Mol Breeding (2023) 43:69
https://doi.org/10.1007/s11032-023-01412-1

Combined strategy employing MutMap and RNA-seq reveals genomic regions and genes associated with complete panicle exsertion in rice

Anil A. Hake · Suneel Ballichatla · Kalyani M. Barbadikar · Nakul Magar · Shubhankar Dutta · CG Gokulan · Komal Awalellu · Hitendra K Patel · Ramesh V. Sonti · Amol S. Phule · Embadi Prashanth Varma · Pradeep Goud Ayeella · Poloju Vamshi · R. M. Sundaram · Sheshu Madhav Maganti

Received: 23 January 2023 / Accepted: 28 July 2023 / Published online: 22 August 2023

Abstract Complete panicle exsertion (CPE) in rice is an important determinant of yield and a desirable trait in breeding. However, the genetic basis of CPE in rice still remains to be completely characterized. An ethyl methane sulfonate (EMS) mutant line of an elite cultivar Samba Mahsuri (BPT 5204), displaying stable and consistent CPE, was identified and named as CPE-110. MutMap and RNA-seq were deployed for unraveling the genomic regions, genes, and markers associated with CPE. Two major genomic intervals, on chromosome 8 (25668481-25750456) and on chromosome 11 (20147154-20190400), were identified to be linked to CPE through MutMap. A non-synonymous SNP (G/A; Chr8:25683828) in the gene *LOC_Os08g40570* encoding pyridoxamine 5′-phosphate oxidase with the SNP index 1 was converted to Kompetitive allele-specific PCR (KASP) marker. This SNP (KASP 8-1) exhibited significant association with CPE and further validated through assay in the F_2 mapping population, released varieties and CPE exhibiting BPT 5204 mutant lines. RNA-seq of the flag leaves at the booting stage, 1100 genes were upregulated and 1305 downregulated differentially in CPE-110 and BPT 5204. Metabolic pathway analysis indicated an enrichment of genes involved in photosynthesis, glyoxylate, dicarboxylate, porphyrin, pyruvate, chlorophyll, carotenoid, and carbon metabolism. Further molecular and functional studies of the candidate genes could reveal the mechanistic aspects of CPE.

Anil A Hake and Suneel Ballichatla contributed equally to this paper.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11032-023-01412-1.

A. A. Hake · S. Ballichatla · K. M. Barbadikar (✉) · N. Magar · A. S. Phule · E. P. Varma · P. G. Ayeella · P. Vamshi · R. M. Sundaram · S. M. Maganti
ICAR-Indian Institute of Rice Research, Hyderabad, Telangana 500030, India
e-mail: kalyaniaau@gmail.com

S. Dutta · C. Gokulan · K. Awalellu · H. K. Patel · R. V. Sonti
CSIR-Centre for Cellular and Molecular Biology, Hyderabad, Telangana 500007, India

S. Dutta
Research and Education in Cancer, Advanced Centre for Treatment, Navi Mumbai, Maharashtra 410210, India

H. K. Patel
Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

R. V. Sonti
International Centre for Genetic Engineering and Biotechnology, New Delhi 110067, India

S. M. Maganti (✉)
ICAR-Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533105, India
e-mail: sheshu24@gmail.com

Keywords Rice · CPE · Mutant · MutMap · Causal SNP · RNA-seq

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop grown worldwide for consumption. Around half of the world's population consumes rice. Considering the increasing rate of population growth, rice grain production needs to be increased exponentially. Rice productivity is not only hampered by biotic and abiotic stresses but also by the morphology of the crop. Among the morphological trait, panicle exsertion (PE) is an important trait that negatively influence yield. Rice yield is reduced when panicle enclosure occurs during grain-filling stages in cultivated varieties and hybrids (Duan et al. 2012). Panicle enclosure describes that panicles are partly or fully enclosed within the flag leaf sheath leading to undernourished and reduced grain size in varieties whereas in hybrids unfilled grains are dominant. The inability of panicles to exsert fully is commonly considered as a genetic defect (Cruz et al. 2008). Panicle enclosure in the rice genotype is mainly triggered due to the subsiding supply of indigenous hormone, gibberellic acid (GA_3) to the panicle, resulting in limited elongation of the upper internodes, subsequently panicle enclosing in the sheath leaf. Spraying GA_3 on the rice plants at the initial heading stage can eliminate the panicle enclosure, but the large amount of GA_3 increases the grain production cost with environmental pollution. Panicle exsertion trait is quantitatively inherited indicating that multiple genes govern the trait. Quantitative trait loci (QTL) mapping (Bao-Jian et al. 2008; Yang et al. 2009; Herlina and Trijatmiko 2016; Zhao et al. 2016, 2018) and genome-wide association mapping (Dang et al. 2017; Zhan et al. 2019) identified genomic regions/markers for panicle exsertion; however, very few have a significant association and utilized in the breeding program.

Development of mutant lines of elite cultivars provides opportunity for mining several traits of interest and understanding the underlying molecular mechanism. Ethyl methane sulphonate mutants of Samba Mahsuri (BPT 5204), an elite popular cultivar, were developed and evaluated for several traits including, agronomic, yield and, abiotic/biotic stress tolerance (Potupureddi et al. 2021). One of the stabilized mutants, CPE-110, was identified as a completely panicle exerted mutant consistently over years. This mutant was considered for mapping the regions associated with CPE through mutation mapping and RNA-seq (transcriptome analysis).

Currently, next-generation sequencing (NGS) technology has revolutionized the field of biological science and offers extraordinary speed with high sequencing accuracy and cost-effectiveness to study genomic and transcriptome data. Mutation mapping or MutMap is one of the gene mapping approaches based on whole-genome resequencing of pooled DNA from a segregating population (usually F_2) that is derived from the cross between mutant and the parent of candidate mutant that allows rapid identification of causal nucleotide changes of mutants (Abe et al. 2012). This NGS-based method has been successfully applied in rice for rapid identification of the QTL as well as candidate genes responsible for important agronomic traits such as pale green leaf (Abe et al. 2012), dwarfism (Abe et al. 2012; Oh et al. 2020), low cadmium accumulation in grain (Cao et al. 2019), tolerance to salt (Takagi et al. 2015), grain size (Yuan et al. 2017), and male sterility (Chen et al. 2014). Rapid pipelines have been developed for the identification of causal region/ SNPs using MutMap (Sugihara et al. 2022).

RNA-seq has emerged as an effective approach for transcriptome profiling with higher coverage and greater resolution which provides precise measurement of gene expression level at a particular condition (Magar et al. 2022). RNA-seq presents the ease of identification and evaluation of thousands of genes in a single analysis as compared to other transcriptome techniques. Beyond quantifying gene expression, RNA-seq enables the discovery of novel transcripts, alternatively spliced genes, and the detection of allele-specific expression (Phule et al. 2019). Recent advances in the RNA-seq workflow, from sample preparation to library construction and data analysis, have facilitated researchers to further elucidate the functional complexity of the transcription (Kukurba and Montgomery 2015). In rice, several genes were identified by transcriptome approach specific to various organs, growth stages, and traits such as developing embryos, cross cells, nucellar epidermis, ovular vascular trace, endosperm and aleurone layer (Wu et al. 2020; Xu et al. 2012), peduncle (Kandpal et al. 2020), pigmented leaf for anthocyanin content (Xu et al. 2021), coleoptile elongation rates under water stress (Hsu and Tung 2017; Phule et al. 2019), tolerance to

saline-alkaline stress (Li et al. 2020), response to nitrogen use efficiency (Neeraja et al. 2021), seed dormancy (Xie et al. 2019), resistance to rice blast (Yang et al. 2021), sheath blight (Das et al. 2021), bacterial blight (Wang et al. 2019), bakanae disease (Cheng et al. 2020), and brown planthopper (Satturu et al. 2021). Several authors reported transcriptome analysis for panicle (Zhang et al. 2018) and panicle-related traits at different stages viz panicle initiation and grain filling (Katara et al. 2020) leading to the identification of several candidate genes for the trait of interest.

The complete exsertion of panicle from flag leaf is one of the major factors contributing to grain yield that subsequently enhances productivity; identification of causal SNPs/genes/markers responsible for CPE became the premise of this study. Keeping this in view, the objectives of the present study were to identify the genomic region governing CPE by using MutMap and identification of differentially expressed genes using the RNA-seq.

Materials and methods

Development and phenotyping of MutMap population

To identify genomic regions for CPE, a mapping population was developed using BPT 5204, having incomplete panicle exsertion, and its stabilized EMS mutant, CPE-110, having CPE. During the wet cropping season 2018–2019, CPE-110 was crossed with its parent, BPT 5204, and generated F_1 plants (true heterozygotes) were confirmed through genotyping using polymorphic simple sequence repeat (SSR) markers. Furthermore, the seeds of true F_1 plants were sown in the field during the wet cropping season 2019–2020 at ICAR-Indian Institute of Rice Research, Hyderabad for F_2 seeds. Total 200 F_2 plants were grown during the wet cropping season 2020–21 in an augmented block design. The F_2 individual plants were phenotyped at the maturity stage for complete panicle exsertion trait. Panicle exsertion was recorded at the maturity stage in terms of panicle enclosure and percent panicle exsertion. We have made three phenotypic classes, completely exserted panicle, intermediate, and incompletely exserted based on panicle exsertion from flag leaf. The panicle exserted completely from the flag leaf was considered as complete panicle exsertion, while a panicle base enclosed 0.1 to 2.0 cm inside the flag leaf was considered as intermediate type; the panicle base enclosed > 2.0 cm in the flag leaf was considered as incompletely exserted panicle. Panicle enclosure was measured in centimeters by observing the extent of coverage of panicles by the flag leaf sheath, and percent panicle exsertion was calculated by the following formula:

$$\text{Percent panicle exsertion} = \frac{\text{Total panicle length} - \text{length of panicle covered in flag leaf}}{\text{Total panicle length}} \times 100$$

MutMap analysis: isolation of DNA, whole genome sequencing, pre-processing, alignment of short reads, and SNP calling

Genomic DNA was extracted from the leaves of BPT 5204, CPE-110 and F_2 individuals of BPT 5204 × CPE-110 using NucleoSpin Plant II kit (Macherey-Nagel, Dren, Germany). An equimolar concentration of DNA from 20 F_2 plants with completely panicle exserted (CPE), i.e., high phenotypic value (100% panicle exsertion) was pooled together and named as CPE bulk. Thus, BPT 5204, CPE-110, and CPE bulk were prepared for whole genome resequencing (WGRS) separately. About 5 µg DNA was used for the preparation of a sequencing library of average insert size 200–500 bp, according to the protocol for the Paired-End DNA Sample PrepKit (Illumina, USA). The library was sequenced to 40× of genome coverage with the Illumina HiSeq 2500 platform (Illumina, USA). The pipeline of MutMap analysis for panicle exsertion has been depicted in Fig. 1. In brief, 106.98, 108.74, and 121.50 million paired-end short reads from BPT 5204, CPE-110, and CPE bulk were used for the analysis. The raw sequencing data were subjected to quality check to ensure high-quality reads for downstream analyses. The reads with a Phred score < 30, base content biasness, and over-represented sequences (PCR-over duplication, poly G and poly X tails, and adapter contamination) were filtered out using fastp version 0.20.1 (Chen et al.

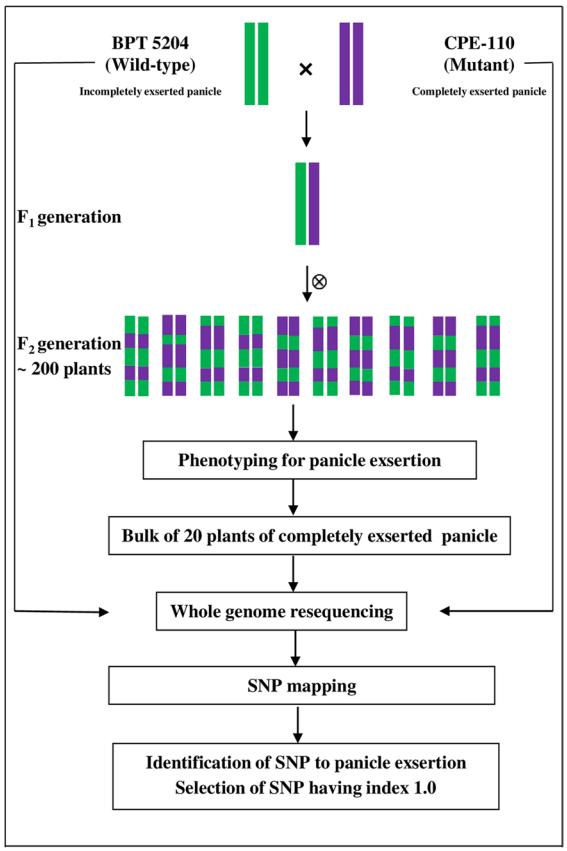


Fig. 1 Pipeline of MutMap analysis for identification of genomic region for complete panicle exsertion

2018). Paired-end sequence reads of CPE bulk were aligned to the R498 reference sequence (Du et al. 2017) using BWA (Li and Durbin 2009), SNPs were identified, and SNP indices were calculated in R and scored as homozygous SNPs (SNP index ≥ 0.9) and heterozygous SNPs (SNP index ≥ 0.3 and < 0.9). The SNP indices were plotted against chromosome coordinates in R using ggplot (https://ggplot2.tidyverse.org/). The window size for moving average was set to 1 Mb with an increment of 10 kb (Abe et al. 2012).

Development and assay of kompetitive allele specific PCR (KASP) marker

To validate the causal SNPs for complete panicle exsertion identified using the MutMap approach, a total of 25 genic SNPs (Supplementary Table S1) of eight genes were selected for developing the KASP marker. The 50 bp left and right-flanking sequences of each SNP site were used to design two allele-specific forward and one common reverse primer. The manufacturing of the KASP assays were performed by LGC genomics (London, UK). The chemical profiling for 5 µL reactions was 0.5 µL of template DNA (10 ng), 2.3 µL of 2× KASP master mix, and 0.14 µL of primer mix. The PCR profile of the KASP reaction was followed as pre-read phase at 30 °C for 1 min, hot start at 94 °C for 15 min, touchdown phase of 10 cycles (94 °C for 20 s and 61–51 °C, (dropping 1 °C per cycle) for 60 s) followed by 26 cycles of amplification (94 °C for 20 s; 55 °C for 60 s). The PCR reaction was run in 384-well formatted Applied Biosystems™ Veriti™ Thermal Cycler. During the pre-read and post-read phases, dyes such as FAMAbs (485 nm), HEXAbs (535 nm), and ROXAbs (575 nm) were utilized to detect fluorescence data.

RNA-seq analysis: RNA isolation, cDNA library preparation, sequencing, pre-processing, and data analysis

The genotypes, BPT 5204 and CPE-110, were utilized for transcriptome analysis. Both the genotypes were grown in the greenhouse (temperature 28 °C, humidity 80%) at ICAR-Indian Institute of Rice Research, Hyderabad during 2021-22. Total RNA was isolated from both the genotypes from flag leaf tissue (middle region) at the panicle initiation stage using the NucleoSpin RNA Plant kit (Macherey-Nagel, Duren, Germany). The quantitative and qualitative assessments of total RNA were conducted using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). The RNA samples with RNA integrity (RIN) value ≥ 8 was pooled in equimolar amounts from three biological replicates prior to the library preparation and subsequent sequencing.

The cDNA libraries were prepared using PrimeScript™ 1st strand cDNA synthesis kit (Takara, Japan) following the manufacturer's instructions. Pair-end sequencing was performed using an Illumina HiSeq 2500 sequencing platform (Illumina, San Diego, CA) at Nucleome Informatics Pvt. Ltd., Hyderabad. The raw sequencing data were subjected to quality check to ensure high-quality reads for downstream analyses. The reads with a Phred score < 30, base content biasness, and overrepresented sequences (PCR-over duplication, poly G and poly X tails, and adapter contamination) were filtered out using Fastp version

0.20.1 (Chen et al. 2018). The reads were mapped on the rice reference genome, R498 using a splice-aware alignment algorithm, HISAT2 (v 2.1.0) (Kim et al. 2019). The cleaned reads of RNA-seq data were deposited in NCBI Sequence Read Archive (SRA) database with the BioProject ID PRJNA687517.

Differential gene expression (DEG), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) term enrichment

The featureCounts program was used to count the reads at the gene level to check the prevalence of the biotypes of RNA (Liao et al. 2014). Subsequently, NOISeq was used to analyze quality control of count data; normalization and low-count filtering; and differential gene expression analysis (Tarazona et al. 2015). GO term enrichments were carried out by topGO using the parent-child Fisher algorithm, which essentially categorizes the terms by their hierarchical order and performs a hypergeometric test (Alexa and Rahnenfuhrer 2010). These enriched terms were filtered on a *p*-value cut-off of 0.05, following which the terms are then visualized in the form of a directed acyclic graph (DAG). KEGG term enrichment analysis was performed using the R package clusterProfiler with the terms hosting Benjamini-Hochberg corrected q values (Yu et al. 2012). This was done by selecting the Entrez gene-id values of DEGs (up- and down-regulated genes) separately through the BioMart package.

Validation of differentially expressed genes (DEGs) by quantitative real time-PCR (qRT-PCR)

To validate the RNA-seq DEG data, qRT-PCR was conducted on candidate genes identified by MutMap and RNA-seq. The total RNA of BPT 5204 and CPE-110 was reverse transcribed using PrimeScriptTM 1st strand cDNA synthesis kit (Takara, Japan). The relative expression was analyzed from three biological replicates using the $2^{-\Delta\Delta Ct}$ method with the rice TPH (tumor protein homolog) and Memp (membrane protein) as reference genes (Phule et al. 2019). The locus ids, function, and primer sequences of the genes are listed in Table S2.

Results

Genetic analysis of F_2 of BPT 5204 × CPE-110 for CPE

A total of 200 F_2 plants along with parents were phenotyped for panicle exsertion during the wet crop season 2019–2020 in the field wet season. The parent, BPT 5204, and its CPE mutant, CPE-110, significantly differed for panicle exsertion (Table 1). The F_2 population displayed high phenotypic variability for the trait. The lines in the F_2 population for panicle enclosing in flag leaf ranged from 0 to 3.79 cm, whereas percent panicles exserted from flag leaf ranged from 78.8 to 100%. The F_2 population exhibited normal distribution for CPE, indicating trait under quantitative control (Fig. 2a, b). Inhibitory gene action was exhibited by the F_2 population of BPT 5204 × CPE-110 where the interaction of the homozygous recessive gene with the homozygous/heterozygous dominant gene produced a completely exserted panicle phenotype (Table 1).

Whole-genome resequencing and alignment of short reads of CPE bulk of MutMap population

The CPE bulk and its parents, BPT 5204 and CPE-110, were sequenced to ~40x genome coverage. The statistical details on the number of raw reads, mapped reads, depth of genome coverage, average coverage, and average quality are presented in Table 2. On aligning, 121.13, 106.5, and 107.5

Table 1 Phenotyping of F_2 population of CPE-110 × BPT 5204 for CPE

Trait	Panicle enclosing in flag leaf (cm)	
Phenotype	Completely exserted	Incompletely exserted
Phenotype class	0 cm	>0.0 cm
Number of **F_2** genotypes	29	171
Trait	Percent panicle exserted	
Phenotype class	100%	<100%
Number of F_2 genotypes	29	171
Chi-square (for 13:3)	2.73	

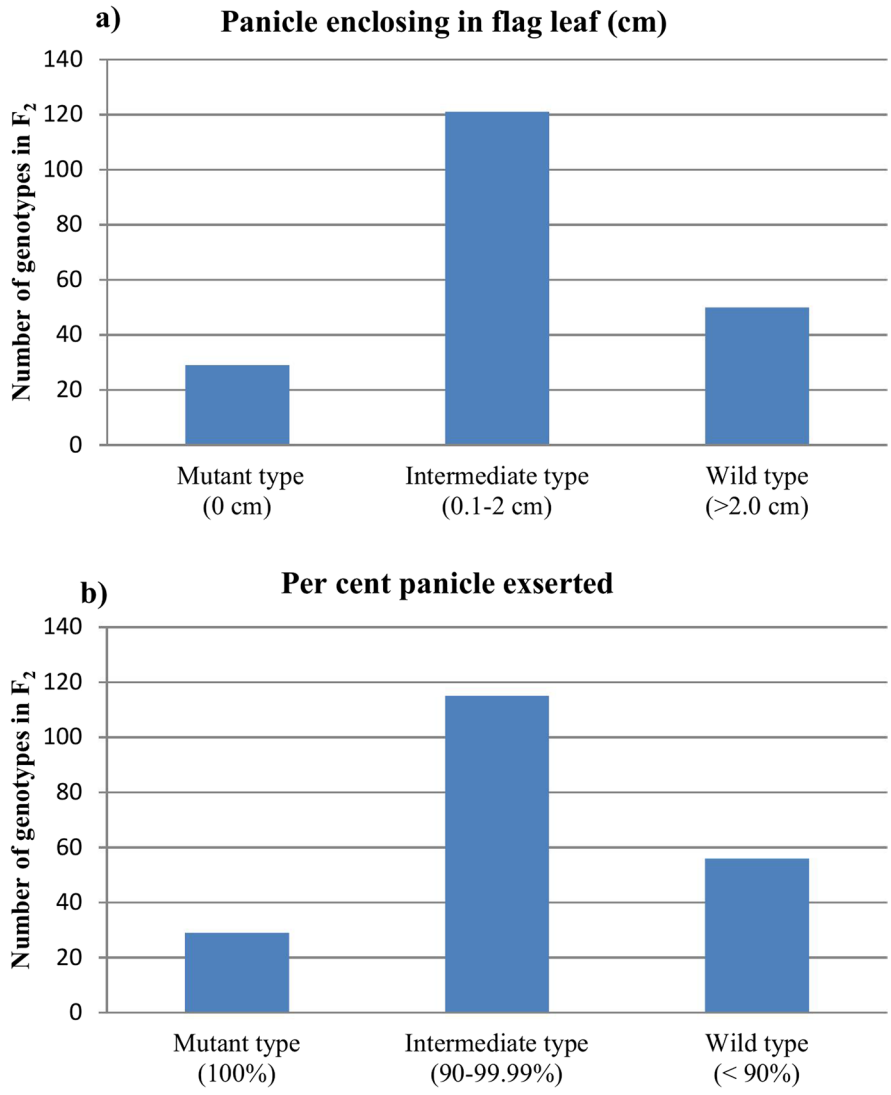


Fig. 2 Distribution pattern of CPE in F_2 population of CPE-110 × BPT 5204 for CPE. **a** Length of panicle choked in flag leaf. **b** Percent panicle exserted

million paired-end reads of CPE bulk, BPT 5204, and CPE-110, respectively, were mapped to the R498 reference genome (Table 2) with genome coverage 97.53, 95.02, and 94.98%, respectively. We obtained 17.85, 15.74, and 15.69 cleaned gigabases for CPE bulk, BPT 5204, and CPE-110, respectively.

Identification of CPE-associated genes and SNPs using MutMap

The Illumina short reads obtained for CPE bulk, BPT 5204, and CPE-110 were separately aligned to the reference sequence of R498 and identified 353547, 630123, and 667155 homozygous SNPs respectively (Table 2). Upon comparison of CPE bulk with wild-type BPT 5204, SNP index plots were generated. Two major peaks, one on chromosome 8 (25668481-25750456) and the other on chromosome 11 (20147154-20190400) with SNP index = 1, were recorded (Fig. 3; Supplementary Fig. 1). We mined the MutMap candidate regions of chromosomes 8 and 11 using the RAP-DB database (http://rapdb.dna.affrc.go.jp/) and identified a total of 15 and ten genes, respectively (Table 3).

Table 2 Summary of QC data BPT 5204, CPE-110, and CPE bulk of F_2 population of BPT 5204 × CPE-110

Genotype/QC description	BPT 5204	CPE-110	Bulk of complete panicle exsertion
Depth of genome coverage (X)	40.25	40.13	45.64
Genome Coverage with R498 reference genome (%)	95.02	94.98	97.53
No. of raw PE reads (million)	106.98	108.74	121.50
number of mapped reads	106.50	107.50	121.13
Total Bases (Gb)	15.74	15.69	17.85
Homozygous SNPs	630123	667155	353547

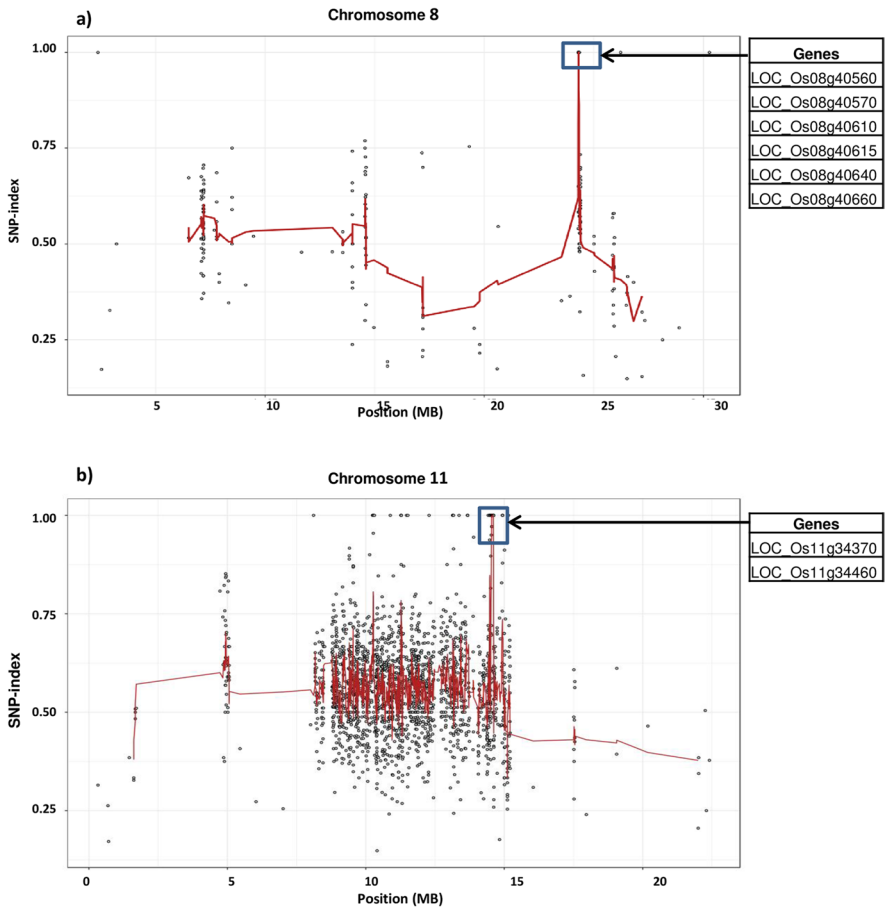


Fig. 3 Genomic region (shown in rectangular shape) on **a** chromosome 8 and **b** chromosome 11 exhibiting for complete panicle exsertion. *X*-axis indicates the physical position of the chromosome, and the *Y*-axis indicates the average SNP-index. SNP index plot regression lines were obtained by averaging SNP indices from a moving window of five consecutive SNPs and shifting the window of one SNP at a time. The *X*-axis value of each averaged SNP index was set at a midpoint between the first and the fifth SNP

Furthermore, upon comparison with BPT 5204, we identified 20 (11 genic and 7 inter-genic) and 37 (14 genic and 23 inter-genic) homozygous SNPs in the MutMap region of chromosome 8 (81.9 kb) and 11 (43.2 kb), respectively. Of the 11 genic SNPs identified in the MutMap region of chromosome 8, three SNPs each were observed in the exon, intron, and promoter region respectively while two SNPs were observed in the 3' UTR region of genes. Out of the three exonic SNPs, each SNP was located in *LOC_Os08g40560*, *LOC_Os08g40640*, and *LOC_Os08g40660* genes, respectively (Table 4). The genes viz, *LOC_Os08g40560*, *LOC_Os08g40570*, *LOC_Os08g40610*, and *LOC_Os08g40615* encoded ZOS8-11-C2H2 zinc finger protein, pyridoxamine 5′-phosphate oxidase family protein, 30S ribosomal protein S16, and

Table 3 List of genes identified from the MutMap region of chromosome 8 and 11 for complete panicle exsertion using F_2 MutMap population of CPE-110 × BPT 5204

SN	Gene	Chromosome	Description	CDS coordinates (5′-3′)
1	LOC_Os08g40550	8	Retrotransposon protein, putative, unclassified, expressed	25673540–25668739
2	LOC_Os08g40555	8	ATPase, E1-E2 type, putative, expressed	25675948–25674428
3	LOC_Os08g40560	8	ZOS8-11 - C2H2 zinc finger protein, expressedX	25682411–25676686
4	LOC_Os08g40570	8	Pyridoxamine 5′-phosphate oxidase family protein, putative, expressed	25686522–25683429
5	LOC_Os08g40580	8	Methyltransferase domain containing protein, expressed	25690627–25687620
6	LOC_Os08g40590	8	Oxysterol-binding protein, putative, expressed	25694808–25699639
7	LOC_Os08g40600	8	Thaumatin, putative, expressed	25701623–25700147
8	LOC_Os08g40610	8	30S ribosomal protein S16, putative, expressed	25704363–25702617
9	LOC_Os08g40615	8	Expressed protein	25707694–25708488
10	LOC_Os08g40620	8	rabGAP/TBC domain-containing protein, putative, expressed	25712814–25717641
11	LOC_Os08g40630	8	mTERF domain containing protein, expressed	25721973–25719257
12	LOC_Os08g40640	8	Retrotransposon protein, putative, Ty1-copia subclass, expressed	25733446 - 25723118
13	LOC_Os08g40650	8	Senescence-induced receptor-like serine/threonine-protein kinase precursor, putative, expressed	25735566–25743180
14	LOC_Os08g40660	8	Retrotransposon protein, putative, unclassified, expressed	25750374–25745440
15	LOC_Os11g34370	11	Phospholipase, patatin family, putative, expressed	20147936–20144591
16	LOC_Os11g34380	11	Retrotransposon protein, putative, unclassified	20148368–20149117
17	LOC_Os11g34390	11	Glycosyltransferase, putative, expressed	20154022–20152496
18	LOC_Os11g34400	11	Retrotransposon protein, putative, unclassified	20157516–20157190
19	LOC_Os11g34410	11	Retrotransposon protein, putative, unclassified, expressed	20158507–20164146
20	LOC_Os11g34420	11	Retrotransposon protein, putative, Ty3-gypsy subclass, expressed	20167991–20164768
21	LOC_Os11g34430	11	Retrotransposon protein, putative, unclassified	20169257–20168931
22	LOC_Os11g34440	11	Phospholipase A2, putative, expressed	20176341–20174651
23	LOC_Os11g34450	11	14-3-3 protein, putative, expressed	20178405–20181583
24	LOC_Os11g34460	11	OsFBO10 - F-box and other domain containing protein, expressed	20187327–20182477

expressed protein respectively while two genes viz, *LOC_Os08g40640* and *LOC_Os08g40660* encoded retrotransposon. Likewise, of 14 genic SNPs identified in the MutMap region of chromosome 11, nine and five SNPs were located in the exon and intron region of two genes respectively. Of nine exonic SNPs, six and three SNPs were observed in *LOC_Os11g34370* and *LOC_Os11g34460*, respectively (Table 4). The gene, *LOC_Os11g34370* encoded for phospholipase, patatin family, is involved in the lipid metabolic process, whereas the gene, F-box, and other domain-containing protein (*LOC_Os11g34460*) have catalytic and protein-binding activity, involved in the signal transduction and biosynthetic processes including nucleobase, nucleoside, nucleotide, and nucleic acid metabolic process as well as in the flower development.

Table 4 Identification of putative candidate genes for complete panicle exsertion using F_2 MutMap population of CPE-110 × BPT 5204

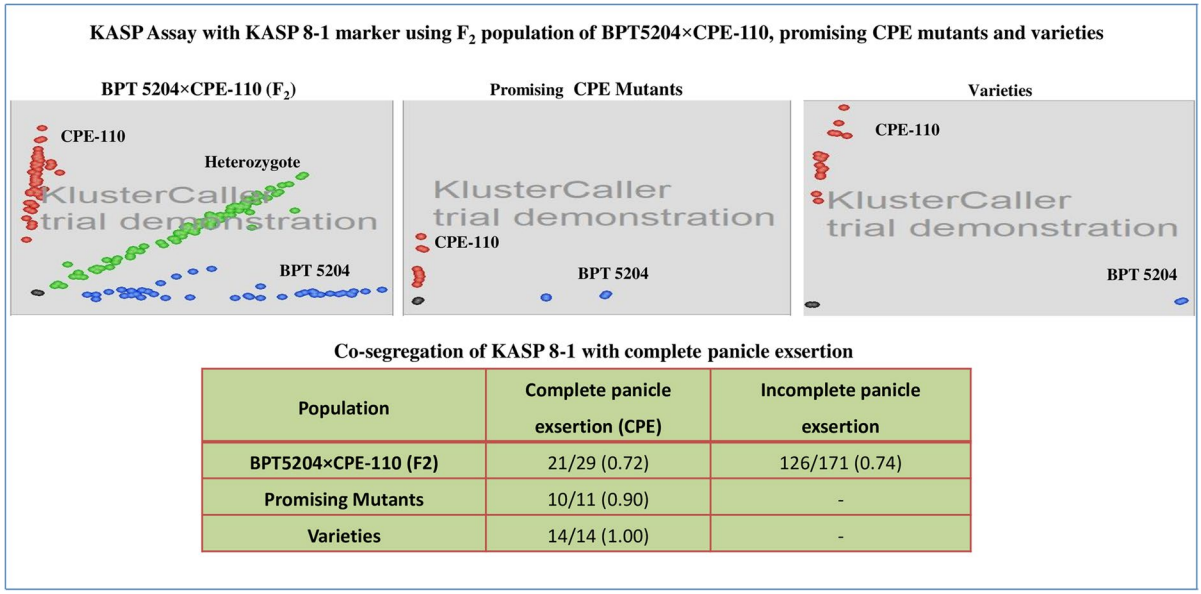
Gene	Chromosome	Position	Location in gene	Ref allele	Alt allele	SNP index	Gene function
LOC_Os08g40560	8	25681951	Intron	G	A	1.0	ZOS8-11-C2H2 zinc finger protein, expressed
		25682026	Exon	G	A	1.0	
LOC_Os08g40570	8	25683828	Promoter	G	A	1.0	Pyridoxamine 5′-phosphate oxidase family protein, putative, expressed
		25683858	Promoter	C	T	1.0	
		25683907	Promoter	C	T	1.0	
LOC_Os08g40610	8	25702666	3′ UTR	C	T	1.0	30S ribosomal protein S16, putative, expressed
LOC_Os08g40615	8	25708269	3′ UTR	G	A	1.0	Expressed protein
LOC_Os08g40640	8	25723394	Intron	C	T	1.0	Retrotransposon protein, putative, Ty1-copia subclass, expressed
		25725623	Exon	C	T	1.0	
LOC_Os08g40660	8	25748406	Intron	G	A	1.0	Retrotransposon protein, putative, unclassified, expressed
		25748564	Exon	G	A	1.0	
LOC_Os11g34370	11	20147154	Intron	C	T	1.0	Phospholipase, patatin family, putative, expressed
		20147420	Intron	G	A	1.0	
		20147484	Exon	C	T	1.0	
		20147595	Exon	C	T	1.0	
		20147655	Exon	G	A	1.0	
		20147736	Exon	C	T	1.0	
		20147770	Exon	G	A	1.0	
		20147800	Exon	C	T	1.0	
		20147929	3′ UTR	G	A	1.0	
LOC_Os11g34460	11	20182992	Exon	G	A	1.0	OsFBO10-F-box and other domain containing protein, expressed
		20183408	Exon	C	T	1.0	
		20186295	Intron	C	T	1.0	
		20186389	Intron	G	A	1.0	
		20186415	Intron	C	T	1.0	

Validation of causal SNP for complete panicle exsertion by kompetitive allele specific PCR (KASP)

To validate the SNP identified for complete panicle exsertion through the MutMap approach, KASP assays were developed for 25 SNPs. The list of developed KASP markers is represented in Supplementary Table S1. Furthermore, 25 KASP markers used for genotyping in the same population, that is, F_2 of BPT 5204 × CPE-110. After genotyping, only one KASP marker, KASP 8-1 (Chr8:25683828; G/A), displayed a strong association with the CPE trait in the F_2 population (Fig. 4). Furthermore, to know the association of KASP 8-1 for CPE, this marker was screened in completely panicle exserted lines such as stabilized mutants ($n = 12$) and released varieties ($n = 15$). The results indicated that KASP 8-1 marker exhibited co-segregation with panicle exsertion (Fig. 4). The KASP 8-1 marker was located in *LOC_Os08g40570* which encodes a Pyridoxamine 5′-phosphate oxidase family protein.

RNA-seq statistics

Using the Illumina sequencing platform, a total of 35.89 million paired-end reads consisting of 5.1 Giga base (Gb) were generated for BPT 5204; of this, a total of 4.8 Gb (95.6%) passed in quality control. Like-wise, for CPE-110, 39.72 million reads comprising 5.6 Gb were generated; of this, a total of 5.4 Gb (95.7%) passed in quality control and retained for further analysis (Table 5). Using HISAT2, a total of 16.64 (92.7%) and 18.20 million paired-end reads (91.7%) were aligned with reference genome for BPT



Co-segregation of KASP 8-1 with complete panicle exsertion

Population	Complete panicle exsertion (CPE)	Incomplete panicle exsertion
BPT5204×CPE-110 (F2)	21/29 (0.72)	126/171 (0.74)
Promising Mutants	10/11 (0.90)	-
Varieties	14/14 (1.00)	-

Fig. 4 Kompetitive allele specific primer assay of KASP 8-1 marker and its co-segregation with complete panicle exsertion

Table 5 Summary of Quality Control (QC) data of CPE-110 and BPT 5204 through RNA-seq

Genotype/QC description	BPT 5204	CPE-110
Number of paired-end reads (million)	35.89	39.72
Total bases (million)	5109.31	5663.27
% duplication	18.45	37.63
Q30 fraction	0.95	0.95
Q30 bases (million)	4884.05	5417.90
GC content fraction	0.56	0.53

5204 and CPE-110, respectively. Of these, 13.43 (74.8%) and 11.61 (58.5%) million paired-end reads from BPT 5204 and CPE-110, respectively, were uniquely mapped (Table 6).

Identification of differentially expressed genes between CPE-110 and BPT 5204

Using NOISeq, genes with counts per million (CPM) > 1 were retained for analyses, leading to the retention of 18,248 genes from a total of 38,978 genes. NOISeq matrix with the 18,248 genes was used for analyzing the level of gene expression using Trimmed Mean of M-values (TMM) normalization, with a $|\log2FC| \geq 1$ and with a probability value of > 0.95. In total, 2469 differentially expressed genes (DEGs) were observed between CPE-110 and BPT 5204 in flag leaf tissue during the panicle initiation stage for complete panicle exsertion (Fig. 5a). Of these, 1100 genes were upregulated while 1305 were downregulated in CPE-110 (Fig. 5b; Supplementary Table

Table 6 Overview of mapping status of RNA-seq data of CPE-110 and BPT 5204

Genotype/mapping description	BPT 5204	CPE-110
Paired-end reads mapped uniquely (million)	13.43	11.61
Paired-end reads mapped discordantly uniquely (million)	0.10	0.07
Paired-end reads one mate mapped uniquely (million)	0.59	0.49
Paired-end reads multimapped (million)	2.44	5.91
Paired-end reads one mate multimapped (million)	0.07	0.11
Paired-end reads neither mate aligned	1.30	1.65
% mapped to Reference genome	92.72	91.68

S3 and S4). The transcriptome analysis between the completely exserted panicle line (CPE-110) and incompletely exserted panicle line (BPT 5204) gave us a deeper insight into the differentially expressed genes (DEGs). In this investigation, several subsets of DEGs were identified which are potentially related to panicle exsertion (Table 7; Fig. 6).

Genes underlying MutMap identified QTLs

In the current study, two genes, namely, *LOC_Os11g34390* and *LOC_Os11g34370*, were identified to be downregulated in CPE-110 at the panicle initiation stage. The genes are located in the region (chrom 11:20147154-20190400) identified on chromosome 11 through MutMap. The gene *LOC_Os11g34390* encodes glycosyltransferase 6, a galactosyl transferase family protein that is involved in the biosynthesis of xyloglucan. Likewise, *LOC_Os11g34370* is encoded by patatin family protein which is involved in the lipid metabolic process (GO:0006629), mainly in triacylglycerol degradation. The downregulation of these genes along with the observed mutations are compelling to speculate a possible role of these genes in complete panicle exsertion in CPE-110.

To validate RNA-seq DEG data, qRT-PCR was conducted on twenty-four functionally relevant genes which were highly up and down-regulated in both the genotypes. The expression of eighteen genes was found to be according to RNA-seq result, while the other six genes, namely, *Os01g0764800*, *Os01g0124200*, *Os10g0100700*, *Os11g0286800*, *Os11g0189600*, and *Os07g0454200*, revealed the opposite expression to RNA-seq (Supplementary Fig. 2; Supplementary Table S3 and S4).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment

Gene Ontology (GO) enrichment was performed for DEGs to gain insights into the enrichment of functional categories that could be associated with CPE. The annotated DEGs were categorized into three major groups viz. biological process (BP), molecular function (MF), and cellular component (CC). The identified GO terms were further classified into down-regulated and up-regulated groups. In downregulated DEGs, a total of 130 GO terms were assigned, including 75, 20, and 35 in BP, CC and MF, respectively, whereas, in up-regulated DEGs, a total of 47 GO terms were assigned, including 26, 8, and 13 in BP, CC, and MF, respectively (Supplementary Table S5-S10). Overall, among the BP category, the significantly

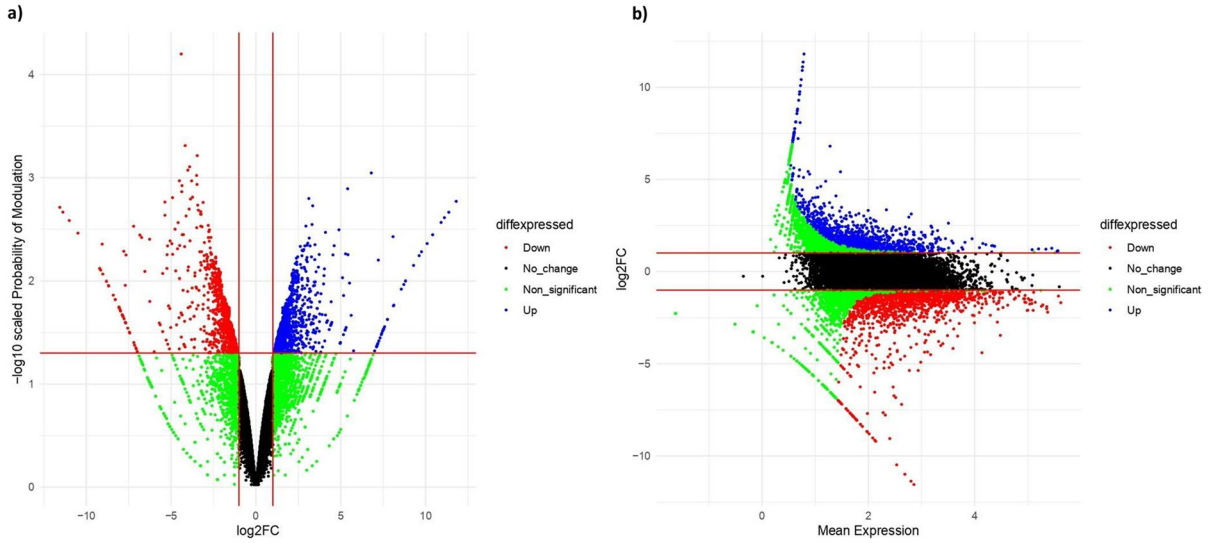


Fig. 5 Study of differential gene expression for complete panicle exsertion by scatter plots **a** volcano plot, created by taking the log2FC on the *X*-axis and the –log (*p*-value) on the *Y*-axis. **b** MA plots created by taking log scaled average expression on the *X*-axis and log2FC on the *Y*-axis

Table 7 List of candidate genes identified through RNA-seq for complete panicle exsertion

SN	RAP gene ID	MSU gene ID	Description
1	Os11g0189600	LOC_Os11g08569	2,3-Oxidosqualene cyclase, triterpene synthase, parkeol synthase
2	Os11g0311100	None	Similar to pantothenate kinase family protein
3	Os07g0454200	LOC_Os07g27120	Similar to hydroxyproline-rich glycoprotein gas29p precursor
4	Os02g0522100	LOC_Os02g32230	Similar to cDNA clone:J013126H19, full insert sequence
5	Os07g0450000	LOC_Os07g26740	Similar to 60S ribosomal protein L44
6	Os07g0472200	LOC_Os07g28910	Similar to zinc-finger protein Lsd1
7	Os04g0511200	LOC_Os04g43200	EFA27 for EF hand, abscisic acid, 27kD
8	Os02g0541500	LOC_Os02g33720	Similar to predicted protein
9	Os07g0663800	LOC_Os07g46852	Similar to oxidoreductase, short chain dehydrogenase/reductase family protein, expressed
10	Os01g0124200	LOC_Os01g03340	Similar to Bowman-Birk trypsin inhibitor
11	Os01g0603800	LOC_Os01g41930	Similar to Triticum sp. (pAWJL3) leucine rich repeat region mRNA (fragment)
12	Os11g0286800	LOC_Os11g18366	Squalene cyclase domain containing protein
13	Os03g0689400	LOC_Os03g48320	Similar to NB-ARC domain containing protein, expressed
14	Os03g0654700	LOC_Os03g45210	Protein of unknown function DUF1637 family protein
15	Os10g0468500	LOC_Os10g33040	Serine/threonine protein kinase-related domain containing protein
16	Os01g0778900	None	Similar to serine-rich protein-related
17	Os10g0446800	LOC_Os10g30970	Similar to predicted protein
18	Os07g0501700	LOC_Os07g31830	C2 calcium-dependent membrane targeting domain containing protein
19	Os09g0466400	LOC_Os09g29130	Zinc finger homeodomain (ZF-HD) class homeobox transcription factor, rice morphogenesis, modulation of leaf rolling
20	Os02g0137700	LOC_Os02g04510	NAD(P)-binding domain containing protein
21	Os04g0578600	LOC_Os04g48930	Similar to H0404F02.15 protein
22	Os07g0249800	LOC_Os07g14600	Similar to IAA-amino acid hydrolase ILR1-like 8
23	Os03g0817100	LOC_Os03g60250	Uncharacterised protein family UPF0497, trans-membrane plant domain containing protein
24	Os02g0136800	LOC_Os02g04420	Protein of unknown function DUF1677, Oryza sativa family protein
25	Os11g0492300	LOC_Os11g29990	Similar to NB-ARC domain containing protein
26	Os01g0764800	LOC_Os01g55940	Indole-3-acetic acid (IAA)-amido synthetase, disease resistance, abiotic stress tolerance
27	Os03g0111300	LOC_Os03g02050	Nonspecific lipid-transfer protein 2 (nsLTP2) (7 kDa lipid transfer protein)
28	Os04g0531750	LOC_Os04g44924	Similar to OSIGBa0125M19.13 protein
29	Os01g0870400	LOC_Os01g65010	Similar to S-domain class receptor-like kinase3
30	Os11g0485200	LOC_Os11g29490	ATPase, P-type, K/Mg/Cd/Cu/Zn/Na/Ca/Na/H-transporter family protein
31	Os09g0558100	LOC_Os09g38560	Similar to low-temperature induced protein lt101.2
32	Os07g0228400	LOC_Os07g12560	Cyclin-like F-box domain containing protein
33	Os02g0147200	LOC_Os02g05400	Similar to cDNA clone:J013125B21, full insert sequence
34	Os10g0469600	LOC_Os10g33130	Similar to leucine-rich repeat family protein, expressed
35	Os05g0177500	LOC_Os05g08480	UDP-glucuronosyl/UDP-glucosyltransferase family protein
36	Os11g0444000	LOC_Os11g25720	Similar to UDP-glucosyltransferase BX8
37	Os07g0689600	LOC_Os07g48980	Nicotianamine synthase 3 (EC 2.5.1.43) (S-adenosyl-L-methionine:S-adenosyl-L-methionine:S-adenosyl-methionine 3-amino-3-carboxypropyltransferase 3
38	Os03g0342100	LOC_Os03g22230	Pollen Ole e 1 allergen/extensin domain containing protein
39	Os03g0197900	LOC_Os03g10150	Protein of unknown function DUF623, plant domain containing protein
40	Os07g0235700	LOC_Os07g13160	Similar to cDNA clone:J023006M12, full insert sequence
41	Os07g0477250	LOC_Os07g29440	Similar to S-adenosylmethionine synthetase 2
42	Os07g0199350	None	Similar to HAT family dimerisation domain-containing protein
43	Os07g0561800	LOC_Os07g37454	Similar to carbohydrate transporter/sugar porter
44	Os10g0100700	LOC_Os10g01080	Vitamin B6 biosynthesis protein family protein

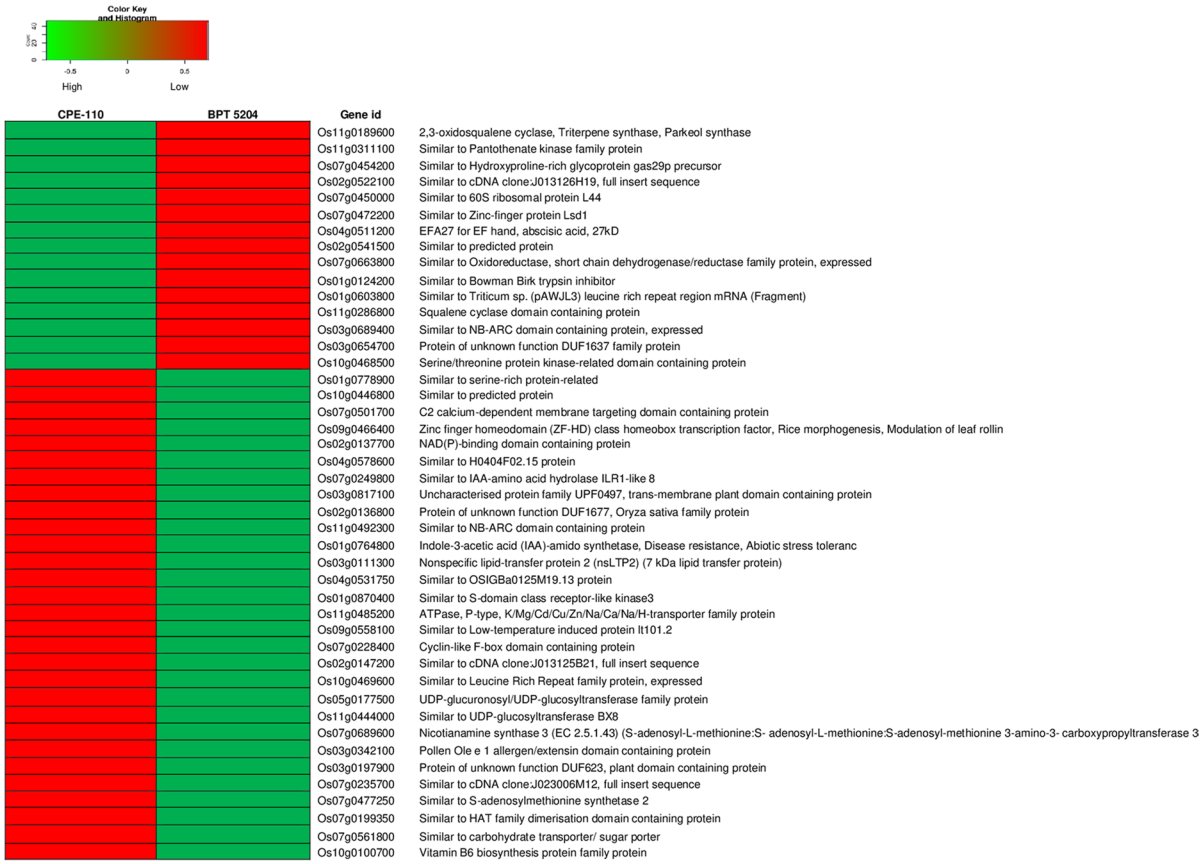


Fig. 6 Heat map of expression profiles of highly significant DEGs related to complete panicle exsertion. Color from red to green indicates that the FPKM values were small to large, red color indicates low level of gene expression, and green color indicates high level of gene expression

upregulated GO terms were "regulation of nucleobase-containing compound metabolic process" followed by "dephosphorylation," "nucleosome organization," and "chromatin assembly or disassembly." In CC category, nucleus," "plasma membrane," "Sm-like protein family complex," "cell periphery," and "small nuclear ribonucleoprotein complex" were the most significant GO terms, whereas, in molecular functions category, phosphatase inhibitor activity, phosphatase regulator activity, and oxidoreductase activity revealed most significant GO terms (Table 8; Fig. 7). Interestingly, in BP category, two GO terms, namely, GO:0009765 and GO:0019684, were specific to down-regulated DEGs and were involved in the "photosynthesis, light harvesting (11 DEGs)" and "photosynthesis, light reaction (11 DEGs)" processes, respectively, suggesting the role of these genes for complete panicle exsertion. Likewise, 11 GO terms were significantly downregulated in a cellular component, mainly in the plastid (58 DEGs) and its components like photosystem (15 DEGs), plastid stroma (2 DEGs), chloroplast thylakoid (83 DEGs), photosystem I reaction center (4 DEGs), photosystem II oxygen-evolving complex (10 DEGs), plastoglobule (2 DEGs), thylakoid lumen (2 DEGs), and photosynthetic membrane (45 DEGs) (Table 9). In the molecular function category, a total of 22 GO terms were downregulated and mostly involved in the oxidoreductase activity (9 DEGs), metallopeptidase activity (9 DEGs), peptidyl-prolyl cis-trans isomerase activity (7 DEGs), protein heterodimerization activity (14 DEGs), etc. (Table 9; Fig. 7).

The KEGG pathway enrichment analysis was performed to determine the metabolic pathways in

Table 8 List of GO terms significantly up regulated in CPE-110 related to cellular component, biological process, and molecular function

GO.ID	Term	DEGs	*p* value
Biological process			
GO:0006413	Translational initiation	3	0.04
GO:0006979	Response to oxidative stress	2	0.02
GO:0009072	Aromatic amino acid family metabolic process	3	0.05
GO:0009741	Response to brassinosteroid	1	0.05
GO:0010119	Regulation of stomatal movement	1	0.03
GO:0014070	Response to organic cyclic compound	1	0.04
GO:0006333	Chromatin assembly or disassembly	3	0.00
GO:0019219	Regulation of nucleobase-containing compound metabolic process	23	0.00
GO:0043446	Cellular alkane metabolic process	1	0.03
GO:0043447	Alkane biosynthetic process	1	0.04
GO:0044786	Cell cycle DNA replication	2	0.01
GO:0065004	Protein-DNA complex assembly	3	0.01
GO:0071248	Cellular response to metal ion	1	0.04
GO:0071824	Protein-DNA complex subunit organization	3	0.02
Cellular component			
GO:0030008	TRAPP complex	2	0.02
GO:0030532	Small nuclear ribonucleoprotein complex	3	0.02
GO:0071944	Cell periphery	22	0.02
GO:0099023	Vesicle tethering complex	2	0.02
GO:0120114	Sm-like protein family complex	3	0.01
GO:1990072	TRAPPIII protein complex	1	0.05
Molecular function			
GO:0003725	Double-stranded RNA binding	1	0.01
GO:0016208	AMP binding	1	0.03
GO:0016624	Oxidoreductase activity, acting on the aldehyde or oxo group of donors, disulfide as acceptor	1	0.04
GO:0019208	Phosphatase regulator activity	5	0.00
GO:0019212	Phosphatase inhibitor activity	5	0.00

which the DEGs were involved. In total, 11 pathways were significantly enriched in both down and up-regulated DEGs where 9 and 2 pathways were specific to down and up-regulated DEGs in CPE-110 as compared to BPT 5204, respectively. The down-regulated DEGs were significantly over-represented in "photosynthesis" (20 genes), photosynthesis-antenna proteins (12 genes), glyoxylate and dicarboxylate metabolism (17 genes), carbon metabolism (30 genes), carbon fixation in photosynthetic organisms (14 genes), porphyrin and chlorophyll metabolism (10 genes), glycine, serine and threonine metabolism (10 genes), pyruvate metabolism (12 genes), and carotenoid biosynthesis (6 genes) (Table 10). In the case of up-regulated DEGs, two pathways were identified, namely, phenylalanine, tyrosine and tryptophan biosynthesis (7 genes), and peroxisome (9 genes) (Table 10).

Discussion

Panicle exsertion is an important agronomic trait that influences grain yield in the rice crop. Shrunken and unfilled grains are generally observed in the incompletely exserted panicle rice genotypes. In this study, we utilized BPT 5204 and its mutant CPE-110 for the

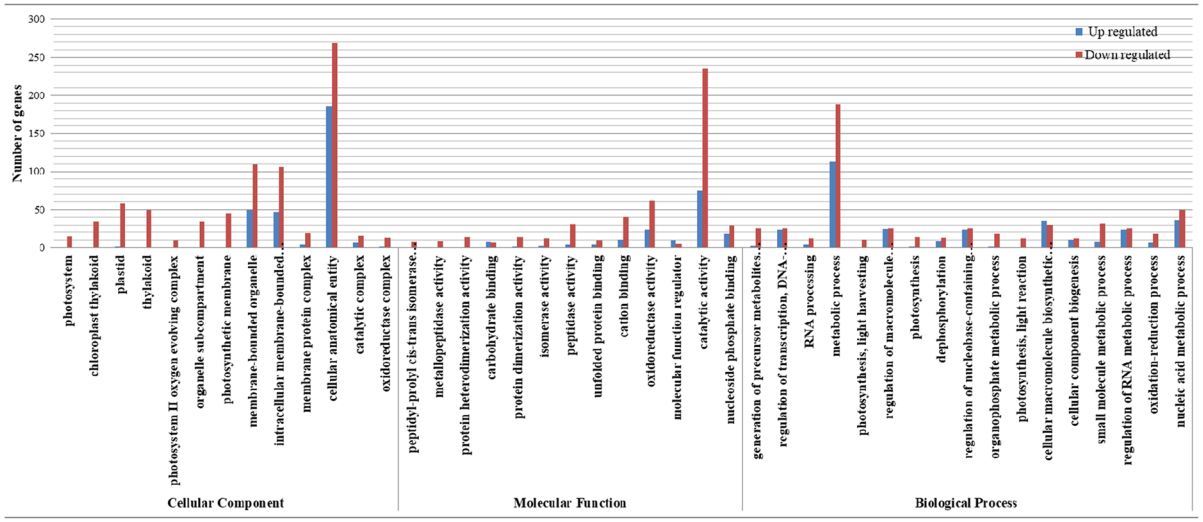


Fig. 7 Gene Ontology enrichment analysis of the DEGs. X-axis represents the sub-categories; Y-axis represents number of genes in each sub-category

identification of genes governing CPE. BPT 5204 is a high yielding medium slender, highly adapted cultivar having incomplete panicle exsertion trait, grown in mostly central and southern regions of India, while CPE-110 is a completely exserted panicle, stabilized mutant, morphologically like BPT 5204. We observed inhibitory genetic inheritance pattern in F_2 generation ($n = 200$) for panicle exsertion indicating the possibility of interaction between one homozygous recessive gene and another dominant homozygous/heterozygous gene leading to CPE. Our result differs with other studies where panicle exsertion was inherited as a dominant (Pandey and Gupta 1993) or recessive trait (Cruz et al. 2008; Zhao et al. 2018). For rapid identification of the gene controlling the panicle exsertion, MutMap, a method based on whole-genome sequencing of bulked DNA of F_2 segregating population derived from BPT 5204 × CPE-110, was used in the present study.

In recent years, wild type and its mutant have emerged as an ideal material for detecting candidate genes because of high genetic similarity which eliminates most genetic background noise. MutMap identifies the SNPs introduced by mutagenesis that can be deployed further for trait improvement. The markers are used to identify the regions harboring the mutation responsible for a given phenotype, and the causal SNPs are readily identified due to sufficient sequence coverage in that region (Abe et al. 2012). In rice, using the MutMap approach, genes have been identified for important agronomic traits. For instance, *08SG2/OsBAK1* for small grain size (Yuan et al. 2017), *OsRR22* for salinity tolerance (Takagi et al. 2015), *WB1* for endosperm development (Wang et al. 2018), *OsNRAMP5* for low Cd uptake and accumulation (Cao et al. 2019), *MER3* for male sterility (Chen et al. 2014), *OsCAO1* for pale green leaf (Abe et al. 2012), and *dwf1* for dwarfism (Oh et al. 2020).

In the present study, we have identified 25 SNPs of eight putative candidate genes of novel genomic region on chromosome 8 (25668481-25750456) and chromosome 11 (20147154-20190400) for CPE using MutMap. After designing the KASP assay for 25 SNPs, one KASP marker, KASP 8-1, exhibited strong co-segregation with the trait. The KASP 8-1 (chrom8: 25683828) is located in the promoter region of *LOC_Os08g40570*, encoded for pyridoxamine 5′-phosphate oxidase family protein. Here, we found an SNP in the cis-acting regulatory DNA element (promoter) of *LOC_Os08g40570* of CPE-110 that could be associated with CPE. The three signal sequences were recognized at SNP of promoter, namely, TATTAG (Fusada et al. 2005) (TA**C**TAG in BPT 5204; TA**T**TAG in CPE-110), GTAC (Kropat et al. 2005) (GTA**C** in BPT 5204; GTA**T** in CPE-110), and YACT (Gowik et al. 2004) (YA**C**T in BPT 5204; YA**T**T in CPE-110), regulating the expression of pyridoxamine 5′-phosphate oxidase. In wild type parent, BPT 5204, pyridoxamine 5′-phosphate oxidase

Table 9 List of GO terms significantly down regulated in CPE-110 related to biological process, cellular component, and molecular function

GO.ID	Term	DEGs	*p*_value
Biological process			
GO:0009765	Photosynthesis, light harvesting	11	2E-06
GO:0019684	Photosynthesis, light reaction	12	5E-05
Cellular component			
GO:0009521	Photosystem	15	0.01
GO:0009532	Plastid stroma	2	0.04
GO:0009534	Chloroplast thylakoid	34	0.03
GO:0009536	Plastid	58	0.00
GO:0009538	Photosystem I reaction center	4	0.00
GO:0009579	Thylakoid	49	0.00
GO:0009654	Photosystem II oxygen evolving complex	10	0.03
GO:0010287	Plastoglobule	2	0.01
GO:0031977	Thylakoid lumen	2	0.01
GO:0031984	Organelle subcompartment	34	0.00
GO:0034357	Photosynthetic membrane	45	0.00
Molecular function			
GO:0003755	Peptidyl-prolyl cis-trans isomerase activity	7	0.00
GO:0004176	ATP-dependent peptidase activity	5	0.01
GO:0004222	Metalloendopeptidase activity	5	0.04
GO:0004417	Hydroxyethylthiazole kinase activity	1	0.04
GO:0008124	4-Alpha-hydroxytetrahydrobiopterin dehydratase activity	2	0.01
GO:0008237	Metallopeptidase activity	9	0.00
GO:0008887	Glycerate kinase activity	1	0.04
GO:0016168	Chlorophyll binding	1	0.05
GO:0016642	Oxidoreductase activity, acting on the CH-NH2 group of donors, disulfide as acceptor	2	0.01
GO:0016667	Oxidoreductase activity, acting on a sulfur group of donors	4	0.02
GO:0016703	Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of one atom of oxygen (internal monooxygenases or internal mixed function oxidases)	3	0.04
GO:0016709	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen	2	0.03
GO:0016765	Transferase activity, transferring alkyl or aryl (other than methyl) groups	4	0.04
GO:0016846	Carbon-sulfur lyase activity	3	0.02
GO:0019156	Isoamylase activity	1	0.03
GO:0030170	Pyridoxal phosphate binding	2	0.02
GO:0030410	Nicotianamine synthase activity	3	0.00
GO:0035251	UDP-glucosyltransferase activity	4	0.01
GO:0043743	LPPG:FO 2-phospho-L-lactate transferase activity	1	0.04
GO:0046982	Protein heterodimerization activity	14	0.00
GO:0070279	Vitamin B6 binding	2	0.02
GO:0102193	Protein-ribulosamine 3-kinase activity	1	0.05

oxidizes pyridoxamine-5-PO_4 (PMP) and pyridoxine-5- PO_4 (PNP) to pyridoxal-5-PO_4. The pyridoxal-5-PO_4 acts as a cofactor for ACC deaminase which catalyzes 1-aminocyclopropane-1-carboxylic acid (ACC) to ammonia and alpha-ketoglutaric acid resulting in lowering the synthesis of ethylene in wild type,

Table 10 Involvement of up and down regulated DEGs in different metabolic pathways for CPE

KEGG ID	Pathway	Gene ratio	*p* value	Gene ID
Involvement of up regulated DEGs in different metabolic pathways in BPT 5204 for CPE				
osa00400	Phenylalanine, tyrosine and tryptophan biosynthesis	7/152	0.00	4328828/4333918/4335756/4341584/4343946/4345872/4349157
osa04146	Peroxisome	9/152	0.00	4324062/4327232/4332347/4337714/4337904/4342124/4345945/4349764/4350881
Involvement of down regulated DEGs in different metabolic pathways in CPE-110 for CPE				
osa00196	Photosynthesis - antenna proteins	12/223	0.00	4324599/4328623/4330828/4333359/4340892/4343583/4343604/4343709/4345663/4346803/4347166/4350176
osa00195	Photosynthesis	20/223	0.00	4324479/4324933/4326537/4327150/4332431/4332745/4334300/4335799/4337500/4339593/4339833/4342192/4342370/4343366/4343515/4343570/4344899/4346326/4351694/4352085
osa00630	Glyoxylate and dicarboxylate metabolism	17/223	0.00	4324401/4326849/4326980/4327981/4329690/4331509/4332108/4334274/4336245/4337051/4337272/4337447/4339682/4343993/4345962/4349114/4350456
osa01200	Carbon metabolism	30/223	0.00	107276220/4324401/4325531/4326849/4326980/4327981/4329690/4330413/4330673/4331130/4331495/4331509/4331761/4332364/4334274/4335227/4336044/4336245/4337051/4338750/4339204/4339682/4341496/4342543/4343993/4345962/4347022/4347204/4349114/4350456
osa00710	Carbon fixation in photosynthetic organisms	14/223	0.00	4325531/4330413/4331495/4331761/4332364/4334274/4335227/4336044/4338750/4339204/4339682/4341496/4342543/4343993
osa00860	Porphyrin and chlorophyll metabolism	10/223	0.00	4326901/4330711/4332843/4333259/4341462/4346136/4348519/4348648/4349004/4349433
osa00260	Glycine, serine and threonine metabolism	10/223	0.00	107276062/4324401/4326849/4326980/4327981/4336624/4337051/4345962/4349114/4350456
osa00620	Pyruvate metabolism	12/223	0.00	4325531/4326849/4326980/4330673/4331130/4334274/4337361/4338750/4339682/4343993/4344858/4347022
osa00906	Carotenoid biosynthesis	6/223	0.00	4328572/4330451/4335984/4336753/4345810/4352846

BPT 5204. It has been reported that ethylene induces gibberellic acid synthesis by inducing kaurene synthase and down-regulating AP2-ERF (Qi et al. 2011). Also, in RNA-seq data, we found that *LOC_Os10g01080* (*Os10g0100700*), a vitamin B6 biosynthesis protein family protein, was highly downregulated in mutant (11 fold change) CPE-110. Thus, it can be predicted that in mutant CPE-110, mutation in pyridoxamine 5′-phosphate oxidase resulted in enhanced ethylene and gibberellic acid that finally result in complete panicle exsertion from flag leaf (Fig. 8). We found the reduced expression of *LOC_Os08g40570* and *LOC_Os10g01080* in mutant CPE-110 via quantitative real time PCR (Fig. 9).

Overall, this study revealed potential genomic regions in rice that could be associated with complete panicle exsertion. The transcriptome profiling points towards a possible involvement of gibberellic acid metabolism and other pathways in determining the extent of panicle exsertion. The study has resulted in the development of an SNP marker (KASP 8-1, pyridoxamine 5′-phosphate oxidase family protein) that could be of potential use in

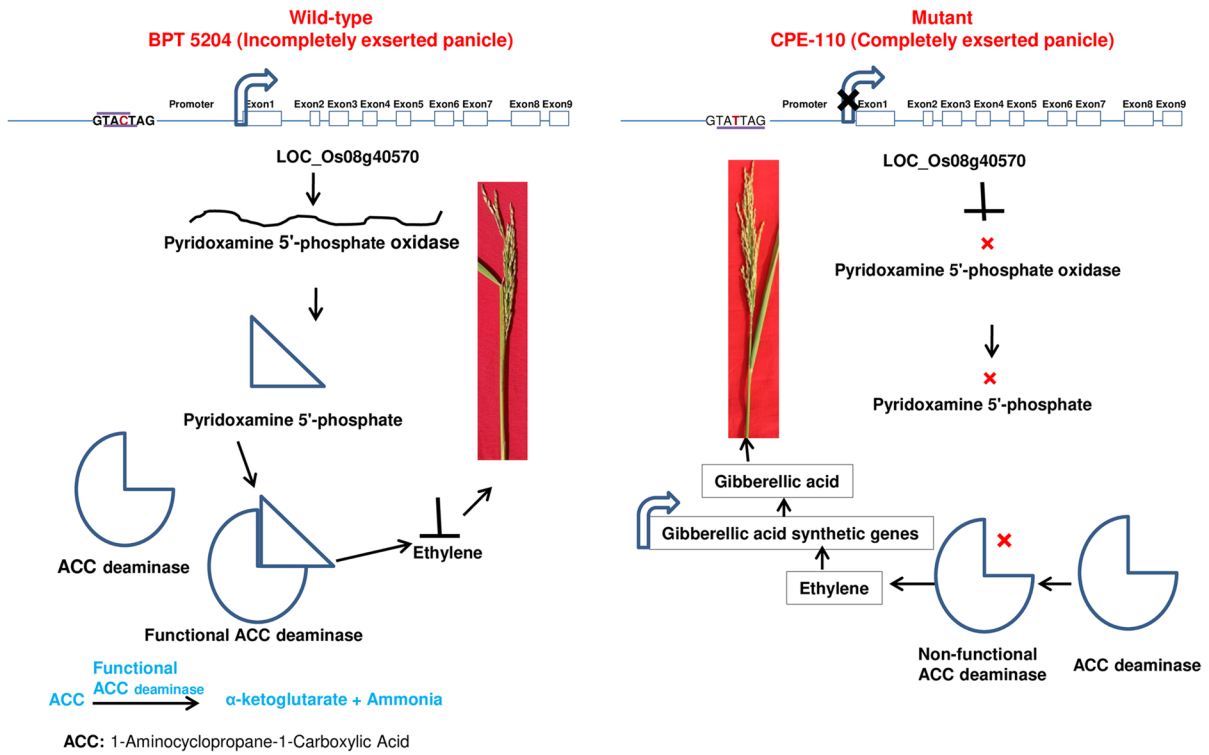


Fig. 8 Role of LOC_Os08g40570 (pyridoxamine 5′-phosphate oxidase) in panicle exsertion

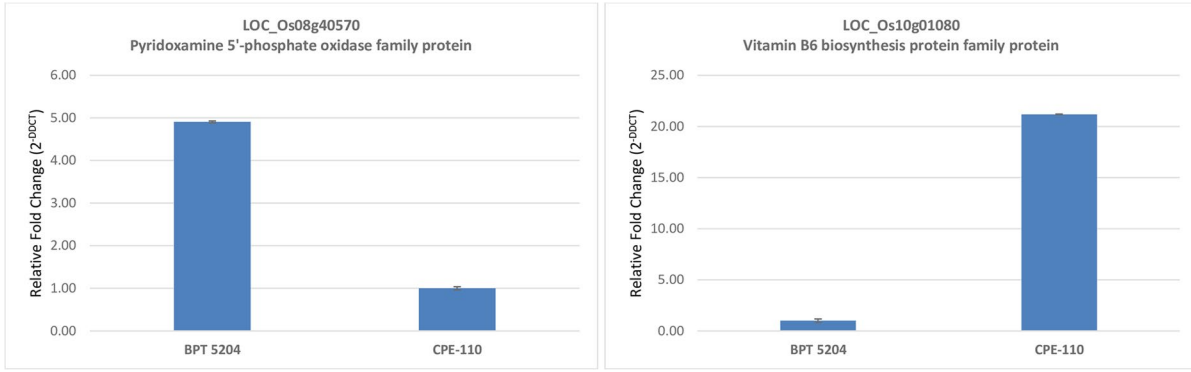


Fig. 9 Reduced expressions of LOC_Os08g40570 and LOC_Os10g01080 demonstrated by quantitative real time PCR

introgressing CPE trait into rice cultivars displaying panicle enclosure and hybrid rice programs. Earlier genomic regions, namely, *qPEL10.2* on chromosome 10 (Dang et al. 2017) and *qPE12* on chromosome 12 (Zhao et al. 2018) related with panicle exsertion, were identified; however, we identified novel genomic region as well as potential candidate gene for complete panicle exsertion in rice. Additionally, candidate genes have been identified, that might be of prime importance in governing CPE in rice, thus providing scope for functional characterization of these genes. The results from this study are expected to accelerate the genetic improvement of rice, especially in hybrid seed production.

Author contribution MSM conceived the study; MSM and KMB planned and designed the experiments; AH, SB, GCG, NM,

AP, EPV, PGA, and PV performed phenotyping, validation experiments, and genotyping. MSM and HKP contributed to the genetic material. SD, GCG, and KA executed scripts for MutMap data. AH, KMB, SB, and NM analyzed the data. AH and KMB drafted the manuscript. MSM, RMS, KMB, and HKP critically revised the manuscript. All authors reviewed and approved the submission.

Funding Authors are thankful to the directors, ICAR-IIRR and CSIR-CCMB and Council of Scientific and Industrial Research, Government of India, for the financial support. This work was supported by the Council of Scientific and Industrial Research, Government of India, Grant No. 34/1/TD-Agri-NutriBiotech/NCP-FBR 2019-RPPBDD/TMD-SeMI to Dr. Maganti S Madhav and Dr. Hitendra K Patel.

Data availability All the experiments and data analyses were conducted in ICAR-Indian Institute of Rice Research Hyderabad, India. MutMap analysis was done at CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India.

Declarations

Ethics approval We declare that these experiments complied with the ethical standards in India.

Consent to participate Not applicable.

Conflict of interest The authors declare no competing interests.

References

Abe A, Kosugi S, Yoshida K et al (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178. https://doi.org/10.1038/nbt.2095

Alexa A, Rahnenfuhrer J (2010) topGO: topGO: Enrichment analysis for Gene Ontology. R Package Version 2(18):0 Search in

Bao-Jian Q, Xiao-Biao ZHU, Ying-Ying W, De-Lin H (2008) Mapping of QTL for three panicle exsertion-related traits in rice under different growing environments. Acta Agron Sin 34:389–396 https://zwxb.chinacrops.org/EN/Y2008/V34/I03/389

Cao ZZ, Lin XY, Yang YJ et al (2019) Gene identification and transcriptome analysis of low cadmium accumulation rice mutant (lcd1) in response to cadmium stress using MutMap and RNA-seq. BMC Plant Biol 19:1–13. https://doi.org/10.1186/s12870-019-1867-y

Chen S, Zhou Y, Chen Y et al (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Chen Z, Yan W, Wang N et al (2014) Cloning of a rice male sterility gene by a modified MutMap method. Yi Chuan 36:85–93 https://www.chinagene.cn/EN/Y2014/V36/I1/85

Cheng A-P, Chen S-Y, Lai M-H et al (2020) Transcriptome analysis of early defenses in rice against Fusarium fujikuroi. Rice 13:1–15. https://doi.org/10.1186/s12284-020-00426-z

Cruz RP, Milach SCK, Federizzi LC (2008) Inheritance of pinacle exsertion in rice. Sci Agric 65:502–507. https://doi.org/10.1590/S0103-90162008000500009

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. Front. Plant Sci 8. https://doi.org/10.3389/fpls.2017.02112

Das A, Mazahar M, Sahu A et al (2021) Differential regulation of rice transcriptome to Rhizoctonia solani infection. bioRxiv. https://doi.org/10.1101/2021.05.05.442799

Du H, Yu Y, Ma Y et al (2017) Sequencing and de novo assembly of a near complete indica rice genome. Nat Commun 8:15324. https://doi.org/10.1038/ncomms15324

Duan YL, Guan HZ, Ming ZHUO et al (2012) Genetic analysis and mapping of an enclosed panicle mutant locus esp1 in rice (Oryza sativa L.). J Integr Agric 1:1933–1939. https://doi.org/10.1016/S2095-3119(12)60449-3

Fusada N, Masuda T, Kuroda H et al (2005) Identification of a novel cis-element exhibiting cytokinin-dependent protein binding in vitro in the 5′-region of NADPH-protochlorophyllide oxidoreductase gene in cucumber. Plant Mol Biol 59:631–645. https://doi.org/10.1007/s11103-005-0579-x

Gowik U, Burscheidt J, Akyildiz M et al (2004) Cis-regulatory elements for mesophyll-specific gene expression in the C4 plant Flaveria trinervia, the promoter of the C4 phosphoenolpyruvate carboxylase gene. Plant Cell 16:1077–1090. https://doi.org/10.1105/tpc.019729

Herlina L, Trijatmiko KR (2016) Identification of quantitative trait loci (QTL) for awn, incomplete panicle exertion and total spikelet number in an F2 population derived from a backcross inbred line, bio-148, and the recurrent parent, IR64. Makara J Sci 20:3. https://doi.org/10.7454/mss.v20i1.5657

Hsu S-K, Tung C-W (2017) RNA-seq analysis of diverse rice genotypes to identify the genes controlling coleoptile growth during submerged germination. Front Plant Sci 762. https://doi.org/10.3389/fpls.2017.00762

Kandpal M, Vishwakarma C, Krishnan K et al (2020) Gene expression dynamics in rice peduncles at the heading stage. Front Genet 11:584678. https://doi.org/10.3389/fgene.2020.584678

Katara JL, Verma RL, Parida M et al (2020) Differential expression of genes at panicle initiation and grain filling stages implied in heterosis of rice hybrids. Int J Mol Sci 21:1080. https://doi.org/10.3390/ijms21031080

Kim D, Paggi JM, Park C et al (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 37:907–915. https://doi.org/10.1038/s41587-019-0201-4

Kropat J, Tottey S, Birkenbihl RP et al (2005) A regulator of nutritional copper signaling in Chlamydomonas is an SBP domain protein that recognizes the GTAC core of copper response element. Proc Natl Acad Sci 102:18730–18735. https://doi.org/10.1073/pnas.0507693102

Kukurba KR, Montgomery SB (2015) RNA sequencing and analysis. Cold Spring Harb Protoc 11:951–969. https://doi.org/10.1101/pdb.top084970

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li Q, Ma C, Tai H et al (2020) Comparative transcriptome analysis of two rice genotypes differing in their tolerance to saline-alkaline stress. PLoS One 15:e0243112. https://doi.org/10.1371/journal.pone.0243112

Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930. https://doi.org/10.1093/bioinformatics/btt656

Magar ND, Shah P, Harish K et al (2022) Gene expression and transcriptome sequencing: basics, analysis, advances. InGene Expression, IntechOpen. https://doi.org/10.5772/intechopen.105929

Neeraja CN, Barbadikar KM, Krishnakanth T et al (2021) Down regulation of transcripts involved in selective metabolic pathways as an acclimation strategy in nitrogen use efficient genotypes of rice under low nitrogen. 3 Biotech 11:80. https://doi.org/10.1007/s13205-020-02631-5

Oh J, Cheon K-S, Kang D-Y et al (2020) MutMap analysis of a rice dwarf mutant line. Korean Soc Breed Sci 52:9–19. https://doi.org/10.9787/KJBS.2020.52.1.9

Pandey DK, Gupta HS (1993) Genetics of panicle exsertion in cold-tolerant rice (Oryza sativa). Plant Breed 111:82–85. https://doi.org/10.1111/j.1439-0523.1993.tb00610.x

Phule AS, Barbadikar KM, Maganti SM et al (2019) RNA-seq reveals the involvement of key genes for aerobic adaptation in rice. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-41703-2

Potupureddi G, Balija V, Ballichatla S et al (2021) Mutation resource of Samba Mahsuri revealed the presence of high extent of variations among key traits for rice improvement. PLoS One 16:e0258816. https://doi.org/10.1371/journal.pone.0258816

Qi W, Sun F, Wang Q et al (2011) Rice ethylene-response AP2/ERF factor OsEATB restricts internode elongation by down-regulating a gibberellin biosynthetic gene. Plant Physiol 157:216–228. https://doi.org/10.1104/pp.111.179945

Satturu V, Kudapa HB, Muthuramalingam P et al (2021) RNA-seq based global transcriptome analysis of rice unravels the key players associated with brown planthopper resistance. Int J Biol Macromol 191:118–128. https://doi.org/10.1016/j.ijbiomac.2021.09.058

Sugihara Y, Young L, Yaegashi H et al (2022) High-performance pipeline for MutMap and QTL-seq. PeerJ 10:e13170. https://doi.org/10.7717/peerj.13170

Takagi H, Tamiru M, Abe A et al (2015) MutMap accelerates breeding of a salt-tolerant rice cultivar. Nat Biotechnol 33:445–449. https://doi.org/10.1038/nbt.3188

Tarazona S, Furió-Tarí P, Turrà D et al (2015) Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. Nucleic Acids Res 43:e140–e140. https://doi.org/10.1093/nar/gkv711

Wang C, Tariq R, Ji Z et al (2019) Transcriptome analysis of a rice cultivar reveals the differentially expressed genes in response to wild and mutant strains of Xanthomonas oryzae pv. oryzae. Sci Rep 9:1–13. https://doi.org/10.1038/s41598-019-39928-2

Wang H, Zhang Y, Sun L et al (2018) WB1, a regulator of endosperm development in rice, is identified by a modified MutMap method. Int J Mol Sci 19:2159. https://doi.org/10.3390/ijms19082159

Wu T-Y, Müller M, Gruissem W et al (2020) Genome wide analysis of the transcriptional profiles in different regions of the developing rice grains. Rice 13:1–19. https://doi.org/10.1186/s12284-020-00421-4

Xie K, Bai J, Yang YY et al (2019) The RNA-seq transcriptome analysis identified genes related to rice seed dormancy. Biol Plant 63:308–313. https://doi.org/10.32615/bp.2019.035

Xu H, Gao Y, Wang J (2012) Transcriptomic analysis of rice (Oryza sativa) developing embryos using the RNA-seq technique. PLoS One 7:e30646. https://doi.org/10.1371/journal.pone.0030646

Xu R, Pan R, Zhang Y et al (2021) RNA-seq-based profiling of pl mutant reveals transcriptional regulation of anthocyanin biosynthesis in rice (Oryza sativa L.). Int J Mol Sci 22:9787 https://doi.org/10.3390/ijms22189787

Yang D, Li S, Xiao Y et al (2021) Transcriptome analysis of rice response to blast fungus identified core genes involved in immunity. Plant Cell Environ 44:3103–3121. https://doi.org/10.1111/pce.14098

Yang D-W, Zhu Z, Zhang Y-D et al (2009) Substitution mapping of QTL for panicle exertion using CSSL in rice (Oryza sativa L.). Yi Chuan 31:741–747. https://doi.org/10.3724/sp.j.1005.2009.00741

Yu G, Wang L-G, Han Y et al (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. Omi a J Integr Biol 16:284–287. https://doi.org/10.1089/omi.2011.0118

Yuan H, Fan S, Huang J et al (2017) 08SG2/OsBAK1 regulates grain size and number, and functions differently in Indica and Japonica backgrounds in rice. Rice 10:1–12. https://doi.org/10.1186/s12284-017-0165-2

Zhan C, Hu J, Pang Q et al (2019) Genome-wide association analysis of panicle exsertion and uppermost internode in rice (Oryza sativa L.). Rice 12:1–13. https://doi.org/10.1186/s12284-019-0330-x

Zhang W, Sun P, He Q et al (2018) Transcriptome analysis of near-isogenic line provides novel insights into genes associated with panicle traits regulation in rice. PLoS One 13:e0199077. https://doi.org/10.1371/journal.pone.0199077

Zhao C, Zhao Q, Zhao L et al (2018) Characterization and fine mapping of qPE12, a new locus controlling rice panicle exsertion. Euphytica 214:1–10. https://doi.org/10.1007/s10681-017-2104-0

Zhao CF, Chen T, Zhao QY et al (2016) Analysis of QTLs for panicle exsertion and its relationship with yield and yield-related traits in rice (Oryza sativa L.). Genet Mol Res 15:gmr.15027423. https://doi.org/10.4238/gmr.15027423