for plant height in NIL36. First, we mapped the QTL to a 31-kb region between the markers Indel12-29 and Indel12-31. The rice genome annotation indicated the presence of three candidate genes in this region. Through gene prediction and cDNA sequencing, we confirmed that the target gene in NIL36 was Osiaa30, hereafter referred to as qPH-iaa30. Further analysis showed that qPH-iaa30 was produced by a 1-bp deletion in the first exon that resulted in the premature termination of OsIAA30. Knockout experiments showed that qPH-IAA30 was responsible for the plant height phenotype. Although qPH-IAA30 from Jiafuzhan showed a higher plant height, the plant also exhibited a longer panicle length, more spikelets and a higher yield. Taken together, our results demonstrate that qPH-IAA30 has good specific application prospects in future rice breeding.

Conclusions: 176 NILs are produced from two rice cultivars, using the 176 NILs, a novel qPH-iaa30 for plant height is identified, and the qPH-IAA30 gene is responsible for the plant height phenotype.

Background

Plant height is an important factor determining the architecture and grain yield of cereal plants[1–2]. The semidwarf genes, which result in a shortened culm, improved lodging resistance and an increased harvest index, contributed to the “Green Revolution” in wheat and rice[3–4]. However, the wide application of dwarf germplasm resources and their narrow genetic range coupled with the excessive use of pesticides and fertilizers have led to serious environmental problems[5–6], and these problems have encouraged the study of genetic and molecular mechanisms for establishing an “ideal” plant structure through height regulation. Most of the identified genes related to plant height, such as semidwarf1 (sd1)[7], GA-insensitive dwarf1 (gid1)[8], GA-insensitive dwarf2 (gid2)[9], BR-deficient dwarf1 (brd1)[10], BR-insensitive mutant (d61)[11], and BR-deficient mutant (osdwarf4-1)[12], are related to the metabolism or signaling of the phytohormones gibberellin (GA) and brassinosteroid (BR)[6].

Although the GA- and BR-related genes associated with plant height have been extensively studied, an increasing number of novel plant height-related genes that rely on pathways other than the GA and BR pathways are being discovered. For instance, the carotenoid-derived phytohormone strigolactone has become a focus of research on plant architecture patterning [13]. Recent studies have shown that OsCKX9, which encodes a cytokinin oxidase that catalyzes the degradation of cytokinin, functions as a primary strigolactone-responsive gene that regulates rice tillering, plant height, and panicle size, likely via
the secondary response gene OsRR5, which encodes a cytokinin-inducible rice type-A response regulator. This pathway demonstrates that strigolactone regulates the rice shoot architecture by enhancing cytokinin catabolism through modulation of the expression of OsCKX9[14].

Auxin exerts pleiotropic effects on plant cell elongation, cell division and differentiation, root initiation, apical dominance, and tropic responses by regulating the expression of the early auxin-responsive auxin/indoleacetic acid (Aux/IAA) genes[15]. By screening the available databases, Jain et al. (2006) identified 31 Aux/IAA genes in rice and found that these genes have different functions[15]. OsIAA1 and OsIAA3 play important roles in the crosstalk between the auxin and brassinosteroid signaling pathways and plant morphogenesis [16, 17]. The gain-of-function mutation in OsIAA11 inhibits lateral root development in rice[18]. OsIAA13-mediated auxin signaling is involved in lateral root initiation in rice[19]. OsIAA6 is involved in drought tolerance and tiller outgrowth[20], and the OsIAA10 protein directly targets the rice dwarf virus P2 protein and enhances viral infection and disease development[21]. Near-isogenic lines (NILs) carry one or more donor chromosome segments, which provides distinct advantages for QTL identification[22]. Moreover, NILs can block background genetic noise, undoubtedly enhance our understanding of complex traits and promote plant genomic studies [22, 23].

In this study, we described the development of NILs of rice through the crossing and back-crossing of two rice cultivars. Here, the japonica cultivar Hui1586 served as the donor, and the indica cultivar Jiafuzhan served as the recipient. Using 176 NILs, we identified the plant height gene, which was designated qPH-iaa30 based on subsequent analyses. qPH-iaa30 exhibits a 1-bp deletion in the first exon that results in premature termination of qPHIAA30, which encodes an auxin-responsive protein. Using CRISPR/Cas9 genome editing, we knocked out qPH-IAA30 in Jiafuzhan and observed that the knockout mutants showed the NIL36 plant height phenotype, which demonstrated that qPH-IAA30 is a functional auxin-responsive protein that regulates the height of rice plants. Moreover, the findings showed that qPH-IAA30 simultaneously affects the plant height, panicle length, spikelets and yield and will have specific application prospects in future rice breeding.

**Methods**

**Plant materials**

The indica rice cultivar Jiafuzhan and the japonica rice cultivar Hui1586 were preserved at the Rice Research Institute, Fujian Academy of Agricultural Sciences, China. Suxiu867 (Food Crops Research Institute, Jiangsu Academy of Agricultural Sciences), a japonica cultivar, was used as the recipient, and Minghui86 (Rice Research Institute, Fujian Academy of Agricultural Sciences), a restorer indica cultivar, was used as the donor. The F₁ plants were generated from Suxiu867 as the female and Minghui86 as the male. The F₁ plants were backcrossed with Suxiu867 to produce the BC₁F₁ generation. These BC₁F₁ plants were backcrossed with Suxiu867 to produce BC₂F₁ plants, and these plants were self-pollinated to produce BC₂F₂ lines. The resulting lines were self-interbred to obtain Six generations, and a stable line named Hui1586 was obtained.
Identification of QTLs for plant height

In autumn 2018, Jiafuzhan, Hui1586 and 176 NILs were planted under natural conditions in the paddy fields of Sanya Experimental Station, Hainan Province. Forty-eight plants of each parent and each NIL were planted in six rows, and four plants in the center of each plot were selected to investigate their plant height characteristics.

QTLs were identified based on significant differences between parents. Plant height, panicle length, effective panicle number, spikelets per panicle, seed setting rate and 1000-grain weight were measured at maturity stage.

All plants were planted in accordance with standard commercial practices, with row spacing ranging from 13.3 cm to 26.4 cm, and field management generally followed normal rice field management practices.

Construction of a genetically mapped population

NIL36 hybridized with Jiafuzhan to form a mapping population. The F\textsubscript{2} location population was constructed by self-crossing of F\textsubscript{1} population. A primary linkage of the QTL for plant height was obtained using 45 recessive plants from the F\textsubscript{2} population, and 1264 recessive plants in the F\textsubscript{2} population were selected for fine localization.

PCR amplification and marker detection analysis

The CTAB method\textsuperscript{[24]} with minor modifications was used for the extraction of plant DNA from frozen leaves of the rice plants. For PCR amplification, each 20-µL reaction mixture contained 30 ng DNA, 0.4 µM primers and 2× Es Tag MasterMix (Dye). The amplification program includes the following procedures: 2 min at 94°C; 33 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and a final extension at 72°C for 2 min. The amplified PCR product underwent 3% agarose gel electrophoresis and was stained with ethidium bromide\textsuperscript{[25]}.

Molecular mapping of QTLs for plant height

We used phenotypic data and SSR markers for the detection of QTLs. Genetic distance was estimated using MapDraw V2.1\textsuperscript{[26]}. The genetic linkage map obtained in this study is basically consistent with that reported by Rahman et al \textsuperscript{[27]}.

Physical map construction and bioinformatics analysis of QTLs for plant height

The physical map of QTLs for plant height was constructed through a bioinformatics analysis using the published sequences of BAC and P1-derived artificial chromosome (PAC) clones of cv. Nipponbare released by the International Rice Genome Sequencing Project (IRGSP, http://rgp.dna.affrc.go.jp/IRGSP/index.html). Target gene linkage markers were used to clone and sequence alignment was performed using the matching Basic Local Alignment Search Tool. According to
the existing sequence annotation database (http://rice.plantbiology.msu.edu/; http://www.tigr.org/).

Candidate genes of the prediction were based on the existing sequence annotation database analysis (http://rice.plantbiology.msu.edu/; http://www.tigr.org/).

**Targeted knockout of OsIAA30 in rice using the CRISPR/Cas9 system**

The first exon of the *OsIAA30* gene in Jiafuzhan was targeted with one gRNA spacer. Highly specific gRNA spacer sequences were designed using CRISPR plant database and website (Supplementary Table 1)[28]. Genome editing mutations of target genes in regenerated plants were analyzed. The deletion and insertion of genes were detected by PCR. PCR products were selected from transgenic CRISPR-edited strains for sequencing to identify specific mutations. The degradation sequence decoding method was used to analyze the double peaks [29]. The primers used in CRISPR/Cas9 studies were shown in Table S1.

**Detection of hormone levels**

Stems of Jiafuzhan (CK), NIL36, *OsIAA30KO*-line1, *OsIAA30KO*-line2 and *OsIAA30KO*-line3 were obtained during the rice jointing stage. The auxin (IAA) contents were then detected using MetWare (http://www.metware.cn/) based on the AB Sciex QTRAP4500 LC-MS/MS platform. Three replicates were performed for each experiment.

**Results**

**NILs development**

For development of the NILs, Jiafuzhan, an *indica* cultivar, was used as the recipient, and the restorer *japonica* cultivar Hui1586 was used as the donor. The F1 plants were generated from Jiafuzhan as the female and Hui1586 as the male, and the F1 plants were back-crossed with Jiafuzhan to produce the BC1F1 generation. These BC1F1 plants were then backcrossed with Jiafuzhan to produce BC2F1 plants. Using the same approach, 118 BC3F1 individuals were obtained, and these plants were self-pollinated to produce the BC3F2 lines. Based on their characteristics, we selected one or two individual plants from each line. As a result, 176 NILs were obtained (Fig. 1).

**Identification and analysis of QTLs for plant height in the NILs**

To evaluate the potential advantages of the NILs for QTL detection, the phenotypic variations in plant height were observed in 176 NILs, and NIL36 exhibited a lower plant height than Jiafuzhan. Further investigations and analyses showed that the plant height of Jiafuzhan was 116.22 cm, whereas that of Jiafuzhan NIL36 was 79.52 cm. The difference in plant height between these lines reached a very significant level (Fig. 2).
The phenotypic comparisons between NIL36 and Jiafuzhan are presented in Table 1. The results showed some significant differences in major agronomic traits, including the plant height, panicle length, spikelets per panicle and yield per plant, between NIL36 and Jiafuzhan. However, no significant difference in the number of effective panicles, seed setting rate, 1,000-grain weight, grain length or grain width was found (Table 1).

### Table 1
Comparison of the main agronomic traits of Jiafuzhan, NIL36 and knockout lines

| Traits                  | Jiafuzhan          | NIL36             | qPH-iaa30KO-Line 1 | qPH-iaa30KO-Line 2 | qPH-iaa30KO-Line 3 |
|-------------------------|--------------------|-------------------|---------------------|---------------------|---------------------|
| Plant height (cm)       | 116.22 ± 2.26      | 79.52 ± 1.72**    | 80.22 ± 1.76**      | 81.02 ± 1.82**      | 80.35 ± 1.81**      |
| Panicle length (cm)     | 27.12 ± 1.12       | 21.26 ± 1.08*     | 22.36 ± 1.24*       | 22.66 ± 1.12*       | 21.96 ± 1.32*       |
| Number of effective panicles | 10.54 ± 1.04       | 10.82 ± 1.08      | 10.42 ± 1.12        | 10.82 ± 1.18        | 10.72 ± 1.02        |
| Spikelets per panicle   | 168.46 ± 4.86      | 125.86 ± 4.32**   | 128.76 ± 4.62**     | 130.12 ± 4.82**     | 122.86 ± 4.22**     |
| Seed setting rate (%)   | 97.52 ± 1.26       | 98.22 ± 1.18      | 97.28 ± 1.28        | 97.38 ± 1.18        | 96.98 ± 1.18        |
| 1,000-grain weight (g)  | 23.32 ± 0.54       | 23.44 ± 0.42      | 23.12 ± 0.48        | 23.54 ± 0.53        | 23.64 ± 0.46        |
| Grain length (mm)       | 10.95 ± 0.13       | 10.72 ± 0.16      | 10.80 ± 0.20        | 10.92 ± 0.214       | 10.88 ± 0.15        |
| Grain width (mm)        | 2.68 ± 0.08        | 2.72 ± 0.05       | 2.70 ± 0.08         | 2.66 ± 0.09         | 2.68 ± 0.07         |
| Yield per plant (g)     | 40.38 ± 1.02       | 31.35 ± 0.98**    | 30.17 ± 1.08**      | 32.33 ± 1.02**      | 31.16 ± 1.08**      |

*P < 0.05 and **P < 0.01 for the differences among Jiafuzhan, NIL36 and knockout lines. The data were derived from the trial performed at the Hainan experimental station in April 2019.

### Genetic analysis of NIL36 in terms of plant height

To determine whether NIL36 was controlled by a single gene, NIL36 was hybridized with Jiafuzhan. F₁ hybridization showed the phenotype of Jiafuzhan, and the F₂ population showed Mendelian segregation (Table 2). Segregation between the Jiafuzhan and NIL36 phenotypes fit a 3:1 segregation ratio in the two F₂ populations ($\chi^2 = 0.134 \sim 0.456, P > 0.5$). The results showed that the NIL36 phenotype for plant height was controlled by a single recessive gene.
Table 2
Segregation of the F₂ populations crossed with NIL36

| Crosses         | F₁ phenotype | F₂ population                      | χ²(3:1) P     |
|-----------------|--------------|------------------------------------|---------------|
|                 |              | Normal type of Jiafuzhan            | Normal type of NIL36 | Total plants |               |
| NIL36/Jiafuzhan | Normal type of Jiafuzhan | 240 | 82 | 322 | 0.456* 0.5–0.75 |
| Jiafuzhan/NIL36 | Normal type of Jiafuzhan | 286 | 90 | 376 | 0.134* > 0.9   |

* The segregation ratio of normal to mutant plants was 3:1 at the 0.05 significance level.

**Linkage analysis of the QTL for plant height in NIL36**

To identify the gene responsible for the NIL36 phenotype, we located the QTL for plant height in NIL36, and a total of 506 SSR markers from the rice molecular map were selected for polymorphism surveys between Hui1586 and Jiafuzhan[30]. Of these, 296 pairs exhibited polymorphism. Based on these 296 primer pairs, 45 recessive plants from the F₂ population (NIL36/Jiafuzhan) were used for a linkage analysis between markers and the QTL. One of these SSR markers, RM3326 on chromosome 12, was found to be linked to the trait in the 45 F₂ individuals.

**Initial localization of the QTL for plant height**

Published markers around RM3326 were used to initially locate the QTL. A genetic linkage analysis revealed that the QTL was located between the molecular markers RM2854 and RM235, which are located at a distance of 7.7 cM (Fig. 3-a).

To determine the location of the QTL within a smaller region, we identified 1264 recessive plants from the F₂ population from Jiafuzhan/NIL36, and six polymorphic indel markers were screened from 18 newly developmental indels (Table 3). Indel markers from the open rice genome sequences were designed and tested to predict the likelihood of polymorphism between NIL36 and Jiafuzhan by comparing sequences from *Nipponbare* (http://rgp.dna.affrc.go.jp/) and the *indica* cultivar 93–11 (http://rice.genomics.org.cn/). The genotyping of all recombinant genes was performed using six polymorphic markers. The results showed that the QTL was located in the 295-kb region between the molecular markers Indel12-7 and Indel12-9 on chromosome 12 (Fig. 3-b and Table 3).
Table 3

| Marker       | Sequence of the forward primer | Sequence of the reverse primer |
|--------------|--------------------------------|--------------------------------|
| RM3326       | CTCTCATCCACATCGTCACCAC         | TCGTCGGGAGAGAGAGAGAG          |
| RM2854       | ATGAGAGAGAGAAAGAGAGT           | AATGGAGAGAAAAAGTATTA          |
| RM235        | AGAAACATAGGTCACCAAGCAC         | TCACCTGGTCAGGCTTCTTTC         |
| Indel12-1    | CACCATGGACAGATTCTCTCTCG        | GATCGATGACGAAGAGAGAGAC        |
| Indel12-4    | GCGAGGTGTGGTGAGGATG            | ACACCTCATCTTGGCCTTCTCG        |
| Indel12-7    | ATCATCGTCATCGACTCTCTCTCC       | CGTCAGTTCTGAGGCGTATAAGG       |
| Indel12-9    | ACGGTGGTGTTGTTGTGGTGTTG       | TTAACCTTTGGCCGGGAGGTG         |
| Indel12-12   | GGTGTTGATTAAGCTGATCTCTCTCC    | GATCAGCAACAAGCAGCTCACG        |
| Indel12-15   | TTGCTACTACACACACACAGGTTCC     | GCAGCCACACGCTTTGAAATAGC       |
| Indel12-20   | CAACACGGGTGAAGAGAGA           | CTTTGTACCTTGTGCTAC           |
| Indel12-23   | TAGAAGATGGGGAAGAGGAA          | TGTTCATTTACATGCAAGG          |
| Indel12-24   | ACATCGATCCTCCTGATGTT          | ACATCACGTGGGTAAATTT          |
| Indel12-26   | TCCATACACACCACACCTCCT         | TTTTCCTGACATTTGGAAC          |
| Indel12-29   | TGCTGAATAATCTGTGTG            | ATCTTTTTCTGGGTTGTA           |
| Indel12-31   | CATACACACAACAAATAGAA         | CGCCATCTTTAAATAGGTT          |
| Indel12-33   | ACACGTCTTTTCTGACAGAT         | GAACGAACATGAAGAGCTA          |
| Indel12-36   | TGGATGCATGGTAAACTAATG        | TGAATTGCTCTCCATGAAAT         |

Fine mapping of the QTL for plant height

For the fine mapping of the QTL, eight polymorphic indel markers were screened from 26 newly developed indels (Table 3). Recombinant screening with eight markers located in a more internal position within the target locus detected 13, 11, eight, six, two, one, two, and five recombinant plants, respectively (Fig. 3-c). Thus, the QTL was precisely located within the 31-kb region between the molecular markers Indel12-29 and Indel12-31.

Candidate genes in the 31-kb region

According to the available sequence annotation databases (http://rice.plantbiology.msu.edu/; http://www.tigr.org/), three annotated genes were located in the 31-kb region (Fig. 3-d), and all had a corresponding full-length cDNA. Among these genes, LOC_Os12g40860 encodes the leucine-rich repeat
family protein, LOC_Os12g40880 encodes the uridine kinase family protein, and LOC_Os12g40890 is the auxin-responsive Aux/IAA gene family member OsIAA30.

**Sequence analyses of the QTL for plant height**

To identify the gene responsible for the observed phenotype, we then sequenced three genes of Jiafuzhan and NIL36. A deletion of only 1 bp (120:C) was found in LOC_Os12g40890 (Fig. 4), and no further difference was observed in the remaining two gene sequences. Thus, we hypothesized that LOC_Os12g40890 and OsIAA30 corresponded to the QTL for plant height in NIL36, and this gene was tentatively designated qPH-iaa30.

The analysis of the open reading frame (ORF) region showed that qPH-IAA30 had five exons. qPH-iaa30 exhibited a 1-bp deletion in the 120th base of the first exon, which resulted in the premature termination of OsIAA30 (Fig. 4).

**qPH-iaa30 is responsible for the plant height phenotype of NIL36**

To confirm that qPH-IAA30 confers a plant height phenotype, we examined whether the knockout of qPH-IAA30 in Jiafuzhan would lead to the NIL36 phenotype. One sequence-specific guide RNA (sgRNA) was designed to knock out qPH-IAA30 using the CRISPR/Cas9 gene editing system. A total of three plants from three independent events (OsIAA30KO-line1, OsIAA30KO-line2 and OsIAA30KO-line3) were obtained, and sequencing confirmed that these plants carry mostly insertions or deletions in the targeted sites (Supplementary Fig. 1).

We then investigated the plant height phenotype of these three homozygous lines at maturity and found that all three lines showed the NIL36 phenotype (Fig. 5 and Table 1). Therefore, the targeted mutation of qPH-IAA30 led to the NIL36 plant height phenotype, which indicated that the loss of function of OsIAA30 was responsible for this phenotype. Most importantly, OsIAA30KO-line1, OsIAA30KO-line2 and OsIAA30KO-line3 showed shorter panicle lengths, fewer spikelets per panicle and lower yields than Jiafuzhan (Fig. 5 and Table 1). Therefore, we hypothesized that the qPH-IAA30 gene not only affected the plant height but also regulated the panicle length, spikelets per panicle and yield in rice.

**Comparative analysis of the hormone content between Jiafuzhan and OsIAA30KO-lines**

To analyze whether qPH-IAA30 affects changes in the Aux/IAA levels, we measured the Aux/IAA content in Jiafuzhan (CK) and OsIAA30KO-line1. The results showed that the Aux/IAA content of OsIAA30KO-line1, OsIAA30KO-line2, OsIAA30KO-line3 and NIL36 was significantly lower than that of Jiafuzhan (CK) (Fig. 6).
Discussion

NILs have been developed and used for genetic studies and the fine mapping of QTLs for genome-wide target traits[22]. For example, each NIL carries one or more donor chromosome segments, which provides distinct advantages for QTL identification, and a QTL can be visualized as a single Mendelian factor by blocking background genetic noise. Several QTLs, such as \( qDTY\,2.2 \)[23], \( Cn1a[31] \), \( GS3[32] \), \( GW2[33] \), \( Ghd7\)[34], \( DEP1\)[35], cold tolerance QTLs[36], 99 putative QTLs[37] and \( qHD19\)[38], have been identified or cloned based on NILs. Moreover, NILs block background genetic noise, undoubtedly enhance the understanding of complex traits and promote plant genomic studies[22, 23]. In the present study, we developed 176 NILs with the genetic background of Jiafuzhan rice. Using these lines, we mapped the plant height gene as the \( qPH-iaa30 \) gene. Map-based cloning and knockout experiments confirmed that the plant height phenotype of NIL36 was caused by loss of function of the \( OsIAA30 \) gene.

Previous studies have indicated that Aux/IAA genes play important roles in plant growth and development by regulating the expression of early auxin-responsive genes[15]. For example, \( OsIAA1 \) and \( OsIAA3 \) affect plant morphogenesis[16, 17], \( OsIAA11 \) and \( OsIAA13 \) affect root development in rice[18, 19], and \( OsIAA6 \) is involved in tiller outgrowth[20]. The present study demonstrated that the \( qPH-iaa30 \) gene significantly reduces plant height in rice, and knockout experiments confirmed that \( qPH-iaa30 \) was responsible for the plant height phenotype. Three homozygous knockout lines also exhibited the NIL36 phenotype, which includes a lower plant height, a shorter panicle length, fewer spikelets per panicle and a lower yield per plant compared with Jiafuzhan (Table 1). Further analysis of the hormone content showed that the Aux/IAA contents of NIL36, \( OsIAA30KO-line1 \), \( OsIAA30KO-line2 \) and \( OsIAA30KO-line3 \) were significantly lower than that of Jiafuzhan (CK) (Fig. 6). Therefore, we hypothesized that \( qPH-IAA30 \) could simultaneously regulate the plant height, panicle length, spikelets per panicle and yield per plant by affecting the auxin levels.

Although \( qPH-IAA30 \) affects certain traits, such as the plant height, panicle length, spikelets per panicle and yield per plant, \( qPH-IAA30 \) has specific application prospects in the improvement of rice breeding. First, to further increase the rice yield, breeders can transform \( qPH-IAA30 \) into excellent material via molecular marker-assisted selection. Second, the \( qPH-iaa30 \) gene is controlled by a single recessive gene. Therefore, to breed a new hybrid rice variety with an ideal plant height, breeders can transfer this gene into both restorer and sterile lines through molecular marker-assisted selection.

Conclusions

In this study, 176 NILs were produced from the crossing and back-crossing of two rice cultivars. Using the 176 NILs, we identified a novel \( qPH-iaa30 \) for plant height in NIL36. Further analysis show that \( qPH-iaa30 \) is produced by a 1-bp deletion in the first exon that results in the premature termination of \( OsIAA30 \) and the \( qPH-IAA30 \) gene is responsible for the plant height phenotype.

Declarations
Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The genome sequence of the *qPH-IAA30 (OsIAA30)* can be found in the NCBI database (http://www.ncbi.nlm.nih.gov/), and the number of GenBank is AK068213.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All the authors contributed to the conception and design of the study. YD planned and performed the experiments. The preparation of the materials was performed by CC and YN. The collection and analysis of the data were performed by HN, HF, ZG, and ZX. The first draft of the manuscript was written by YD, HN, and CC and YN prepared figures 1-2, HF, ZG prepared figures 3-5, ZX prepared figure 6. All the authors read and approved the final manuscript.

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References

1. Wang B, Smith SM, Li JY. Genetic regulation of shoot architecture. *Annu Rev Plant Biol*. 2018; 69: 437–468.

2. Liao ZG, Yu H, Duan JB, Yuan K, Yu CJ, Meng XB, Kou LQ, Chen MJ, Jing YH, Liu GF, Smith SM, Li JY. SLR1 inhibits MOC1 degradation to coordinate tiller number and plant height in rice. *Nat Commun*. 2019; 10: 2738.

3. Spielmeyer W, Ellis MH, Chandler PM. Semidwarf (sd-1), “green revolution” rice, contains a defective gibberellin 20-oxidase gene. *Proc Natl Acad Sci U S A*. 2002; 99: 9043–9048.

4. Peng JF, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP. ‘Green revolution’ genes encode mutant gibberellin response modulators. *Nature*. 1999; 400: 256–261.

5. Pimentel, D. Green revolution agriculture and chemical hazards. *The Science of the Total Environment*. 1996; 188: S86-S98.

6. Deng WJ, Li RQ, Xu YW, Mao RY, Chen SF, Chen LB, Chen LT, Liu YG, Chen YL. A lipid transfer protein variant with a mutant eight-cysteine motif causes photoperiod- and thermo-sensitive dwarfism in rice. *J Exp Bot*. 2019; 71: 1294–1305.

7. Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M. A mutant gibberellin-synthesis gene in rice. *Nature*. 2002; 416: 701–702.

8. Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YIC, Kitano H, Yamaguchi I. *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature*. 2005; 437(7059): 693–698.

9. Hirano K, Asano K, Tsuji H, Kawamura M, Mori H, Kitano H, Ueguchi-Tanaka M, Matsuoka M. Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. *Plant Cell*. 2010; 22: 2680–2696.

10. Mori M, Nomura T, Ooka H, Ishizaka M, Yokota T, Sugimoto K, Okabe K, Kajiwara H, Satoh K, Yamamoto K, Hirochika H, Kikuchi S. Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis. *Plant Physiol*. 2002, 130(3): 1152–1161.

11. Hong Z, Ueguchi-Tanaka M, Umemura K, Uozu S, Fujioka S, Takatsuto S, Yoshida S, Ashikari M, Kitano H, Matsuoka M. A rice brassinosteroid-Deficient mutant, ebisu dwarf (d2), is caused by a loss of function of a new member of cytochrome P450. *Plant Cell*. 2003; 15(12): 2900–2910.

12. Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S, Tanaka H, Kitano H, Matsuoka M. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nat Biotechnol*. 2006, 24: 105–109.

13. Zhou F, Lin QB, Zhu LH. D14-SCF (D3)-dependent degradation of D53 regulates strigolactone signalling. *Nature*. 2013; 504: 406–410.
14. Duan JB, YuH, Yuan K, Liao ZG, Meng XB, Jing YH, Liu GF, Chu JF, Li JY. Strigolactone promotes cytokinin degradation through transcriptional activation of CYTOKININOXIDASE/DEHYDROGENASE 9 in rice. Proc Natl Acad Sci U S A. 2019; 116(28): 14319–14324.

15. Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP. Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (Oryza sativa). Funct Integr Genomi. 2006; 6: 47–59.

16. Thakur JK, Tyagi AK, Khurana JP. OsIAA1, an Aux/IAA cDNA from rice, and changes in its expression as influenced by auxin and light. DNA Research. 2001; 8: 193–203.

17. Nakamura A, Umemura I, Gomi KJ, Hasegawa Y, Kitano H, Sazuka T, Matsuoka M. Production and characterization of auxin-insensitive rice by overexpression of a mutagenized rice IAA protein. Plant J. 2006; 46: 297–306.

18. Zhu ZX, Liu Y, Liu SJ, Mao CZ, Wu YR, Wu P. A gain-of-function mutation in OsIAA11 affects lateral root development in rice. Mol Plant. 2012; 5: 154–161.

19. Kitomi Y, Inahashi H, Takehisa H, Sato Y, Inukai Y. OsIAA13-mediated auxin signaling is involved in lateral root initiation in rice. Plant Sci. 2012; 190: 116–122.

20. Jung H, Lee DK, Choi DY, Kim JK. OsIAA6, a member of the rice Aux/IAA gene family, is involved in drought tolerance and tiller outgrowth. Plant Sci. 2015; 236: 304–312.

21. Jin L, Qin QQ, Wang Y, Pu YY, Liu LF, Wen X, Ji SY, Wu JG, Wei CH, Ding B, Li Y. Rice dwarf virus P2 protein hijacks auxin signaling by directly targeting the rice OsIAA10 protein, enhancing viral infection and disease development. PLOS Pathog. 2016; 12(9): e1005847.

22. Yang DW, Ye XF, Zheng XH, Cheng CP, Ye N, Huang FH. Development and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of the whole wild rice genome. Front Plant Sci. 2016; 7: 01737.

23. Henry A, Swamy BPM, Dixit S, Torres RD, Batoto TC, Manalili M, Anantha MS, Mandal NP, Kumar A. Physiological mechanisms contributing to the QTL-combination effects on improved performance of IR64 rice NILs under drought. J Exp Bot. 2015; 66(7): 1787–1799.

24. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 1980; 8(19): 4321–4325.

25. Panaud O, Chen X, Mccouch SR. Development of microsatellite and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.). Mol Gen Genet. 1996; 252: 597–607.

26. Liu RH, Meng JL. MapDraw: a microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas (Beijing). 2003; 25: 317–321.

27. Rahman ML, Chu SH, Choi MS, Qiao YL, Jiang WZ, Piao RH, Khanam S, Cho YI, Jeung JU, Jena K, Koh HJ. Identification of QTLs for some agronomic traits in rice using an introgression line from Oryza minuta. Mo Cell. 2007; 24(1): 16–26.

28. Xie KB, Zhang JW, Yang YN. Genome-wide prediction of highly specific guide RNA spacers for CRISPR-Cas9-mediated genome editing in model plants and major crops. Mol Plant. 2014; 7(5): 923–926.
29. Ma XL, Chen LT, Zhu QL, Chen YL, Liu YG. Rapid decoding of sequence-specific nuclease-induced heterozygous and biallelic mutations by direct sequencing of PCR products. *Mol Plant.* 2015; 8(8): 1285–1287.

30. Mccouch SR, Teytelma L, Xu YB, Lobos KB, Clare K, Walton M, Fu BY, Maghirang R, Li ZK, Xing YZ, Zhang QF, Kono I, Yano M, Jellstrom RF, de Clerck G, Schneider D, Cartinhour S, Ware D, Stein L. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.), *DNA Research.* 2002; 9: 199–207.

31. Ashikari M, Sakakibara H, Lin S Y, Yamamoto T, Takashi T, Nishimura A, Angeles E R, Qian Q, Kitano H, Matsuoka M. Cytokinin oxidase regulates rice grain production. *Science.* 2005; 309: 741–745.

32. Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet.* 2006; 112: 1164–1171.

33. Song XJ, Huang W, Shi M, Zhu MZ, Lin HX. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet.* 2007; 39: 623–630.

34. Xue W Y, Xing Y Z, Weng X Y, Zhao Y, Tang W J, Wang L, Zhou H J, Yu S B, Xu C G, Li X H, Zhang Q F. 2008. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nat Genet,* 40: 761–767.

35. Huang X Z, Qian Q, Liu Z B, Sun H Y, He S Y, Luo D, Xia G M, Chu C C, Li J Y, Fu X D. 2009. Natural variation at the DEP1 locus enhances grain yield in rice. *Nat Genet,* 41: 494–497.

36. Zhou L, Zeng Y W, Hu G L, Pan Y H, Yang S M, You A Q, Zhang H L, Li J J, Li Z C. 2012. Characterization and identification of cold tolerant near-isogenic lines in rice. *Breed Sci,* 62: 196–201.

37. Furuta, T, Uehara K, Rosalyn B, Angeles-Shim R B, Shim J, Ashikari M, Takashi T. 2014. Development and evaluation of chromosome segment substitution lines (CSSLs) carrying chromosome segments derived from *Oryza rufipogon* in the genetic background of *Oryza sativa* L. *Breed Sci,* 63(5): 468–475.

38. Yang D W, Cheng C P, Zheng X H, Ye X F, Ye N, Huang F H. 2020. Identification and fine mapping of a major QTL, qHD19, that plays pleiotropic roles in regulating the heading date in rice. *Mol Breeding,* 40:234.

**Figures**
Flowchart of the development of NILs in the present study. Jiafuzhan was used as the recipient, and Hui1586 was used as the donor. The F1 plants were continuously backcrossed with Jiafuzhan to produce BC1F1, BC2F1 and BC3F1 plants.

Figure 1
Figure 2

Phenotypic comparison of Jiafuzhan and NIL36. The phenotypes of Jiafuzhan and NIL36 at the mature period are shown.
Figure 3

Genetic and physical maps of qPH-iaa30. a: Primary mapping of qPH-iaa30. The gene was mapped to the region between the markers RM2854 and RM235. b: Further mapping of qPH-iaa30. The gene was mapped to the region between markers Indel12-7 and Indel12-9. c: Fine mapping of qPH-iaa30. qPH-iaa30 was localized to a 31-kb region between the markers Indel12-29 and Indel12-31, and the recombinant number between the markers and target genes is indicated under the linkage map. d: Candidate genes in the 31-kb target region.
Figure 4

Structural comparison between OsIAA30 and qPH-iaa30. OsIAA30 has five exons, and qPH-iaa30 exhibits a 1-bp deletion in the first exon.
OsIAA30-knockout mutants showing the NIL36 phenotype. The three knockout lines generated using CRISPR/Cas9 technology exhibit the NIL36 phenotype.
Figure 6

Comparison of Aux/IAA levels between Jiafuzhan and OsIAA30KO-line1. The Aux/IAA contents of OsIAA30KO-line1, OsIAA30KO-line2, OsIAA30KO-line3 and NIL36 were significantly lower than those of Jiafuzhan (CK). Three experimental replicates of each line were included. *, Statistical significance (P < 0.01) determined using Student’s t-test

Supplementary Files

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