

eXtra Botany

Insight

Stripping off the rice panicle: induced genetic variation awakens the sheathed spikelet for a better yield

Jitendra K. Mohanty and Swarup K. Parida*

National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110067, India

* Correspondence: swarup@nipgr.ac.in or swarupdbt@gmail.com

This article comments on:

Ballichatla S, Gokulan CG, Barbadikar KM, Hake AA, Potupureddi G, Guha PK, Phule AS, Magar ND, Baliya V, Awalellu K, Kokku P, Golla S, Sundaram RM, Padmakumari AP, Laha GS, Senguttuvel P, Lella Venkata SR, Patel HK, Sonti RV, Maganti SM. 2024. Impairment in a member of AP2/ERF and F-box family protein enhances complete panicle exertion in rice. *Journal of Experimental Botany* **75**, <https://doi.org/10.1093/jxb/erae244>.

The rice panicle is the reproductive structure that contains the seeds, so a productive spikelet in a panicle contributes to increased yield. A sheathed panicle covers a significant portion of spikelets with the flag leaf and impairs effective pollination which subsequently compromises the yield. Through induced mutagenesis and integrated molecular breeding strategies, Ballichatla et al. (2024) uncovered the intricate regulation of complete panicle exertion (CPE) in rice. This study also delineates a set of high-confidence novel genomic loci and superior gene alleles which have a substantial translational significance in rice crop improvement towards future food security.

Complete panicle exertion: tailoring the rice panicle architecture for agronomic and economic advantage

The panicle architecture plays a vital role in determining the yield and productivity of rice. Panicle exertion is one such quantitative architectural trait, which is defined as the distance between the neck node of the panicle and the leaf cushion of the flag leaf in rice. CPE out of the flag leaf results in an unshathed panicle with more reproductive success, whereas impaired panicle exertion results in a sheathed panicle,

affecting grain filling and subsequently crop yield in rice (Ma et al., 2002; Cruz et al., 2008; Liu et al., 2008). Although the phenomenon of panicle exertion is observed to varying degrees in diverse germplasm accessions of rice, cytoplasmic male sterile (CMS) lines more frequently exhibit incomplete panicle exertion (IPE). The spikelets in the partially enclosed panicle of CMS lines are impaired in effective pollination, leading to low fertilization and seed setting efficiency, which consequently causes a significant loss in rice seed yield (Shen et al., 1987; Yang et al., 2002; Yin et al., 2007; Cruz et al., 2008; Guan et al., 2011; Luo et al., 2013). Thus, the issue of IPE poses a significant threat to grain yield in general and to hybrid rice seed production in particular. This highlights the importance of CPE as a trait of both agronomic and economic value in the global rice trade and commerce. A pioneering effort to tackle this issue in rice has identified a phenotypic modification distinct from CPE, known as elongated uppermost internode (EUI), which has been shown to enhance panicle exertion by elongating the uppermost internodes (Fig. 1A). Few forward genetics strategies have identified major quantitative trait loci (QTLs) and causal genes that govern the complex quantitative traits of EUI by modulating the gibberellin (GA) biosynthetic pathways. (Zhu et al., 2011; Ji et al., 2014; Gao et al., 2016; Zhan et al., 2019). However, these findings have not yet been translated efficiently to develop rice lines with desirable CPE phenotypes. Further, the EUI as compared with the CPE phenotype is associated with various adverse agronomic implications such as panicle breakage and a higher incidence of neck blast disease, thus affecting the overall grain yield in rice (Kalia and Rathour, 2019). Identifying the molecular genetic basis of CPE with limited internode elongation and fewer agronomic adversities is essential. However, the limited availability of natural accessions and breeding lines with contrasting CPE traits, along with the lack of genetic markers tightly linked to QTLs and genes governing CPE, hinders the genomics-assisted improvement of high-yield rice varieties. (Suneel et al., 2020). In

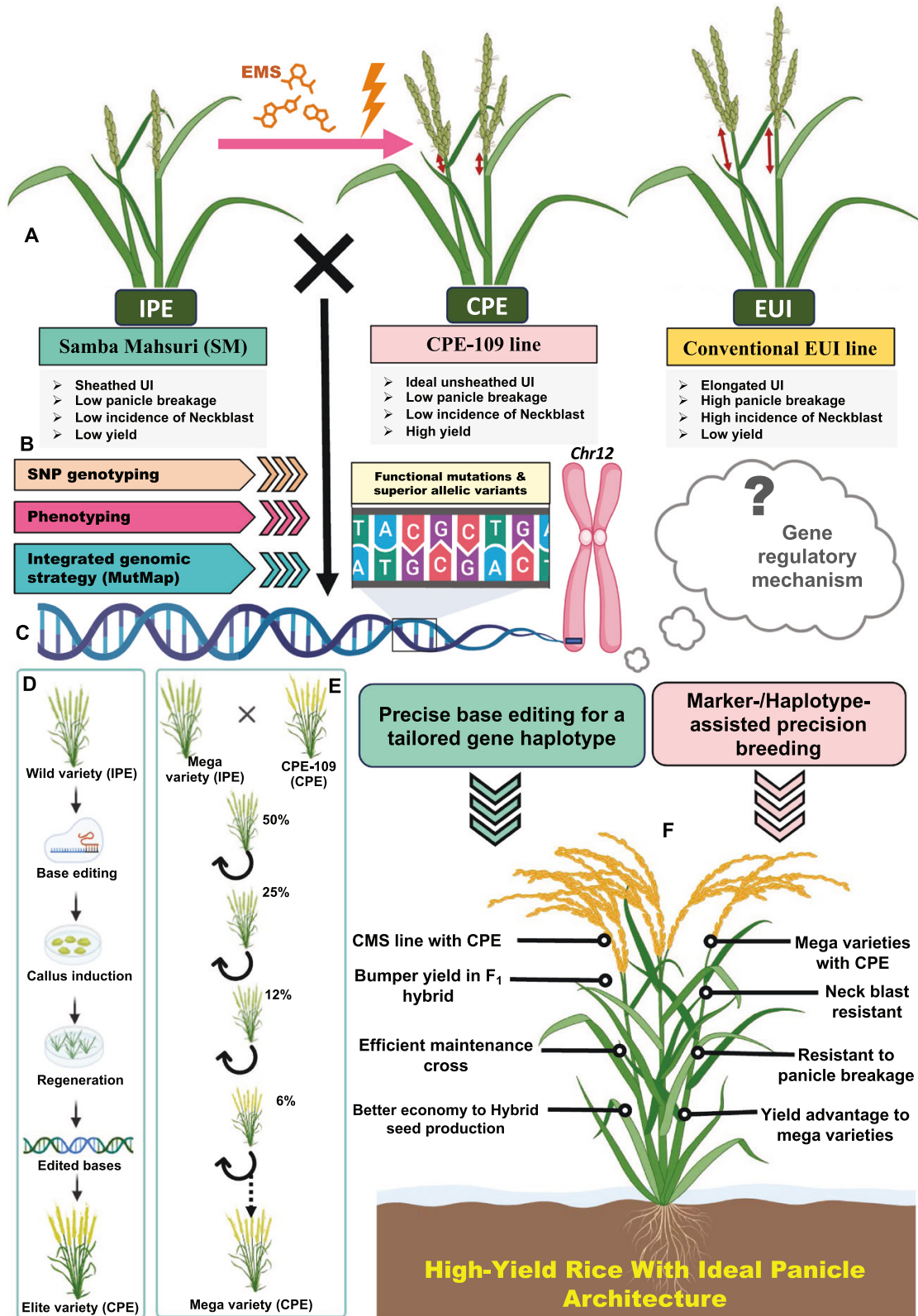


Fig. 1. Induced mutagenesis-based genomic strategy to dissect the complete panicle exertion (CPE) trait for rice crop improvement. (A) Schematic representation of different panicle architectures, such as incomplete panicle exertion (IPE), CPE, elongated uppermost internode (EUI), and their associated concerns in rice crop production. Ballichatla et al. (2024) exposed the IPE-type Samba Mahsuri (SM) to EMS-induced mutagenesis to develop

a CPE-109 line, which was subsequently crossed with the parental SM to generate a mapping population. (B) Large-scale phenotyping and genome-wide SNP genotyping deployed in an integrated genomic strategy (including MutMap) delineated potential genomic regions controlling the CPE trait in rice. (C) The delineated functional mutations and the allelic variants regulate CPE by modulating several hormonal biosynthetic process; however, the intricate gene regulatory mechanism is yet to be deciphered. The identified functional loci have a great significance in translational genomics (genome editing and marker-assisted breeding) for rice improvement targeting the CPE trait. (D) Precise/targeted editing of the genetic background of wild-type landraces (IPE) to develop elite varieties with CPE. (E) Marker-/haplotype-assisted introgression breeding to improve the existing mega rice varieties with the CPE trait. (F) Induced mutagenesis-based integrated genomic strategy led to development of high-yielding rice varieties restructured with desirable panicle architecture (CPE) which has high agronomical and economic importance. The image of the crops are created with Biorender.com.

this context, utilization of induced mutagenesis-mediated forward genetics strategies for generating novel functional mutations controlling agronomically important traits appears to be an attractive solution. This approach facilitates the quantitative dissection of the complex CPE trait and accelerates the genetic enhancement of rice.

Induced mutagenesis: delineating untapped causative functional mutations to decipher the complex genetic architecture of CPE in rice

Induced mutagenesis for breeding, trait dissection, and crop genetic improvement is a widely accepted strategy that creates heritable genetic variations. It is particularly useful in cases where naturally occurring crop germplasm accessions lack sufficient trait variations. This is a well-established strategy that is used to identify novel mutations, genes, alleles, and gene functions of agronomic importance (Jankowicz-Cieslak *et al.*, 2017). The current study by Ballichatla *et al.* (2024) along with their previous findings (Suneel *et al.*, 2020; Potupureddi *et al.*, 2021) utilized the power of the ethylmethane sulfonate (EMS)-induced mutagenesis strategy to develop a novel rice mutant (CPE-109) with CPE phenotype in the genetic background of a popular mega rice variety Samba Mahsuri (SM) (Fig. 1A). The mutant CPE-109 showed high genetic similarity as well as distinctness, uniformity, and stability (DUS) trait homology with its SM parent, except for the CPE trait. This suggests that CPE-109 can be directly introduced into the marker-assisted varietal breeding trial to develop an improved SM rice variety with a completely unsheathed panicle architecture and enhanced grain yield.

To accomplish this, the authors primarily deployed combinatorial molecular breeding and functional genomic strategies by integrating the traditional linkage (QTL) mapping and next-generation sequencing (NGS)-based bulk segregant (QTL-seq) assay with differential gene expression profiling (Fig. 1B). Briefly, a divergent RPHR-1005 line and wild-type SM displaying IPE were inter-crossed independently with CPE-109 to generate two different F₂ mapping populations (i.e. CPE-109×RPHR-1005 and CPE-109×SM). The F₁ plants from both of the mapping population crosses exhibit an IPE phenotype, indicating CPE as a recessive trait in rice. Continuous normal frequency distribution of exerted panicle length in the F₂ individuals from both mapping populations highlights the quantitative trait genetic inheritance pattern of CPE. Further,

QTL mapping and QTL-seq assays were deployed in the two mapping populations, and two overlapping major QTL genomic regions on chromosomes (Chr) 4 and 12 were deciphered that govern CPE in rice.

An extensive integrated genomic analysis identified two distinct single nucleotide polymorphisms (SNPs): KASP 1-12 (T→C; located at Chr 12:1269983) and KASP 12-16 (G→A; located at Chr 12:1515198) (Fig. 1C). These SNPs, found in the genes *Os12g0126300* and *Os12g0131400*, respectively, explained 81% and 60% of the phenotypic trait variation for CPE. The SNP KASP 12-12 (T→C), identified in the sixth exon of the AP2/ethylene-responsive transcription factor (*AP2/ERF*) gene (*Os12g0126300*), resulted in a missense mutation, changing methionine to valine at the 385th amino acid. In contrast, the SNP KASP 12-16 (G→A), located in the third exon of the F-box protein-coding gene (*Os12g0131400*), led to a nonsense mutation, causing premature termination at the 183rd amino acid. Differential gene expression profiling between CPE-109 and SM showed down-regulation of *AP2/ERF* in the CPE-109 mutant. This down-regulation may be due to an altered SNP (KASP 12-17) in the target gene promoter sequence, which co-segregates with the CPE trait in rice. Hence, the upstream regulatory mutation in the promoter, along with the missense mutations in coding regions of the *AP2/ERF* gene, can lead to reduced expression and functionality of this gene, respectively. This compromise in transcript abundance and functionality of the *AP2/ERF* gene is subsequently reported to down-regulate the ethylene biosynthesis process and up-regulate the GA and cytokinin biosynthesis cascades relaying the CPE phenotype. Similarly, the impaired F-box protein in CPE-109 can down-regulate the cytokinin biosynthetic repressor (*OsRR1*), resulting in up-regulation of cytokinin which subsequently leads to the CPE phenotype.

Novel superior alleles for translational genomics: restructuring panicle architecture with CPE for developing high-yield future rice crop

The current study investigated a new panicle architectural trait, CPE, that exerted the panicle completely from the leaf sheath without elongating the uppermost internode significantly in rice. The article highlights the agronomic implications of the CPE trait and identifies key candidate genes and their associated functional mutations/alleles that influence this important trait,

paving the way for future genetic improvement of rice. CPE is a recessive and quantitative complex trait controlled by two QTLs and its associated gene alleles. It is interesting to observe the significance of these induced mutations in genes with differential regulatory control over CPE, distinguishing them from EUI, despite both being influenced by GA signaling. Further, the combination of these alleles in the SM background with no pleiotropy indicates that CPE-109 can be directly introduced in the marker-assisted varietal trial for developing an improved SM with CPE phenotypes to impart a higher grain yield in rice. The functionally relevant mutations and superior alleles delineated in the genes can serve as targets for precise base editing by CRISPR (clustered regularly interspaced palindromic repeats) in several improved mega rice varieties to augment their yield advantage by the CPE trait (Fig. 1D). The identified KASP (Kompetitive allele-specific PCR) markers tightly linked to the QTLs and gene alleles with high PVE (phenotypic variance explained) for CPE have significance in rapid introgression of CPE into the popular cultivars through marker-assisted breeding for developing high-yielding rice varieties (Fig. 1E).

The salient findings of the study on CPE appear to be highly beneficial for the hybrid rice industry, enhancing the efficiency of commercial hybrid seed production and emphasizing its value in rice trade and commerce. CMS lines are frequently being used as female parents in rice hybrid seed production; however, the IPE phenotype is a common concern in these lines. The yield of the F₁ hybrid or the multiplication of the male sterile line clearly depends on the efficient pollination of the CMS line by the restorer and maintainer line, respectively, which is being compromised by the partially opened panicle of the CMS line. Tailoring the genomic background of conventional CMS line with the discovered superior CPE trait-associated mutations and gene alleles, by marker-assisted breeding and/or using precise genome editing, can solve the common but major issue of incomplete panicle exertion in rice. The genetically tailored rice varieties, restructured with the CPE trait through translational genomic intervention (marker-assisted breeding and genome editing), will address two key agronomic problems simultaneously in rice. Firstly, they can enhance the grain yield by exposing the sheathed spikelet for pollination. Secondly, unlike EUI, these improved rice varieties will not face the risk of panicle breakage and neck blast disease due to a normal uppermost internode (Fig. 1F). The novel genetic and genomic insight, particularly regarding panicle architectural traits, will be useful in opening up new avenues for translational genomic research for developing future high-yield hybrid rice that can contribute to future food security.

Acknowledgements

SKP acknowledges the S. Ramachandran National Bioscience Award for Career Development (BT/HRD/NBA-NWB/39/2020–21) from the Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India. JKM acknowledges the DBT for a Research Fellowship Award.

Conflict of interest

The authors declare that they have no competing interests.

Keywords: CPE, mutagenesis, panicle, QTL, rice.

References

- Ballichatla S, Gokulan CG, Barbadikar KM, et al.** 2024. Impairment in a member of AP2/ERF and F-box family protein enhances complete panicle exertion in rice. *Journal of Experimental Botany* **75**, 5611–5626.
- Cruz RPD, Milach SCK, Federizzi LC.** 2008. Inheritance of pinnacle exertion in rice. *Scientia Agricola* **65**, 502–507.
- Gao S, Fang J, Xu F, Wang W, Chu C.** 2016. Rice HOX12 regulates panicle exertion by directly modulating the expression of ELONGATED UPPERMOST INTERNODE1. *The Plant Cell* **28**, 680–695.
- 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.
- Jankowicz-Cieslak J, Mba C, Till BJ.** 2017. Mutagenesis for crop breeding and functional genomics. In: Jankowicz-Cieslak J, Tai T, Kumlhehn J, Till B, eds. *Biotechnologies for plant mutation breeding*. Cham: Springer, 3–18.
- 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.
- Kalia S, Rathour R.** 2019. Current status on mapping of genes for resistance to leaf- and neck-blast disease in rice. *3 Biotech* **9**, 209.
- Liu GL, Mei HW, Yu XQ, Zou GH, Liu HY, Hu SP, Li MS, Wu JH, Chen L, Luo LJ.** 2008. QTL analysis of panicle neck diameter, a trait highly correlated with panicle size, under well-watered and drought conditions in rice (*Oryza sativa* L.). *Plant Science* **174**, 71–77.
- Luo D, Xu H, Liu Z, et al.** 2013. A detrimental mitochondrial–nuclear interaction causes cytoplasmic male sterility in rice. *Nature Genetics* **45**, 573–577.
- Ma J, Zhou KD, Ma WB, Wang XD, Tian YH, Ming DF, Xu FY.** 2002. The characteristics of the tissues of the first internode and their relations to the grain-filling for the different panicle types of rice. *Acta Agronomica Sinica* **28**, 215–220.
- 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.
- Shen ZT, Yang CD, He ZH.** 1987. Studies on eliminating panicle enclosure in WA-type MS line of rice (*Oryza sativa* subsp. *indica*). *Chinese Journal of Rice Science* **1**, 95–99.
- Suneel B, Laha GS, Padmakumari AP, Sundaram RM, Rao LS, Sudhakar P, Satya AK, Madhav MS.** 2020. Morphological and molecular characterization of complete panicle emergence mutant lines for assessing genetic relatedness. *International Journal of Current Microbiology and Applied Sciences* **9**, 549–561.
- Yang RC, Zhang SB, Huang RH, Yang SL, Zhang QQ.** 2002. Breeding technology of eui-hybrids of rice. *Agricultural Sciences in China* **1**, 359–363.
- Yin C, Gan L, Ng D, Zhou X, Xia K.** 2007. Decreased panicle-derived indole-3-acetic acid reduces gibberellin A1 level in the uppermost internode, causing panicle enclosure in male sterile rice Zhenshan 97A. *Journal of Experimental Botany* **58**, 2441–2449.
- 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 exertion and uppermost internode in rice (*Oryza sativa* L.). *Rice* **12**, 72.
- Zhu L, Hu J, Zhu K, et al.** 2011. Identification and characterization of SHORTENED UPPERMOST INTERNODE 1, a gene negatively regulating uppermost internode elongation in rice. *Plant Molecular Biology* **77**, 475–487.