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A Dysfunction in a Member of the Ap2/Erf And F-Box Protein Family Increases the Full Emergence of Panicles in Rice

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ABSTRACT

Complete panicle exsertion (CPE) is a significant quantitative trait that influences grain production in rice. We utilized a comprehensive strategy to investigate the molecular basis of CPE by employing a stable ethyl methane sulfonate mutant line, CPE-109 of the Samba Mahsuri (SM) rice variety (Oryza sativa), which demonstrates CPE. Two stable genomic regions associated with CPE were discovered through quantitative trait locus (QTL) mapping [qCPE-4 (28.24–31.22 Mb) and qCPE-12 (2.30–3.18 Mb)] as well as QTL-sequencing [chr 4 (31.21–33.69 Mb) and chr 12 (0.12–3.15 Mb)]. Two non-synonymous single nucleotide polymorphisms, specifically KASP 12–12 (T→C; chr12:1269983) in Os12g0126300, which encodes an AP2/ERF transcription factor, and KASP 12–16 (G→A; chr12:1515198) in Os12g0131400, encoding a protein with an F-box domain, accounted for 81.05% and 59.61% of the phenotypic variance, respectively.

I. INTRODUCTION

Rice (Oryza sativa L.) is an essential staple food crop that greatly affects the culture, diet, and economy of more than half of the world's population. The panicle is vital for grain production as its structure significantly influences the yield potential of rice. Complete panicle exsertion (CPE) is an important quantitative trait that impacts how the panicle extends beyond the flag leaf, which improves grain filling and subsequently boosts yield. In contrast, incomplete panicle exsertion, a frequent adverse occurrence, describes panicles that are partially obscured by the flag leaf (3–4 cm), resulting in a 10–20% reduction in overall crop yield (Guan et al., 2011). A phenotype known as elongated uppermost internode (EUI) promotes panicle exsertion by extending the upper internode. The main distinction between EUI and CPE is that elongation of the uppermost internode is not present in CPE. The CPE trait is essential for hybrid seed production, as numerous cytoplasmic male sterile lines display incomplete panicle exsertion, which can lead to less effective pollination. There are limited genetic resources and molecular markers related to CPE, constraining focused breeding initiatives.

The QTL-sequencing (QTL-seq) technique, based on next-generation sequencing, has emerged as an efficient method for pinpointing specific genomic regions in rice and various other plant species. Takagi et al. (2013) pioneered this approach and successfully identified QTLs associated with resistance to blast disease and seedling vigor in rice. Using the QTL-seq method, several QTLs have been discovered, including those related to rice grain length (qGL3), grain weight (qTGW3.1 and qTGW3.3), the dwarfing gene that affects plant height (asd1), and mesocotyl length (Wang et al., 2021). RNA-seq, a transcriptome analysis technique based on next-generation sequencing, has been widely utilized to explore the genes responsible for specific economically important traits in various spatial and temporal contexts.

II. Plant material and development of mapping population

In 2015, the stabilised mutant line CPE-109 was crossed with RPHR-1005, a divergent line with incomplete panicle exsertion, to create a bi-parental mapping population (F2) for QTL mapping and QTL-seq analysis. Both populations (n=200) were raised in the wet season of 2016 at ICAR- Indian Institute of Rice Research's experimental field in Hyderabad, following suggested agronomic approach (Guha et al., 2024).

III. Phenotyping for panicle exsertion

The exsertion of panicles of each line of the F2 mapping populations (*n*=200) derived from CPE-109×RPHR-1005 and CPE-109×SM was manually measured using a 30 cm ruler at the reproductive maturity stage. The phenotype in which the panicle was fully enclosed (2–4 cm covered with the flag leaf) was classified as Samba Mahsuri/RPHR 1005 type and considered non-CPE with a score of '0'. The phenotype with panicles completely exserted (3–4 cm) from the flag leaf was classified as a mutant type and considered CPE with a score of '1'. The phenotype with panicles exerted by 1–2 cm from flag leaf was classified as an intermediate type with a score of '2'.

IV. DNA isolation

Genomic DNA was isolated using cetyl-tri-methyl ammonium bromide method (Gumi *et al.*, 2018). The quality of isolated DNA was assessed using 0.8% agarose gel electrophoresis and quantified using a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA).

V. Genotyping and quantitative trait locus analysis

Genotyping of 200 F2 lines from CPE-109×RPHR-1005 was conducted using simple sequence repeat (SSR) markers, following a previously outlined protocol (Aglawe et al., 2018). A total of 835 SSR markers, spread evenly across the 12 rice chromosomes, were utilized to assess the parental polymorphism between CPE-109 and RPHR-1005, resulting in the identification of 135 polymorphic SSRs. These 135 SSR markers were then used to genotype the F2 population of CPE-109×RPHR-1005. The amplified products were separated electrophoretically on 3% agarose gel (Lonza, Rockland, ME, USA) alongside a 100 bp DNA ladder to determine the size of the products for each primer pair. The amplified SSR fragments from each F2 individual were recorded and assigned scores of '0' (for CPE-109 allele), '1' (for RPHR-1005 allele), '2' (representing heterozygosity), and −1 (indicating absence of amplicons) for each primer. QTL analysis was conducted using QTL IciMapping v. 4.2 (which includes composite interval mapping) with a logarithm of the odds (LOD) threshold set at 2.5 to identify significant QTLs (Aglawe et al., 2018). The underlying genes for the identified QTLs were retrieved from the RAP-DB database (http://rapdb.dna.affrc.go.jp/) and classified based on their functional annotations.

VI. Sequencing analysis for Quantitive trait locus

In the F2 population derived from CPE-109×SM, CPE phenotyping facilitated the creation of two distinct bulks: one comprising 20 F2 individuals exhibiting the mutant phenotype (designated as CPE bulk), and the other consisting of 20 individuals with the SM phenotype (identified as non-CPE bulk). Genomic DNA was isolated from 100 mg of fresh leaves of selected F2 individuals utilizing the DNeasy Plant Mini Kit (Qiagen), and the DNA concentration was measured using the Quant-iT PicoGreen dsDNA reagent and kits (Thermo Fisher Scientific). The pooled DNA from both extreme bulks, along with the genomic DNA from the parental lines, was processed for whole genome re-sequencing. A DNA-seq library was prepared using the NEB Next Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, USA) by adhering to the manufacturer's instructions and sequenced on an Illumina HiSeq 2500 system (Illumina, USA) employing the 2 × 150 bp paired-end read mode, achieving an approximate sequencing depth of 40× for each sample. The tasks of library preparation and sequencing were contracted out to Nucleome Informatics Pvt Ltd, located in Hyderabad, India. The raw '.fastq' data generated from the sequencing process were evaluated with FastQC v0.11.9 (Andrews, 2010). Subsequently, the data were utilized directly as input for the Python-based QTL-seq pipeline with the command: qtlseq -r RICE_MSUv7.fasta -p wildtype_1.fq.gz,wildtype_2.fq.gz -b1 non-cpe_bulk_1.fq.gz,non-cpe_bulk_2.fq.gz -b2 cpe_bulk_1.fq.gz,cpe_bulk_2.fq.gz -n1 20 -n2 20 -o cpe_output (Sugihara et al., 2022). The alignment file statistics and variant file statistics were generated using samtools v1.9 and bcftools v1.9, respectively. The single nucleotide polymorphisms (SNPs) within the QTL interval were annotated through the variant effect predictor (VEP) in plant Ensembl release 51 (McLaren et al., 2016).

VII. KASP markers and assays development

A selection of homozygous SNPs located in genomic areas pinpointed by linkage mapping and QTL-seq was chosen for verification with Kompetitive Allele-Specific PCR (KASP) primers. A cohort consisting of advanced breeding lines (BC1F6; n=29), F2 populations from CPE-109×SM (n=200) and CPE-109×RPHR-1005 (n=200), Samba Mahsuri CPE mutant lines (n=12), and approved rice varieties (n=15) was utilized for KASP genotyping. The genotyping procedures were carried out in a Bio-Rad qRT-PCR 384-well format, conducted as 10 µl reactions with 4.86 µl of template DNA (10 ng), 5.0 µl of 2× KASP master mix, and 0.14 µl of primer mix. PCR amplification was performed on a StepOne Plus machine (Thermo Fisher Scientific, USA) according to the protocol: a pre-read phase at 30 °C for 1 minute, followed by a hot start at 94 °C for 15 minutes, then 10 touchdown cycles (94 °C for 20 seconds; touchdown at 61 °C, decreasing 1 °C each cycle for 60 seconds), followed by 26 amplification cycles (94 °C for 20 seconds; 55 °C for 60 seconds). The dyes FAM_{Abs} (485 nm), HEX_{Abs} (535 nm), and ROX_{Abs} (575 nm) were used to capture fluorescence data during both the pre-read and post-read phases (30 °C for 1 minute). The association between markers and traits was evaluated using single marker analysis with QTL IciMapping v. 4.2, as well as through single-factor ANOVA in Microsoft Excel.

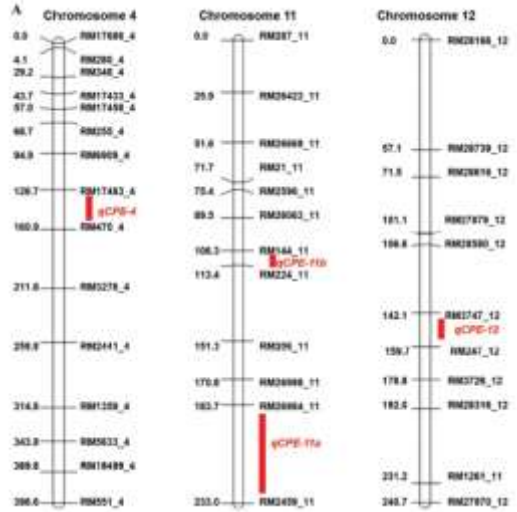
VIII. Genomic regions identified for CPE through bi-parental mapping

The mutant CPE-109 displayed comparable distinctness, uniformity, and stability (DUS) traits to SM, differing only in the

CPE characteristic (Figure 1).

Figure 1 Panicle exsertion patterns among CPE-109 (A), Samba Mahsuri (B), and RPHR-1005 (C).

An F2 mapping population made up of 200 lines, derived from CPE-109 × RPHR-1005, was used for the QTL analysis of CPE. A linkage map was created using 135 polymorphic SSR markers, and the QTL analysis was performed with phenotyping data through QTL IciMapping v. 4.2. The QTL analysis revealed four genomic regions associated with CPE: qCPE-4, qCPE-11a, qCPE-11b, and qCPE-12. The individual QTL intervals qCPE-4, qCPE-11a, and qCPE-12 accounted for 13.23%, 34.91%, and 27.13% of the phenotypic variation, respectively, while qCPE-11b had the least contribution at 6.19% to the overall panicle exsertion (Figure 2).



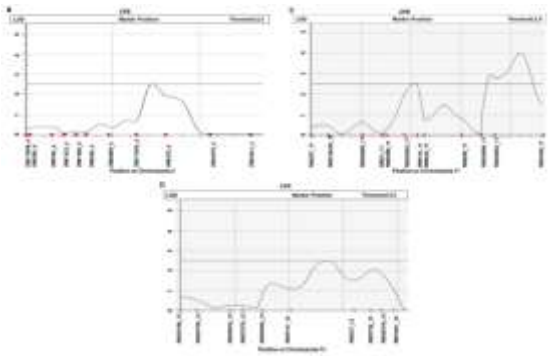


Figure 2 Details of QTLs identified for complete panicle exsertion using F2 mapping population of CPE-109×RPHR-1005. (A) QTLs identified for CPE using F2 mapping population of CPE-109×RPHR-1005. Marker loci indicated to the right side of the chromosome are used for construction of the linkage map. Genetic distance for each of the marker is provided in cM to the left side of the chromosomes. Region marked with red colour indicate location of QTLs responsible for CPE. (B–D) Details of QTLs identified on chromosome 4, 11, and 12 using F2 mapping population, screened for the complete panicle exsertion. (B) QTL identified on chromosome 4. (C) QTLs identified on chromosome 11. (D) QTL identified on chromosome 12. LOD, logarithm of the odds.

IX. Genomic regions identified for CPE through QTL-seq analysis

The results showed that four genomic regions on chr 4 (31.21-33.69 Mb), chr 5 (0.65-4.19 Mb), chr 9 (21.50-22.73 Mb), and chr 12 (0.12-3.15 Mb) were connected to the trait (Figure 3). The chromosomal areas chr 4 (31.21-33.69 Mb) and chr 12 (0.12-3.15 Mb) coincided with identified QTL regions qCPE-4 (28.24-31.22Mb) and qCPE-12 (2.30-3.18Mb) (Figure 4). Approximately 2176 genes were discovered in the genomic areas utilising the RAPDB database (http://rapdb.dna.affrc.go.jp/).

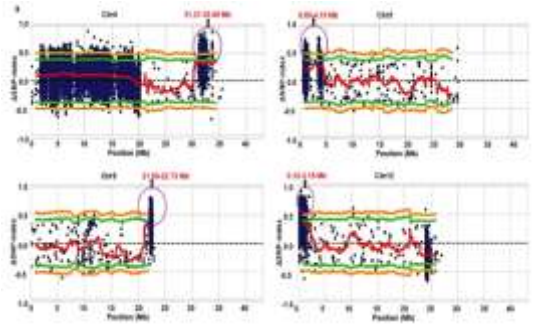


Figure 3 QTL-Seq analysis and heat map of the differentially expressed genes specific to the QTL-seq regions. (A) QTL-seq analysis: single nucleotide polymorphism (SNP) index of chromosomes 4, 5, 9, 10, and 12. Plots of SNP index of SNPs in the wild type-bulk and mutant type-bulk and plots of the Δ (SNP index) of the two bulks. Sliding window plots of the average SNP index, with a 500 kb window size and 5 kb steps presented as red lines. The pairs of orange and green lines in the Δ (SNP index) plots represent the 95% and 99% confidence intervals, respectively. The blue colour and pink colour circles highlight the detected QTL region.

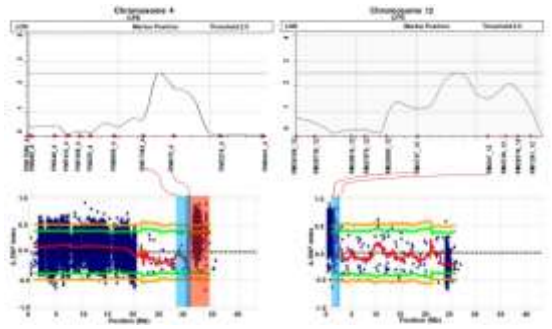


Figure 4 QTL intervals identified to be common between the linkage analysis and QTL-seq analysis. QTL analysis revealed two QTL intervals in chromosomes 4 and 12 to be overlapping between the classical linkage analysis and the QTL-seq analysis. The dotted lines in black represent the relative position of the flanking markers in the QTL-seq data. Blue and red shaded boxes indicate the QTL regions identified through linkage and QTL-seq analyses, respectively. The blue-dotted border indicates the overlapping regions from both analyses. LOD, logarithm of the odds; QTL, quantitative trait locus; SNP, single nucleotide piolymorphism.

X. Validation of causal SNPs in consistent QTL identified through QTL linkage mapping and QTL-seq

SNPs were picked from QTL-seq and QTL areas of chromosomes 4, 5, 9, 12, and qCPE-11a regions and tested for parental polymorphism. KASP 12-12 (chr12:1269983) found an SNP in Apetala2/ethylene-responsive element binding protein (AP2/ERF) (Os12g0126300), whereas KASP 12-16 (chr12:1515198) found an SNP in F-box domain-containing protein (Os12g0131400). These two showed a strong correlation with the CPE, characterized by high phenotypic percentage variation and low P-values. This was also confirmed in a group of rice lines that included advanced breeding lines (BC1F6; n=29), the F2 populations of CPE-109×SM (n=200) and CPE-109×RPHR-1005 (n=200), promising mutant lines for CPE (n=12), and released rice varieties that demonstrate CPE (n=15) (Figure 5). As a result, Os12g0126300 and Os12g0131400 were identified as potential candidate genes for the CPE phenotype. A mutation (single nucleotide variation; T→C: missense) was detected in the sixth exon of the AP2/ERF gene (Os12g0126300), leading to a conversion from methionine to valine at the 385th position of the amino acid sequence. In the gene that codes for an F-box family protein (Os12g0131400), a G→A mutation was found in the third exon, resulting in a premature stop codon at the 183rd amino acid position.

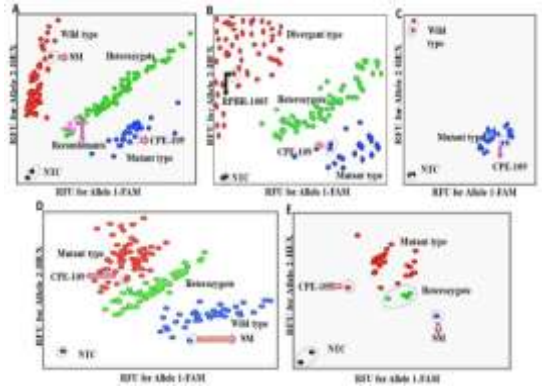


Figure 5 Genotyping of various rice population for CPE by KASP markers. (A–C) Allelic discrimination by KASP 12–12 with F2 population of CPE109×SM (A), F2 population of CPE109×RPHR-1005 (B), and advanced breeding lines (BC1F6) generation (C). Genotypes are shown in different colours, using supplementary software for the veriti 384 well thermal cycler. Blue ovals correspond to plants showing mutant type genotype; red ovals plants showing wild type genotype, green ovals plants showing HZ (heterozygous) genotype, pink ovals plants showing recombinants from wild type and black colour ovals represents non-template control (NTC). (D, E) Allelic discrimination by KASP 12–16 with F2 population of CPE-109×SM (D) and advanced breeding lines (BC1F6) generation (E). *x*- and *y*-axes show relative amplification units (FAM for allele 1 and HEX for allele 2). Blue ovals correspond to plants showing wild type genotype; red ovals plants showing mutant type genotype, green ovals plants showing HZ (heterozygous) genotype, and black ovals represent NTC. RFU, relative fluorescence unit.

XI. DISCUSSION

Panicle exsertion in rice is a complicated feature that manifests as full panicle exsertion (CPE) of the flag leaf. The CPE trait provides benefits over EUI since plants with EUI have a greater incidence of neck blast and panicle breakage (Kalia and Rathour, 2019). Introducing CPE in elite rice cultivars offers two benefits: (i) enhanced seed setting and (ii) as a breeding line. This study identified three new QTLs (qCPE-4, qCPE-11a, and qCPE-12) by linkage mapping. The QTL qCPE11b (19.50-28.28 Mb) overlapped with a previously found QTL for panicle exsertion known as qPE-11 (19.18-26.50 Mb). Interestingly, both QTLs contribute 6-7% of the phenotypic variation (Zhao et al., 2016).

Our findings supported earlier research demonstrating the quantitative character of panicle exsertion (Zhan et al., 2019). Furthermore, consistent genomic areas on chromosomes 4 and 12 have been discovered for CPE using two methods: linkage mapping and QTL-seq. Earlier research identified multiple QTLs for panicle exsertion on chromosomes 1, 2, 3, 4, 6, 8, 9, 11, and 12. The combination of QTL, QTL-seq, and RNA-seq data identified 47 shared DEGs linked with CPE.

QTL identification and precise mapping of the EUI trait in rice mutants have been reported, but not for panicle exsertion. Zhan et al. (2019) found that oslis-l1 or cyp734a4 mutants were considerably shorter than the wild type. This suggests that OsLIS-L1 and CYP734A4 genes play a crucial role in pancreatic exsertion and EUI. Although a few QTLs have been shown to contribute to panicle exsertion, no large impact QTLs have been identified or employed to enhance CPE. Additionally, the associated genes have not yet been cloned (Dang et al., 2017).

Six mutants related to panicle exsertion and EUI have been identified in rice till date, with their respective genes either mapped or cloned (Zhan et al., 2019). The well-characterized EUIDA mutant (eui1) demonstrated an accumulation of gibberellins (GAs), which resulted in the complete exsertion of the panicle from the sheath of the flag leaf. The mutation causing EUI has been linked to the impaired negative regulation of GA synthesis by the homeodomain-leucine

zipper transcription factor, HOX12. Furthermore, enzymes involved in the development of cells and tissues have been shown to contribute to EUI in the shortened uppermost internode (sui 1 and sui 4) mutants (Ji et al., 2014). In the enclosed shorter panicle 2 (esp2) mutants, the panicles remain fully enclosed within the flag leaf sheath, and the uppermost internode is nearly entirely degenerated, with the mutation associated with a putative phosphatidylserine synthase gene (Guan et al., 2011). Zhan et al. (2019) examined 205 rice accessions from various japonica and indica groups, identifying a total of 22 QTLs related to panicle exsertion through a genome-wide association study. In this study, we utilized an integrated approach incorporating linkage mapping, QTL-seq, RNA-seq, and differential gene expression to pinpoint genomic regions, genes, markers, and the molecular mechanism underlying CPE.

Two SNP markers, KASP 12-12 and KASP 12-16, showed a substantial correlation with the CPE. KASP 12-12 targets a T/C variant in the AP2/ERF gene's coding sequence. Os12g0126300 (AP2/ERF) was down-regulated in CPE-109 compared to SM in both RNA-seq and qRT-PCR. This allele may impact Os12g0126300's ability to bind to DNA and/or perform transcription. We propose a mechanism for CPE based on the substantial co-segregation of KASP 12-12 (Os12g0126300) and divergent gene expression patterns.

It is suggested that in CPE-109, a mutation in Os12g0126300 (AP2/ERF) causes down-regulation of ethylene biosynthesis genes, namely Os01g0192900 (ACC synthase) and Os05g0497300 (ethylene-responsive factor-2). Rashid et al. (2012) classified Os12g0126300 as part of the Ia AP2/ERF gene family, which produces proteins important in seed and flowering properties. Qi et al. (2011) found that OsEATB, a transcription factor from the AP2/ERF family, reduces ethylene-induced gibberellin responsiveness during internode elongation by inhibiting the gibberellin biosynthetic gene ent-kaurene synthase A.

Interestingly, in CPE-109, we detected an up-regulation of the gene for ent-kaurene synthase (Os06g0569900), which might be owing to a mutation in the AP2/ERF gene, resulting in an increase in gibberellic acid concentration. According to Gao et al. (2016), EUI is caused by increased bioactive gibberellic acid in the upper internode, which leads to increased cell proliferation and elongation.

In our study, we observed the down-regulation of the gene for A-Type Response Regulator 1 (Os04g0442300), a negative regulator of cytokinin signalling, and the up-regulation of two cytokinin biosynthesis genes, Os07g0486700 (cytokinin-O-glucosyltransferase 2) and Os10g0479500 (similar to carboxylase). This is likely to cause a rise in cytokinin concentration in the flag leaf. Previous research has shown that the wild-type AP2/ERF transcription factor CROWNROOTLESS5 induces OsRR1 and OsRR2, resulting in lower cytokinin levels and higher auxin levels (Kitomi et al., 2011).

We propose that complete panicle exsertion is due to mutation in the gene for an AP2/ERF, which leads to an increase in gibberellic acid content (due to up-regulation of the gene for ent-kaurene synthase) and cytokinin content (due to up-regulation of cytokinin biosynthesis genes for cytokinin riboside 5C-monophosphate phosphoribohydrolase and cytokinin-O-glucosyltransferase 2) and decrease in ethylene content (due to down-regulation of the gene for ACC syntha Panicle exsertion affects nutrition delivery to grains and has been linked to hormonal interactions (Chen et al., 2013).

Another SNP, KASP 12-16, in the F-box protein gene Os12g0131400, co-segregated with CPE. The SNP is projected to introduce a premature termination codon in the coding region, leading to a shortened protein. He et al. (2018) discovered that overexpression of the F-Box protein gene (OsAFB6) in rice delayed heading, dramatically increased the number of spikelets per panicle and primary branch, and enhanced grain output by 50%. OsAFB6, like AP2/ERF, inhibits blooming by increasing OsRR1, and it is mostly expressed in immature tissues.

CONCLUSION

We propose an impairing Os12g0131400 (F-box protein) in CPE-109 might result in down-regulation of OsRR1 and an increase in cytokinin content owing to upregulation of two cytokinin biosynthesis genes (Os07g0486700, Os10g047950).

REFERENCE

Guan H, Duan Y, Liu H, *et al*. 2011. Genetic analysis and fine mapping of an enclosed panicle mutant *esp2* in rice (*Oryza sativa* L.). Chinese Science Bulletin 56, 1476–1480.

Takagi H, Abe A, Yoshida K, *et al*. 2013. QTL‐seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. The Plant Journal 74, 174–183.

Wang Y, Liu J, Meng Y, Liu H, Liu C, Ye G.

2021. Rapid identification of QTL for mesocotyl length in rice through combining QTL-seq and genome-wide association analysis. Frontiers in Genetics 12, 713446.

Guha, P.K., Magar, N.D., Kommana, M., Barbadikar, K.M., Suneel, B., Gokulan, C., Lakshmi, D.V., Patel, H.K., Sonti, R.V., Sundaram, R.M., Madhav, M.S., 2024 Apr. Strong culm: a crucial trait for developing next-generation climate-resilient rice lines. Physiol. Mol. Biol. Plants 30 (4), 665–686.

Aglawe SB, Bangale U, Devi SJS, Balija V, Bhadana VP, Sharma SK, Kumar S, Prasad MS, Madhav MS. 2018. Characterization of Akhanaphou, a unique landrace from North-East India and its RIL population for rice leaf and neck blast resistance. Current Trends in Biotechnology and Pharmacy 12, 118–127.

Gumi, A.M., Guha, P.K., Mazumder, A., Jayaswal, P., Mondal, T.K., 2018 Feb. Characterization of *OglDREB2A* gene from African rice (*Oryza glaberrima*),

comparative analysis and its transcriptional regulation under salinity stress. 3 Biotech 8 (2), 91.

Sugihara Y, Young L, Yaegashi H, Natsume S, Shea DJ, Takagi H, Booker H, Innan H, Terauchi R, Abe A. 2022. High-performance pipeline for MutMap and QTL-seq. PeerJ 10, e13170.

McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. 2016. The Ensembl variant effect predictor. Genome Biology 17, 122.

Zhao C, Chen T, Zhao QY, Zhou LH, Zhao L, Zhang YD, Zhu Z, Yao S, Wang CL. 2016. Analysis of QTLs for panicle exsertion and its relationship with yield and yield-related traits in rice (*Oryza sativa* L.). Genetics and Molecular Research 15, gmr-15027423.

Zhan C, Hu J, Pang Q, Yang B, Cheng Y, Xu E, Zhu P, Li Y, Zhang H, Cheng J. 2019. Genome-wide association analysis of panicle exsertion and uppermost internode in rice (*Oryza sativa* L.). Rice 12, 72.

Dang X, Fang B, Chen X, *et al*. 2017. Favorable marker alleles for panicle exsertion length in rice (*Oryza sativa* L.) mined by association mapping and the RSTEP-LRT method. Frontiers in Plant Science 8, 2112.

Ji H, Kim H, Yun DW, Yoon UH, Kim TH, Eun MY, Lee GS. 2014. Characterization and fine mapping of a shortened uppermost internode mutant in rice. Plant Biotechnology Reports 8, 125–134.

Guan H, Duan Y, Liu H, *et al*. 2011. Genetic analysis and fine mapping of an enclosed panicle mutant *esp2* in rice (*Oryza sativa* L.). Chinese Science Bulletin 56, 1476–1480.

Rashid M, Guangyuan H, Guangxiao Y, Hussain J, Xu Y. 2012. AP2/ ERF transcription factor in rice: genome-wide canvas and syntenic relationships between monocots and eudicots. Evolutionary Bioinformatics, 8, EBO.S9369.

Qi W, Sun F, Wang Q, Chen M, Huang Y, Feng YQ, Luo X, Yang J. 2011. Rice ethylene-response AP2/ERF factor OsEATB restricts internode elongation by down-regulating a gibberellin biosynthetic gene. Plant Physiology 157, 216–228.

Gao S, Fang J, Xu F, Wang W, Chu C. 2016. Rice HOX12 regulates panicle exsertion by directly modulating the expression of *ELONGATED UPPERMOST INTERNODE1*. The Plant Cell 28, 680–695.

Kitomi Y, Ito H, Hobo T, Aya K, Kitano H, Inukai Y. 2011. The auxin responsive AP2/ERF transcription factor CROWN ROOTLESS5 is involved in crown root initiation in rice through the induction of OsRR1, a type - A response regulator of cytokinin signaling. The Plant Journal 67, 472–484.

Chen H, Jiang S, Zheng J, Lin Y. 2013. Improving panicle exsertion of rice cytoplasmic male sterile line by combination of artificial microRNA and artificial target mimic. Plant Biotechnology Journal 11, 336–343.

He Q, Yang L, Hu W, Zhang J, Xing Y. 2018. Overexpression of an auxin receptor OsAFB6 significantly enhanced grain yield by increasing cytokinin and decreasing auxin concentrations in rice panicle. Scientific Reports 8, 14051.