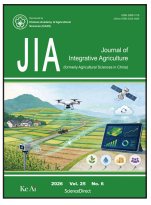
Journal of Integrative Agriculture 2026, 25(6): 2623–2626

Available online at ScienceDirect

Journal of Integrative Agriculture



LETTER

A novel labor-saving strategy for hybrid rice seed production

Quan Gan, Ran Zhou, Hao Yu, Cuixiang Lin, Bin Teng, Fengshun Song#, Dahu Ni#

Rice Research Institute, Anhui Academy of Agricultural Sciences/Key Laboratory of Rice Germplasm Innovation and Molecular Improvement in Anhui Province, Hefei 230001, China

Highlights

- *OsGATA15* plays an important role in regulating the growth and development of the rice flag leaf.
- The small flag leaf restorer line NP27 offers a novel breeding strategy and germplasm resource for hybrid rice seed production without leaf-cutting.

Rice is one of the main food crops globally. With the continuous increase in population and the decrease in effective arable land area, increasing rice yield is necessary to ensure the food supply (Wing *et al.* 2018). The purpose of hybrid rice is to utilize the genetic differences between different rice varieties and artificially control the hybridization process so that the hybrid offspring can combine the excellent traits of both parents, thereby exhibiting hybrid vigor (Xie *et al.* 2019). This advantage is reflected mainly in a greater growth potential, higher yield, and better stress resistance. In the hybridization process, male sterile lines (unable to reproduce on their own) are usually used as maternal parents to hybridize with restorer lines (paternal parents) with excellent traits, thereby obtaining first-generation hybrids (F_1 generation) with hybrid vigor. This F_1 hybrid can significantly increase the yield and quality of rice during cultivation (Chen *et al.* 2023).

Simplified and effective F_1 seed production methods are a development trend of hybrid rice. Years of hybrid rice seed production practices have shown that smaller flag leaves can maximize the exposure of the entire plant to sunlight, thereby increasing the leaf photosynthetic area and light energy utilization efficiency (Guo *et al.* 2007). This trait can increase rice yield and the seed-setting rate, reduce pollination obstacles during seed production, and effectively prevent some diseases and pests caused by insufficient lighting and ventilation (Huang *et al.* 2021). Therefore, it is usually necessary to manually cut off one-third to half of the top of the flag leaf at an appropriate time. However, this method not only requires high labor intensity and operational skills but also causes some damage to young panicles that have been pulled out, reducing seed production yield. Thus, cultivating small flag leaf varieties and avoiding manual leaf cutting are important directions for the future development of hybrid rice seed production (Wang and Wang 2022).

We used ^{60}Co-$_{\gamma}$ radiation-induced mutagenesis to treat *indica* rice 93–11 and obtained a stable small flag leaf mutant, *ym66*. From the seedling stage to the heading stage, the plant height of the *ym66* mutant was always slightly higher than that of the wild-type (WT) 93–11 (Fig. 1-A-a and B-a). Compared with 93–11, the *ym66* mutant had an additional upper internode (UPI) (Fig. 1-A-d). The basal internodes (UPI-V to UPI-VII) were longer than those of the WT, whereas the uppermost internode (UPI-I) was significantly shorter than that of 93–11 (Appendix A). These findings indicated that the origination and elongation patterns of the UPIs were altered in the *ym66* mutant. The panicles of *ym66* were also shorter than those of 93–11 before flowering (Fig. 1-A-b and B-b). This phenomenon occurred because the number of branches-I was less than that of 93–11 and resulted in fewer spikelets (Fig. 1-B-d; Appendix A). The most notable phenotype in *ym66* was the extremely short, narrow, and stiff flag leaves (Fig. 1-A-c). Compared with those of 93–11, the length of *ym66* was 6.1 cm and substantially decreased by almost 80%, the flag leaf width was 0.36 cm and decreased by almost 85% (Fig. 1-B-f and g), and the flag leaf angle was also significantly reduced (Fig. 1-B-h). Similarly, the lengths of the second and third leaves of *ym66* decreased slightly, whereas the widths of the second and third leaves did not significantly differ (Appendix A). To further examine the cellular changes associated with the small flag leaf phenotype in *ym66*, we performed safranin solid green staining and

Received 18 November 2025; Received in revised form 28 January 2026; Accepted 2 February 2026; Available online 10 March 2026
#Correspondence Dahu Ni, E-mail: dahuni1974@163.com; Fengshun Song, E-mail: sfs108@163.com

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2026.03.019

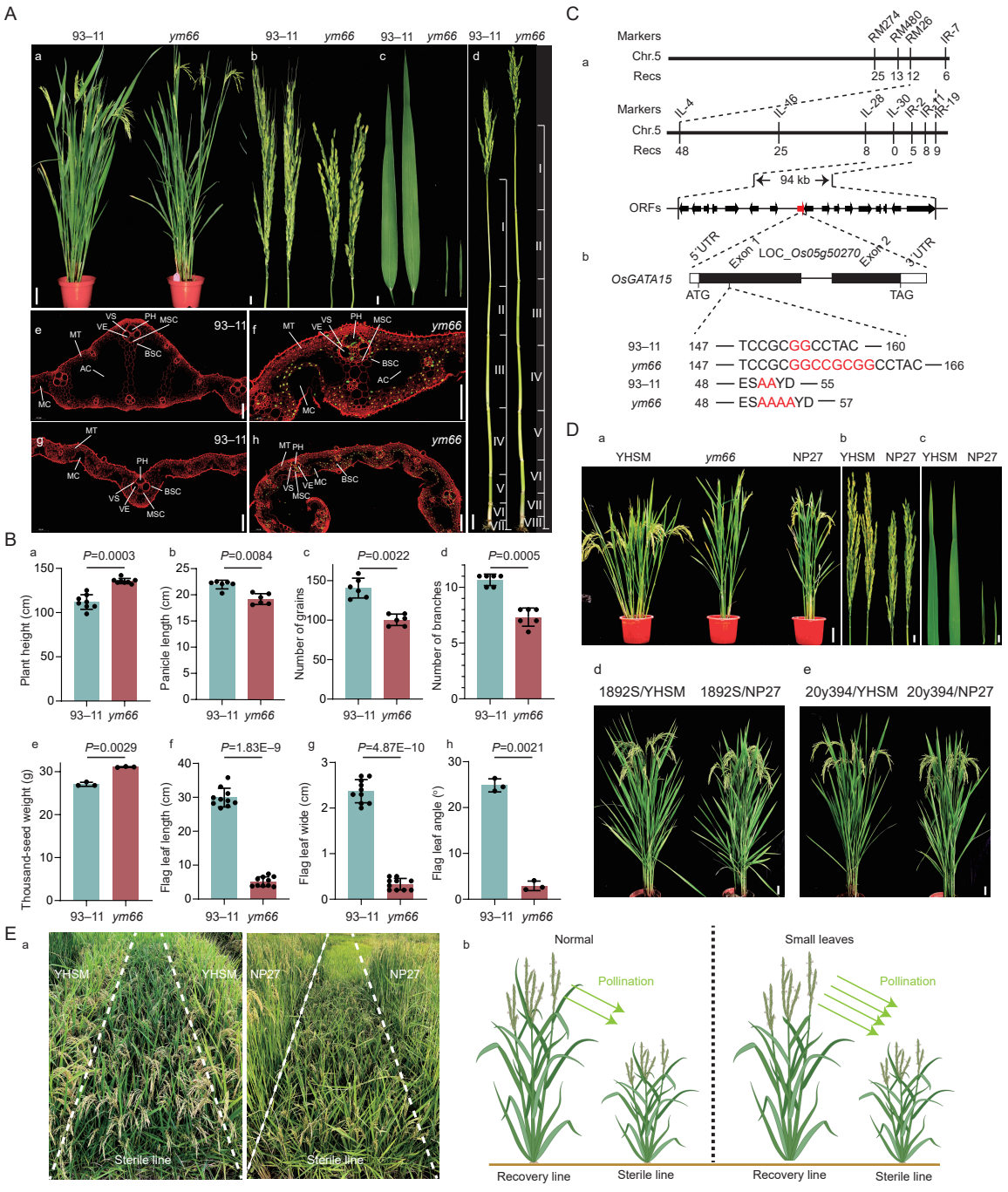


Fig. 1 Phenotypic characterization, map-based cloning and applications of the small flag leaf mutant. A, phenotypic characterization of wild-type (WT) 93–11 and the *ym66* mutant. a, plant type at the end of the heading stage. Bar=10 cm. b, panicle type before flowering. Bar=1 cm. c, the flag leaf type before flowering. Bar=1 cm. d, a culm of WT 93–11 (left) and mutant *ym66* (right) before flowering; vegetative leaves were removed. The transverse lines indicate the nodes that separate the sequential elongated upper internode (UPI); UPI-I to UPI-VIII represent different UPIs from the uppermost to the basal vegetative phytomers. Bar=5 cm. e–h, Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay of flag leaves before flowering. e and f, longitudinal axis center of the flag leaf in 93–11/mutant *ym66* plants. g and h, longitudinal axis lateral to the flag leaf in 93–11/mutant *ym66*. Vascular bundles; MSC, mestome sheath cells; VE, vessels; PH, phloem; MT, mesophyll tissue; MC, mother cells; AC, air cavity; BSC, bundle sheath cells. Bars=100 μm. B, agronomic traits of WT 93–11 and the *ym66* mutant. a, plant height. b, panicle length. c, number of grains. d, number of branches. e, thousand-seed weight. f, flag leaf length. g, flag leaf width. h, flag leaf angle. Bars mean SE (n=3). C, map-based cloning of *ym66* and the gene structure of *OsGATA15*. a, fine mapping of *ym66*. The molecular markers are indicated above the black line, which indicates chromosome 5, and the corresponding numbers of recombinants are indicated below the black line. The candidate open reading frames (ORFs) is highlighted in red. b, gene exon–intron structure of *OsGATA15*. The black rectangles indicate different exons, the black lines represent introns, and the white rectangles represent 5′ and 3′ untranslated regions (UTRs). The mutation site in *ym66* is located in exon 1, and the specific sequences are listed below. D, development of small flag leaf restorer lines NP27 and their application in hybrid seed production. a–c, the plant, panicle, and flag leaf types are at the end of the heading stage of YHSM and NP27. Bars=10, 1, and 1 cm, respectively. d and e, plant type of F_1 progenies from the restorer lines YHSM and NP27 hybridizing with 1892S and 20y394. E, field cultivation scene pictures (a) and model diagram of small flag leaf restorer line hybrid rice seed production (b). The green arrow represents the pollination process.

transmission electron microscope (TEM) analysis of the middle part of *ym66* flag leaves before flowering. The mutant *ym66* presented fewer veins than 93–11 did. Given that the vascular system found in veins is thought to be directly associated with leaf size (Wang *et al.* 2017), this finding might be the reason for the narrower width of the mutant flag leaf. In addition, darker red staining, which suggested severe lignification, surrounded the flag leaf of the mutant, especially at the edge of the transverse section of *ym66*. In terms of structure, the small air cavity (AC) in the flag leaves of the mutant did not support the microstructure. This issue led to some important structures being squeezed together, such as mestome sheath cells (MSCs), bundle sheath cells (BSCs), and mesophyll tissue (MT). TEM analysis of the vascular system revealed abnormal cell morphology and organelles in the *ym66* mutant (Appendix B). These structures likely prevent the transportation of some essential nutrients and induce cell death. Thus, we assessed the degenerated cells of mutant *ym66* in flag leaves *via* a Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay. A strong fluorescence signal (green fluorescence) was observed in *ym66*, but in 93–11, no evident signs of apoptosis were observed (Fig. 1-A-e–h). These experimental findings confirmed that cell death occurs in the flag leaves of the *ym66* mutant.

To further investigate the causal gene for the short and narrow flag leaf phenotype in *ym66*, we generated an F_2 mapping population by hybridizing the *ym66* mutant and the *indica* cultivar YHSM. In the F_2 population, approximately a quarter of the individuals (983/4,128) presented a small flag leaf phenotype, indicating that this abnormal flag leaf phenotype was regulated by a single recessive gene (chi-square test: $\chi^2=2.327<\chi^2_{0.05}=3.841$). Then, these 983 mutated individuals were used for mapping. The *ym66* locus was initially mapped to the long arm of chromosome 5 between markers RM26 and IR-7 (Fig. 1-C-a). After fine mapping, the positioning interval further dwindled to a 94 kb genomic region between the markers IL-28 and IR-2, in which 17 open reading frames (ORFs) were annotated (Fig. 1-C-b). To further identify the target gene, we sequenced the genomes of the 17 ORFs one by one and then compared them with the sequences of 93–11. After sequence comparison, between nucleotides 153 and 154, the 6-base sequence GCCGCG was inserted into the first exon of *LOC_Os05g50270* (*OsGATA15*) in the *ym66* mutant, and the corresponding two amino acid AA were inserted. Transgenic knockout and overexpression were performed to verify whether the variation in the insertion of *OsGATA15* was responsible for short and narrow flag leaves in the *ym66* mutant. For the analysis of transgenic knockout, the target sequence of CATCAGGACGTCGCTGCAGC was chosen as the sgRNA, and the CRISPR/Cas9 recombinant plasmid was introduced into strain 93–11. Two transgenic knockout lines (*cas9-1* and *cas9-2*) were obtained, and all individuals presented a small flag leaf phenotype similar to that of *ym66* (Appendix C). A comparison of the sequences of *cas9-1*, *cas9-2*, and 93–11 indicated that dozens of nucleotides were deleted close to the sgRNA. For the analysis of transgenic overexpression, the CDS region of the *OsGATA15* plus the UBI promotor was cloned and inserted into pCAMBIA1300, and the recombinant plasmid was introduced into the mutant *ym66*. Two transgenic overexpression lines (*oe-1* and *oe-2*) were obtained, and the small size of the flag leaf phenotype recovered considerably (Appendix C). qRT-PCR revealed that the relative expression of *OsGATA15* in the transgenic overexpression lines was greater than that in *ym66* (*P*-value<0.05). These findings suggest that short and narrow flag leaves are attributed to variations in *OsGATA15*. GATA factors are essential in various plant development processes, including the regulation of metal-binding complexes, nitrogen metabolism, carbon metabolism, and the light response (Schwechheimer *et al.* 2022). Previous reports indicated that *OsGATA15* played important roles in bract growth, flowering, and panicle development in *japonica* rice (Wang *et al.* 2009). The small flag leaf phenotype caused by the loss-of-function of *OsGATA15* in *indica* rice was not observed in *japonica* rice, suggesting that GATA15 might have played different roles in *indica* and *japonica* rice. As a transcription factor, *OsGATA15* could not only interact with the leaf initiation gene *PLA1* (Wang *et al.* 2009), but our transcriptomic and yeast one-hybrid results also indicated that it regulated the expression of multiple other genes related to leaf development in rice (data not shown). This suggested that the small flag leaf phenotype in the mutant might have been collectively caused by abnormal expression of a series of leaf development-related genes regulated by *OsGATA15* in rice.

Previous phenotypic investigations revealed that the flag leaves of the *ym66* mutant were shorter and narrower than those of 93–11, with the second and third leaves becoming shorter and the others remaining the same length. These leaf-type distribution features are highly suitable for simplified seed production and will help avoid the leaf-cutting process in seed production in two-line hybrid rice. Thus, we screened single plants with short flag leaves and outstanding agronomic traits from the F_2 population of YHSM/*ym66*. After years of continuous backcrossing and breeding, the excellent small flag leaf restoration line NP27 was cultivated. Compared with those of YHSM, the plant height of NP27 increased, the plant type became more compact, and the grain weight and stem thickness significantly improved (Fig. 1-D-a–c; Appendix D). The flag leaf length, width, and UPI length of NP27 were similar to those of the *ym66* mutant. Although the panicle length (19.36 cm) and number of branches of the short flag leaf restoring line NP27 were significantly lower than those of YHSM (panicle length 23.86 cm), the amount of pollen was sufficient to ensure the progress of seed production.

We subsequently performed hybrid rice seed production tests using the sterile lines 1892s and 20y394. The flag leaf cuttings 1892s/YHSM and 20y394/YHSM were used as blank controls. A no flag leaf-cutting combination of 1892s/YHSM and 20y394/YHSM was used as the negative control. The experimental groups included no flag leaf-cutting combinations of 1892s/NP27 and 20y394/NP27. As a result, in both the 1892s and 20y394 lines, the seed production yield of the no flag leaf-cutting combination using the short flag leaf restoring line NP27 as the male parent was greater than that of the no flag leaf-cutting combination using YHSM as the male parent and similar to that of flag leaf-cutting hybrid

combinations with YHSM as the male parent (Fig. 1-D-d and e; Appendix E). These results indicated that hybrid rice seed production using the short-leaf restoring line NP27 as the male parent did not require the flag leaf cutting step and could simplify the seed production process (Fig. 1-E). Moreover, we investigated the seed viability and agronomic traits of F_1 hybrids from 1892s&20y394 and YHSM&NP27. In terms of seed viability, the different hybrid combinations involving YHSM and NP27 did not show significant differences, with germination rates all above 82% and emergence rates also performing normally (Appendix E). Regardless of whether the maternal parent was 1892s or 20y394, the hybrid with NP27 as the paternal parent had a better plant type than did YHSM, presenting a more compact plant type and the same horizontal level of panicles (Fig. 1-D-d and e). In addition, NP27 combinations presented greater rice quality, especially in the degree of chalkiness (Appendix F).

This study provided a natural "leaf-cutting-free" solution for hybrid rice seed production. In the traditional seed production process, manual cutting of flag leaves was necessary to improve pollination conditions, but this operation required high labor intensity and technical skill, and could easily damage panicles, affecting yield. The small-flag-leaf restorer line NP27, developed utilizing the loss-of-function allele of *OsGATA15*, could maintain or even increase seed production yield without leaf cutting, significantly simplifying the production process, reducing labor requirements, and lowering seed production costs by approximately 150–180 CNY per mu (1 ha=15 mu). Furthermore, hybrid offspring bred based on this gene exhibited excellent performance in terms of plant compactness, panicle uniformity, and grain quality, indicating that the *OsGATA15* allele offered a dual advantage in improving the comprehensive traits of hybrid rice.

Acknowledgements

This research was supported by the Key Research and Development Program of Anhui Province, China (2023h11020006 and 2023n06020006), the National Natural Science Foundation of China (32301738), the Science and Technology Innovation and Breakthrough Plan Program of Anhui Province, China (202423m10050002), and the Jianghuai Granary Program, China (2026YL009).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2026.03.019

References

Chen Z C, Wu J X, Deng X W, Tang X Y. 2023. Establishment and advances of third-generation hybrid rice technology: A review. *Rice*, **16**, 56.

Guo Z W, Li H S, Wang R Z, Xiao L T. 2007. Photosynthesis of the flag leaf blade and its sheath in high-yielding hybrid rice 'Liangyoupeijiu'. *Journal of Plant Physiology & Molecular Biology*, **33**, 531–537.

Huang G Q, Hu H, Meene A V, Zhang J, Dong L, Zhang S, Zhang F L, Betts N S, Liang W Q, Bennett M J. 2021. AUXIN RESPONSE FACTORS 6 and 17 control the flag leaf angle in rice by regulating secondary cell wall biosynthesis of lamina joints. *Plant Cell*, **33**, 3120–3133.

Schwechheimer C, Schröder P M and Blaby-Haas C E. 2022. Plant GATA factors: Their biology, phylogeny, and phylogenomics. *Annual Review Plant Biology*, **73**, 123–148.

Wang L P, Yin H F, Qian Q, Yang J, Huang C F, Hu X H, Luo D. 2009. *NECK LEAF 1*, a GATA type transcription factor, modulates organogenesis by regulating the expression of multiple regulatory genes during reproductive development in rice. *Cell Research*, **19**, 598–611.

Wang M Y, Wang J B. 2022. Transcriptome and DNA methylome analyses provide insight into the heterosis in flag leaf of inter-subspecific hybrid rice. *Plant Molecular Biology*, **108**, 105–125.

Wang P, Karki S, Biswal A K, Lin H C, Dionora M J, Rizal G, Yin X J, Schuler M L, Hughes T, Fouracre J P, Abu Jamous B, Sedelnikova O, Lo S F, Bandyopadhyay A, Yu S M, Kelly S, Quick W P, Langdale J A. 2017. Candidate regulators of early leaf development in maize perturb hormone signalling and secondary cell wall formation when constitutively expressed in rice. *Scientific Reports*, **7**, 4535.

Wing R A, Purugganan M D, Zhang Q F. 2018. The rice genome revolution: From an ancient grain to Green Super Rice. *Nature Reviews Genetics*, **19**, 505–517.

Xie Y Y, Shen R X, Chen L T, Liu Y G. 2019. Molecular mechanisms of hybrid sterility in rice. *Science China-Life Sciences*, **62**, 737–743.

Executive Editor-in-Chief Xueyong Zhang
Managing Editor Ning Wang