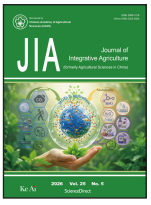
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REVIEW

Advances in rice synthetic biology: Toward a better staple crop and beyond

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Highlights

- Expansion of synthetic biology into rice reflects its growing role in addressing global challenges in agriculture and human health.
- Substantial advancements in rice synthetic biology have enhanced yield, nutritional quality, stress resistance, and resource-use efficiency.
- Synthetic production of high-value micronutrients, pharmaceutical compounds, and therapeutic proteins or peptides in the endosperm positions rice for roles extending beyond food security.

Abstract

Synthetic biology is an interdisciplinary field that applies engineering principles to design and construct novel biological systems or organisms. Initially focused on microbial systems, its applications have expanded to include plants. Plant synthetic biology offers promising solutions to pressing global challenges in agriculture and human health. As a staple crop for much of the world's population and a model species in plant science, rice has emerged as a pivotal platform in this domain. Significant progress has been achieved in genome engineering through multiplex genome editing, synthetic hybrid rice systems, induction of apomixis, reconstruction of photosynthesis and nitrogen-fixation pathways, and biosynthesis of micronutrients, pharmaceuticals, and therapeutic proteins or peptides. This review summarizes recent advances in rice synthetic biology, outlines current developments, and discusses future research directions.

Keywords: rice synthetic biology, multiplex genome editing, synthetic hybrid rice, engineered pathways, endosperm bioreactor

1. Introduction

Synthetic biology is an emerging interdisciplinary field that integrates modern biology, systems science, and synthetic science, guided by engineering principles such as standardization, modularity, and designability (Zhao 2018). Its core objective is to design and construct standardized biological components and to rationally reengineer or even synthesize artificial life forms with predefined functions. The synthetic biology approach spans the redesign, modification, and construction of biological systems, enabling both mechanistic insights into life processes (understanding through construction) and transformative applications in biotechnology (application through construction) (Zhang 2019). While initially focused on microorganisms, synthetic biology has increasingly expanded to plants. Plant synthetic biology aims to design and develop novel biological parts, devices, and systems, as well as reprogram endogenous pathways, with the ultimate goal of endowing plants with traits beyond their naturally evolved capacities (Ye *et al.* 2024; Chai *et al.* 2025; Liu *et al.* 2025).

Rice (Oryza *sativa* L.) is a staple food for more than half of the global population and plays a central role in food security. With continued population growth and intensifying climate change, there is an urgent need to enhance rice productivity and resilience (Chen R *et al.* 2022). Current trends in rice consumption reflect increasing demand for both quantity and

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quality, with a growing preference for nutrient-dense and health-promoting varieties. Despite advances in breeding, including dwarf breeding, hybrid rice development, and super rice breeding, rice yield has plateaued (Zheng *et al.* 2024). Rice production faces ongoing challenges, including limited land and water resources, salinity–alkalinity stress, and climate variability (Rezaei *et al.* 2023). At the same time, demand for rice with enhanced nutritional and health benefits is increasing, posing significant pressures on current production systems.

Rice has a well-characterized genetic background and serves as a model organism for cereal crops. It was the first monocotyledon species to have a high-quality reference genome (Sasaki *et al.* 2005). Highly efficient genetic transformation systems have been developed (Hiei *et al.* 2008). Rice also possesses abundant germplasm resources with pan-genome information (Wang *et al.* 2018). A vast amount of multi-omics data, including transcriptomic, proteomic, metabolomic, and epigenomic datasets, has been accumulated and utilized (Hong *et al.* 2019; Saeed *et al.* 2025). Significant progress has been made in elucidating the molecular mechanisms underlying key agronomic traits and their regulatory networks (Chen R *et al.* 2022). Advanced tools such as multi-gene stacking (Zhu *et al.* 2017; Hathwaik *et al.* 2021) and multiplex genome editing (MGE) (Huang *et al.* 2021; Tan *et al.* 2024) have been successfully applied in rice. These developments establish a solid foundation for rice to serve as an ideal chassis system for synthetic biology applications.

In this review, we summarize recent advances in rice synthetic biology, highlighting key breakthroughs including synthetic genomes *via* MGE, synthetic hybrid rice, apomixis, engineered photosynthesis and nitrogen-fixation pathways, and the biosynthesis of micronutrients, pharmaceutical compounds, and high-value proteins and peptides. We also discuss the current state of the field and explore future prospects.

2. Roadmap and technologies of synthetic biology

The technology roadmap of synthetic biology follows the Design-Build-Test-Learn (DBTL) cycle (Zhao 2018; Zhang 2019). This iterative framework aims to unlock the full potential of biology as a true engineering discipline.

The Design phase applies engineering principles such as abstraction and standardization, enabling the modular assembly of complex genetic circuits from simpler, well-characterized parts. This phase leverages extensive knowledge resources for rice, including reference genome sequences (Sasaki *et al.* 2005), pan-genome information (Wang *et al.* 2018), multi-omics data (Hong *et al.* 2019), molecular mechanisms of traits and regulatory networks (Chen R *et al.* 2022), and functional genetic components and control circuits from other species. Computational tools and modeling software are used to simulate system behavior in silico and predict its function before physical construction.

The Build phase in rice synthetic biology has been revolutionized by the development of highly efficient genetic transformation systems (Hiei *et al.* 2008). For introducing large synthetic pathways, multi-gene stacking systems have been successfully implemented (Zhu *et al.* 2017; Hathwaik *et al.* 2021). A wide range of CRISPR-based genome-editing tools, including nucleases, base editors, transposases, recombinases, and prime editors, have been developed and widely applied in rice (Anzalone *et al.* 2020). Moreover, MGE strategies have been well established and extensively used to simultaneously modify multiple genes or genomic regions, accelerating the improvement of rice varieties (Huang *et al.* 2021; Tan *et al.* 2024; Zhong *et al.* 2024).

In the Test phase, engineered rice lines undergo rigorous molecular characterization and extensive phenotyping. Transgenic and edited lines are cultivated in controlled environments and field trials to evaluate agronomic performance and other key traits. Analytical chemistry techniques are used to quantify the levels of target compounds in grains. This step measures outputs such as protein expression, metabolic flux, or the dynamic behavior of genetic circuits, providing essential empirical data.

Finally, the Learn phase closes the DBTL loop. Data obtained from the Test phase are analyzed to determine the causes of system success or failure. Discrepancies between predicted and actual performance guide the refinement of computational models. This newly acquired knowledge is then fed back into the Design phase, initiating a new and improved iteration of the cycle. Such an iterative process is essential for debugging biological systems and achieving robust, predictable performance.

3. Synthetic genomes *via* MGE

3.1. Agronomic and quality traits

MGE has enabled precise modulation of gene expression levels by targeting regulatory regions. For instance, editing upstream and downstream sequences of *TAC1* generated rice lines with various deletion or inversion sizes, resulting in gradient tiller angles and fine-tuned trait control (Yin *et al.* 2025). Similarly, editing the *cis*-regulatory regions of *Hd1*, *Ghd7*, and *DTH8* produced germplasm with quantitative variation in heading date, allowing selection of region-specific elite lines (Zhou *et al.* 2024). Modulation of upstream open reading frames (uORFs) in *DEP1* and *GIF1* improved yield-related traits such as grain size (Yang *et al.* 2024).

To overcome the agronomic limitations of FXZ, a high-quality but late-maturing variety with undesirable leaf morphology, an MGE-based rapid directional improvement (MRDI) strategy was developed. This approach combined precise gene editing with field-based phenotypic selection to simultaneously edit 12 genes involved in heading date, plant architecture, panicle structure, and grain quality. Backcrossing preserved elite background traits while introducing favorable alleles, yielding early-heading, compact plants with increased panicle number (Wei *et al.* 2024).

In Nangeng 9108, a high-quality but low-yielding cultivar, a comparative genomics-based MGE (CG-MGE) approach identified inferior alleles at *Gn1a*, *GW2*, *TGW3*, and *Chalk5*. Simultaneous CRISPR/Cas9-mediated editing of these four genes significantly enhanced plant architecture, grain yield,

and milling quality (Liu *et al.* 2022).

The salt-tolerant landrace Sea Rice 86 (SR86) was improved through MGE targeting nine genes associated with plant architecture, photoperiod sensitivity, grain number, grain length, fragrance, and nitrogen-use efficiency (NUE). The edited line SR86M exhibited modern-type agronomic traits while retaining elite salt tolerance (Hao *et al.* 2025).

Black and red rice are whole grains rich in diverse nutrients and exhibit a range of health-promoting effects (Yang Z *et al.* 2025). However, many black and red rice varieties have poor cooking and eating quality due to their firm texture. A soft, waxy endosperm, produced by mutations in the *Wx* gene, is required for the palatability of whole-grain rice (Tian *et al.* 2024). Similarly, the pleasant aroma of fragrant rice is attributed to the accumulation of 2-acetyl-1-pyrroline (2AP), a flavor compound that accumulates due to *BADH2* mutations. This trait is highly preferred by consumers (Prodhan and Shu 2020). Knockout of *Wx* and *BADH2* in red rice varieties Xiahuangzhan, Piguzhan, and Niuweizhan, as well as the black rice variety Heimi, simultaneously improved texture and aroma, developing high-quality black and red rice varieties (Wu W *et al.* 2025). This study provided a new strategy for improving colored whole-grain rice.

Resistant starch (RS), a prebiotic dietary fiber, is determined by key enzymes in starch biosynthesis pathways (Yang *et al.* 2016; Zhou *et al.* 2016; Bao *et al.* 2017; Teng *et al.* 2018; Yi and Li 2022; Wang A *et al.* 2023). Fine-tuning of RS content was achieved by promoter editing of *SBEIIb* (Sang *et al.* 2025). Moreover, multiplex editing of starch branching and synthase genes (*sbeI*, *sbeIIb*, *ssIIIa*, *ssIIIb* and *sbeI*, *ssIVb*, *ssIIIa*, *ssIIIb*) generated lines with RS contents exceeding 18% (Wang A *et al.* 2025).

For patients with chronic kidney disease (CKD) or phenylketonuria (PKU), rice with reduced glutelin content is highly desirable. In rice, 12 glutelin genes are classified into four groups: *GluA*, *GluB*, *GluC*, and *GluD* (He *et al.* 2021). Multiplex knockout of 7–9 glutelin genes significantly lowered both glutelin and total protein levels (Chen Z *et al.* 2022; Yang *et al.* 2022; Wakasa *et al.* 2024).

Heavy metal contamination, particularly cadmium (Cd) and arsenic (As), poses a major threat to rice safety. Knockout of *Natural Resistance-Associated Macrophage Protein 5* (*Nramp5*) and mutagenesis of the C-terminal domain of *Low silicon 2* (*Lsi2*) significantly reduced grain Cd and As accumulation without affecting yield under field conditions (Hu *et al.* 2024).

3.2. Stress resistance

Genome editing enables the development of disease-resistant rice varieties without yield penalties by either disrupting susceptibility genes or generating resistant alleles (Wang C *et al.* 2024). Broad-spectrum resistance to bacterial blight was achieved by editing TALE-binding elements in the promoters of *SWEET11*, *SWEET13*, and *SWEET14* (Oliva *et al.* 2019; Xu *et al.* 2019). Elite rice lines with durable resistance were further developed by pyramiding key resistance genes and simultaneously editing susceptibility loci such as *Pi21*, *Bsr-d1*, and *Xa5* (Tao H *et al.* 2025).

Triple knockout mutants of the ABA receptor *PYL1/4/6* exhibited enhanced thermotolerance (Miao *et al.* 2018). High cold tolerance in *indica* rice was achieved by editing *OsWRKY53* and *OsWRKY63* using a SpG-mediated MGE system (Tan *et al.* 2025a).

3.3. Photosynthesis and nutrient utilization efficiency

Multiplex CRISPR-Cas9 was employed to enhance *Photosystem II (PSII) subunit S1* (*PSBS1*) expression by editing its upstream regulatory sequences. Edited lines with elevated PsbS protein levels showed improved non-photochemical quenching (NPQ) and water-use efficiency (WUE) (Patel-Tupper *et al.* 2024).

In the low NUE *japonica* variety Jinxiangyu 1, a combination of base editing and prime editing was used to convert *OsNRT1.1B* and *OsNR2* alleles to their *indica* counterparts. Additionally, *OsTCP19* and *OsDNR1* were knocked out. These edits collectively enhanced NUE and yield stability across varying nitrogen regimes (Tao Y *et al.* 2025).

4. Synthetic hybrid rice

4.1. Synthetic novel sterile and restore lines

The cytoplasmic male sterility (CMS) and photoperiod/thermosensitive genic male sterility (PTGMS) systems are widely used in current commercial hybrid rice production (Zheng *et al.* 2024). However, the limited availability of *Restorer Fertility* (*Rf*) genes, which restore fertility in CMS lines, occurs at a frequency of only 2–5% in rice germplasm and significantly restricts the development of restorer lines (Liao *et al.* 2021). Moreover, generating new CMS and restorer lines is labor-intensive and time-consuming, impeding the broader application of CMS-based hybrid systems (Chen *et al.* 2023). The PTGMS system overcomes the strict maintainer-restorer dependency, thereby expanding hybrid combination possibilities. However, its environmental sensitivity introduces risks in seed propagation and F_1 hybrid production (Lei *et al.* 2013).

Genic male sterility (GMS), caused by mutations in nuclear genes, offers stable sterility and flexible genetic compatibility. Yet, the inability to mass-propagate pure GMS lines has hindered its practical use. To address this, several transgenic strategies have been developed. In 1993, a hemizygous transgenic maintainer system was proposed, combining a fertility-restoration gene with a pollen-lethal gene under a pollen-specific promoter to selectively eliminate transgenic pollen while enabling propagation of non-transgenic male-sterile seeds (Williams and Leemans 1993). In 2002, an alternative approach introduced a fertility-restoration gene linked to a seed-color marker gene into GMS lines, allowing visual separation of fertile (colored) and sterile (non-colored) progeny at a 1:1 ratio (Perez-Prat *et al.* 2002). These strategies were later integrated into maize to develop a transgenic maintainer line carrying the *MS45* fertility gene, a pollen-specific promoter-driven *ZmAA1* (pollen-lethal), and the aleurone-specific promoter-driven *DsRed* (fluorescent marker), enabling efficient mechanical sorting based on red fluorescence. This system produced high-purity male-sterile

seeds and genetically modified organism (GMO)-free F_1 hybrids (Albertsen *et al.* 2006; Wu *et al.* 2016).

In rice, similar approaches have been applied (Fig. 1). In 2010, the *MS26/OsCYP704B2*-based system (Li *et al.* 2010) was established in a *japonica* background. The male-sterile *oscyp704b2* mutant was complemented with *OsCYP704B2*, together with *ZmAA1* and *DsRed* under tissue-specific promoters, enabling self-pollination to produce 50% fluorescent fertile seeds and 50% non-fluorescent male-sterile seeds, which could be efficiently sorted (https://www.most.gov.cn/kjbgz/201009/t20100920_82153.html; Deng *et al.* 2013). In 2016, the *OsNP1*-based system generated male-sterile lines that, when crossed with over 1,000 germplasms, yielded ~10% superior hybrids, demonstrating strong potential for hybrid rice breeding (Chang *et al.* 2016). Further advances include the OsCYP703A3-mediated male-sterility system (Yang *et al.* 2014). Using CRISPR/Cas9, a fertility restoration (*CYP703A3*)-deficient mutant in the *indica* variety 9311 was created and introduced fertility restoration (*CYP703A3*), pollen lethality (*orfH79*), and a fluorescent marker (*DsRed2*), yielding a stable male-sterile maintainer line (9311-3B). This enabled large-scale propagation of male-sterile lines and the generation of high-performing hybrid combinations (Song *et al.* 2021). Recently, the traditional three-line CMS system was converted into an efficient "new two-line" system by introducing a fertility restoration module (Double *Rf4-Rf20*), a pollen-lethal gene (*ZmAA1*), and a fluorescent marker (*DsRed2*) into CMS lines, significantly shortening the breeding cycle (Xu N *et al.* 2025).

A dominant male-sterility system was developed using the maize *ZmMs7* gene (Fig. 2). Ectopic expression, driven by the anther-specific p5126 promoter, resulted in complete male sterility while maintaining female fertility. When coupled with the mCherry fluorescence marker, this system enabled GMO-free hybrid seed production (An *et al.* 2020).

To improve the efficiency of hybrid seed production, a mechanized mixed-planting and mixed-harvesting strategy was implemented using a female-sterile restore line derived from genic female sterility (GFS) mutants (Fig. 3). By integrating *PTB1* (conferring female fertility) (Li *et al.* 2013), *ZmAA1*, and *RsRed* into a female-sterile *ptb1* mutant, transgenic fertile and non-transgenic sterile seeds were generated in a 1:1 segregation ratio, enabling scalable hybrid seed production (Xia *et al.* 2019). In a similar approach, a female maintainer system based on the Bs-group MADS-box protein gene *FST* (Lee *et al.* 2013) enabled herbicide-mediated selection of pure female-sterile lines (Li W *et al.* 2022). A BTZ-RNA interference (RNAi) construct, designed to suppress *CYP81A6* expression and confer sensitivity to the herbicide bentazon (Lu *et al.* 2017), was linked to the *FST* genomic DNA and introduced into homozygous *fst* mutants. Self-pollination of these transgenic plants yielded progeny with a 3:1 segregation ratio of transgene-positive to transgene-negative individuals. Subsequent application of bentazon selectively eliminated transgene-positive seedlings, thereby enriching the population for pure, transgene-free female-sterile lines. Pilot field trials demonstrated that this system significantly enhances the efficiency of hybrid rice seed production compared to conventional strip-planting methods (Li W *et al.* 2022).

Two major male-sterile lines have been developed to date: *OsNP1*-based Zhen20A/Zhen21A (Chang *et al.* 2016) and *OsCYP703A3*-based 9311-3A (Song *et al.* 2021).

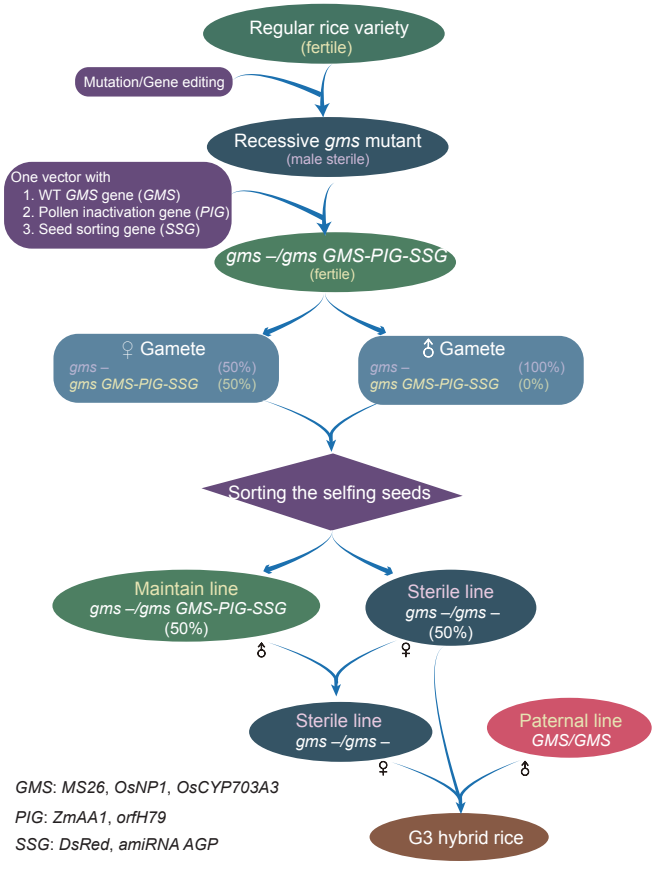


Fig. 1 Synthetic sterile lines based on the genic male sterility (GMS) mutant.

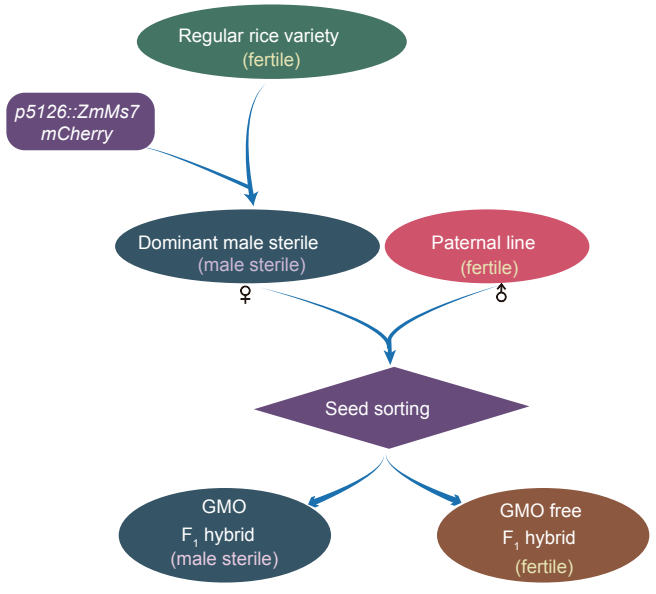


Fig. 2 Synthetic sterile lines based on a dominant male-sterility system. GMO, genetically modified organism.

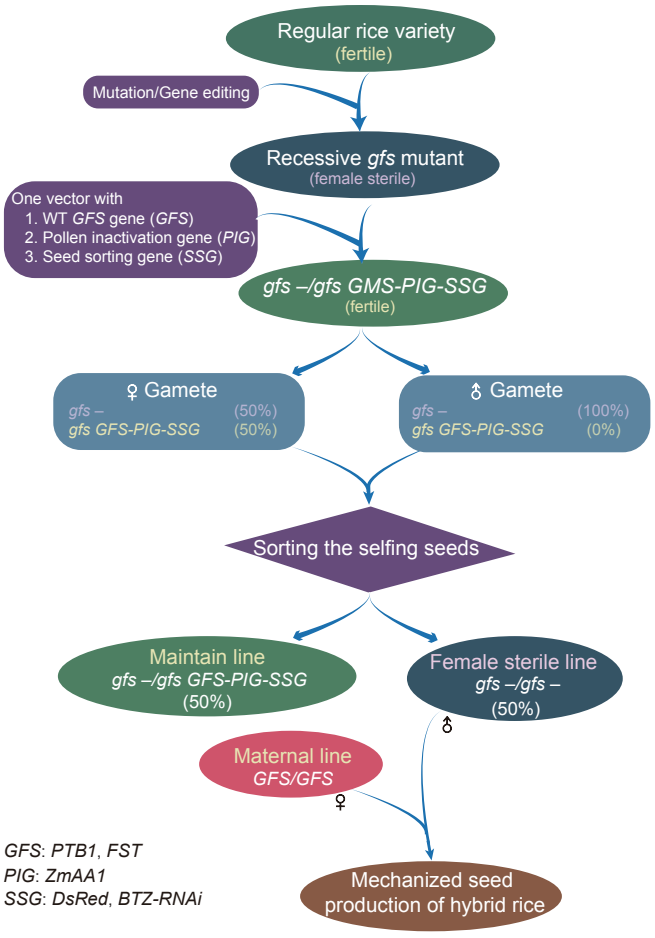


Fig. 3 Synthetic female-sterile restore lines based on GFS mutants. WT, wild type.

Notably, the hybrid Sanyou 1, derived from 9311-3A, exhibits exceptional yield, highlighting the potential of third-generation hybrid rice technology (Liao *et al.* 2021; Chen *et al.* 2023; Li *et al.* 2024). However, existing female-sterility systems often exhibit incomplete sterility or leaky phenotypes, limiting their utility in commercial breeding (Xia *et al.* 2019; Li W *et al.* 2022). Future improvements should therefore focus on novel promoters and gene targets to ensure complete pollen inactivation and prevent transgene escape (Liao *et al.* 2021). Additionally, endosperm-specific artificial miRNAs targeting starch biosynthesis genes have been employed to enable seed sorting by weight (Wu *et al.* 2021). Alternative sorting strategies include the use of herbicide resistance genes (An *et al.* 2019) and coleoptile-specific anthocyanin biosynthesis genes (Du *et al.* 2022).

4.2. Synthetic apomixis for one-line hybrid rice

Hybrid rice cannot be stably propagated through seed due to genetic segregation in heterozygotes, necessitating annual hybrid seed production by breeders and seed companies, a process that demands substantial labor, resources, and infrastructure. Consequently, farmers must repurchase new seeds annually. Apomixis, an asexual reproductive mechanism that enables clonal propagation through seeds (Koltunow and Grossniklaus 2003), offers a promising solution by fixing heterosis and eliminating the need for recurrent hybridization (Calzada *et al.* 1996). In 1987, Prof. Longping Yuan proposed the concept of one-line hybrid rice based on apomixis (Yuan 1987).

Apomixis encompasses three major developmental pathways: apospory, diplospory, and adventitious embryony. Apospory involves the direct development of somatic cells (e.g., nucellar or pericycle cells) into embryos. Diplospory occurs when the megaspore mother cell bypasses meiosis and undergoes mitotic division to form a diploid embryo sac. Adventitious embryony refers to the formation of embryos from somatic cells, typically nucellar cells, independently of the embryo sac and often occurring alongside sexual reproduction (Ozias-Akins and Van Dijk 2007).

In 2009, the meiosis-to-mitosis transition was achieved in *Arabidopsis thaliana* by generating the MiMe (Mitosis instead of Meiosis) genotype through mutations in three key meiotic genes: *AtSPO11-1*, *AtREC8*, and *AtOSD1* (D'Erfurth *et al.* 2009). This enabled the production of genetically identical diploid gametes. The MiMe system was subsequently adapted to rice using multiplex CRISPR/Cas9 to target *REC8*, *OSD1*, and *PAIR1* — the functional analog of *SPO11-1* in *Arabidopsis* (Mieulet *et al.* 2016).

By 2019, three independent studies successfully developed apomictic rice lines capable of producing maternal-genotype clonal seeds. These were achieved by combining the MiMe genotype with strategies to induce parthenogenesis. For instance, ectopic expression (EE) of *BBM1*, a paternally expressed AP2-family transcription factor, in egg cells bypassed fertilization checkpoints and induced embryo development. Integration of MiMe with *BBM1* overexpression yielded two stable S-Apo lines (MiMe+ *BBM1 EE*) that exhibited heritable asexual reproduction across multiple generations (Khanday *et al.* 2019). Another approach combined MiMe with paternal genome elimination via knockout of the pollen-specific phospholipase gene *MTL* (Yao *et al.* 2018) in the F_1 hybrid Chunyou 84, resulting in the Fix1 line, which produced diploid clonal seeds stably propagated to the T_4 generation with conserved genomic, methylomic, transcriptomic, and allelic profiles (Wang *et al.* 2019; Liu *et al.* 2023). Similar results were reported in the japonica variety Yangdao 8 (Xie *et al.* 2019).

Despite these advances, synthetic apomixis in rice faces two major limitations: low clonal seed frequencies (ranging from 6.2 to 29%) and reduced fertility, which hinder agricultural application. To address these challenges, recent efforts have focused on enhancing clonal induction rates and restoring fertility. Co-introduction of MiMe mutations and *BBM1* overexpression in the hybrid BRS-CIRAD 302 achieved over 95% clonal progeny across generations, although seed set remained suboptimal under greenhouse conditions (27.0–35.5% *vs.* 44.5% in wild-type) (Vernet *et al.* 2022). Functional analysis of *OsBBM* homologs revealed that *OsBBM4* could induce parthenogenesis, generating 3.2% haploid progeny. Combining *OsBBM4* with MiMe resulted in the Fix2 line, which showed normal fertility but only 1.7% clonal seed frequency (Wei *et al.* 2023). Ectopic expression of *OsWUS/MOC3* together with MiMe increased clonal progeny frequency to ~21.7% in the Fix3 line (Huang Y *et al.*

2025). Introducing the PAR gene from *Taraxacum officinale* into hybrid rice varieties Jiaheyou 7245 and Jiafengyou 2 significantly improved clonal seed rates to 42.9–67.7% and 43.3–62.5%, respectively (Song *et al.* 2024b). Co-expression of *OsWOX9A*, a zygotic gene enhancing *OsBBM1*-mediated parthenogenesis, boosted induction rates to 86–91%, representing a 4- to 15-fold increase over *OsBBM1* alone (Ren *et al.* 2024). To improve fertility without compromising clonal efficiency, fusion promoters (*AtMYB98_AtDD1_OsECA1-like1*) (Susaki *et al.* 2021) were used to drive *WUS* expression in synergids, antipodals, and egg cells, combined with *BBM1* under the *AtDD45* promoter and MiMe in Yongyou 4949. This strategy increased field fertility, elevating seed set from 68.16 to 83.67% (Dan *et al.* 2024). Additionally, SunTag (Tanenbaum *et al.* 2014; Papikian *et al.* 2019)-mediated enhancement of *BBM1* expression in Yongyou 4949 and Yongyou 1538 generated hybrids with normal fertility and high apomixis penetrance (Song *et al.* 2024a). Knockout of the novel haploid inducer gene *OsPLDα2* together with MiMe generated synthetic apomictic hybrids with normal seed setting but limited clonal efficiency (Hu *et al.* 2025). Expression of the *PpPAR* gene from *Pilosella piloselloides* under the *AtDD45* promoter achieved a maximum clonal seed rate of 84.6%, with seed set ranging from 52.1 to 72.6% (Xiong *et al.* 2025).

A critical challenge remains the accurate discrimination between clonal and zygotic embryos. Recently, a dual-fluorescence labeling system driven by a pollen-specific gene switch and incorporating the Cre/LoxP+FRT recombination system was integrated into the synthetic apomixis framework. This innovation enabled reliable identification of zygotic seeds based on red fluorescence emission, achieving sorting accuracy of 80.2–88.9% (Zhan *et al.* 2025).

5. Synthetic photosynthesis and nitrogen-fixation pathways

Photosynthesis is the primary determinant of crop yield. However, the current efficiency of light-to-biomass conversion in most crops remains below 1%, indicating substantial room for improvement (Leister 2023). The emergence of synthetic biology, which focuses on pathway optimization, system integration, and functional enhancement, has introduced novel strategies for engineering photosynthetic pathways, offering a promising avenue for improving agricultural productivity.

5.1. Synthetic photorespiratory bypass

Photorespiration is initiated when RuBisCO oxygenates ribulose-1,5-bisphosphate instead of fixing CO_2, leading to carbon loss and reducing C_3 crop yields by 20–50%. To mitigate this, six synthetic photorespiratory bypasses have been engineered and introduced into rice to enhance 2-phosphoglycolate (2-PG) metabolism and elevate chloroplast CO_2 concentration (Table 1; Fig. 4).

The GOC bypass, composed of *OsGLO3*, *OsOXO3*, and *OsCATC*, was engineered to convert 2-phosphoglycolate (2-PG) into CO_2 within chloroplasts. Transgenic rice lines demonstrated enhanced photosynthetic efficiency, increased biomass, and elevated nitrogen content under both greenhouse and field conditions, particularly in high-light environments. These improvements were primarily attributed to a chloroplast CO_2-concentrating mechanism rather than optimization of energy balance (Shen *et al.* 2019).

The GCGT bypass integrates OsGLO1, *EcCAT*, *EcGCL*, and *EcTSR* to redirect 75% of glycolate-derived carbon back into the Calvin cycle via glycerate and CO_2. This synthetic pathway significantly enhanced photosynthesis, leading to increased biomass and grain yield (Wang *et al.* 2020).

To address reduced seed-setting rates associated with excessive stem carbohydrate accumulation, the GMA bypass was designed. By converting 2-PG into malate through the action of *OsGLO1*, *CmMS*, and *OsAPX7*, and subsequently metabolizing malate to CO_2 endogenously, GMA plants maintained moderate photosynthate levels while preserving reproductive output (Xu *et al.* 2023).

To avoid H_2O_2 production by glycolate oxidase, the GMS bypass employed *CrGDH* instead of *OsGLO1* or *OsGLO3*. In transgenic rice backgrounds Zhonghua 11 and *osplgg1b*, the GMS pathway improved photosynthesis and biomass, increasing straw yield by 16.0 and 85.7%, respectively, and grain yield by 22.0–34.7% under field conditions (Chen X *et al.* 2025).

To couple carbon and nitrogen metabolism, the GCBG bypass was developed using *CrGDH*, *BhcC*, *BhcB*, and *OsGGT*. This pathway links glycolate-derived oxaloacetate to the GS2/Fd-GOGAT cycle, thereby enhancing amino acid synthesis, energy metabolism, and sugar production. Under natural conditions, GCBG plants exhibited a 19.0% increase in yield compared to wild-type plants and showed improved nitrogen uptake, which mitigated 44.1% of yield losses under nitrogen-deficient conditions (Chen G *et al.* 2025).

A recently developed imGS pathway introduces a light-inducible glycine methylation route in mitochondria using *ApGSMT* and *ApSDMT*. Although imGS plants exhibited enhanced CO_2 fixation, increased plant height, greater tillering, and higher grain number per panicle, low seed-setting rates and reduced thousand-grain weight resulted in lower overall yield (Mo *et al.* 2025).

Table 1 Synthetic photorespiratory bypass in rice

Bypasses	Transferred gene	Product	Advantage	Disadvantage	Reference
GOC	*OsGLO3*, *OsOXO3*, *OsCATC*	CO_2, H_2O, O_2		Produce H_2O_2	Shen *et al.* (2019)
GCGT	*OsGLO1*, *EcCAT*, *EcGCL*, *EcTSR*	Glycerate, CO_2, H_2O, O_2		Produce H_2O_2, reduced seed-setting rates	Wang *et al.* (2020)
GMA	*OsGLO1*, *CmMS*, *OsAPX7*	Malate, H_2O, O_2		Produce H_2O_2	Xu *et al.* (2023)
GMS	*CrGDH*, *CmMS*	Malate	No H_2O_2 produce		Chen X *et al.* (2025)
GCBG	*CrGDH*, *BhcC*, *BhcB*, *OsGGT*	Oxaloacetic acid, H_2O	Couple carbon and nitrogen metabolism		Chen G *et al.* (2025)
imGS	*ApGSMT*, *ApSDM*	Glycine betaie		Reduce grain yield	Mo *et al.* (2025)

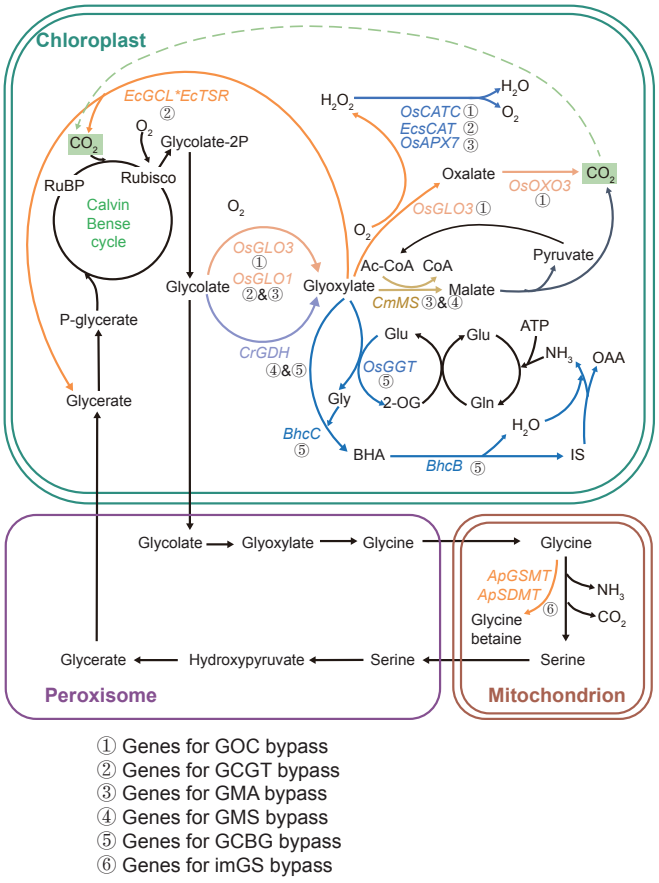


Fig. 4 Synthetic photorespiratory bypasses. Black lines indicate the endogenous pathways. Colors indicate the synthetic photorespiratory bypasses.

5.2. Synthetic crassulacean acid metabolism (CAM)-like pathway

CAM is a water-efficient photosynthetic pathway characterized by nocturnal CO_2 fixation and daytime decarboxylation. A synthetic facultative CAM bypass (CBP) was established in rice by integrating four modules: nocturnal carboxylation, comprising *AcoCA1* and *AcoPEPC1*; daytime decarboxylation, including *AcoMDH1*, *AcoPEPCK1*, and *AcoPPDK1*; malate transport, mediated by *AtALMT9*; and stomatal regulation *via* knockout of *OsSLAC1*. Genetic, transcriptomic, proteomic, and enzymatic analyses confirmed successful CBP assembly. Nighttime malate accumulation and subsequent daytime utilization improved the photosynthetic rate and carboxylation efficiency by 21 and 27%, respectively. Two-year field trials demonstrated approximately 20% increases in both grain yield and biomass (Wu S *et al.* 2025).

5.3. Synthetic D1 nucleus expression pathway

The D1 protein, encoded by the chloroplast gene *psbA*, is a critical component of photosystem II (PSII) and is highly susceptible to photodamage. Its rapid turnover is essential for PSII repair (Dong *et al.* 2016). A synthetic nuclear-encoded pathway was developed by expressing *psbA* cDNA under a heat-responsive promoter. This D1 supplementation preserves PSII integrity under heat stress, significantly improving survival and growth in transgenic *Arabidopsis*, tobacco, and rice. Field trials revealed a 20% increase in grain yield in transgenic rice, demonstrating the potential of this strategy to enhance photosynthetic efficiency and productivity under both normal and heat-stress conditions (Chen *et al.* 2020).

5.4. Synthetic nitrogen fixation in rice

Unlike legumes, cereal crops such as rice lack the root nodule symbiosis system required for biological nitrogen fixation and therefore rely heavily on chemical nitrogen fertilizers to maintain high and stable yields. Engineering plants to perform autonomous nitrogen fixation through synthetic biology represents a promising strategy to reduce this dependency (Guo *et al.* 2023). In a recent study, 13 nitrogenase-encoding genes (i.e., 11 from *Paenibacillus polymyxa* (*nifH*, *nifD*, *nifK*, *nifX*, *nifE*, *nifB*, *nifN*, *nifV*, *hesA*, *groES*, and *groEL*) and two from *Klebsiella oxytoca* (*nifU*, *nifS*)) were successfully introduced into rice using multi-gene assembly, single transformation, co-transformation, and hybridization techniques. Among 256 F_4 transgenic rice lines analyzed, 55 were confirmed by PCR and RT-PCR to contain and express all 13 genes. Quantitative RT-PCR verified transcription of all target genes, and Western blot analysis detected 11 corresponding nitrogenase proteins, excluding NifU and GroES. Notably, NifD and NifK formed stable NifDK tetramers in the rice cytoplasm, an essential structural component for nitrogenase activity. However, NifH exhibited instability in the plant cytoplasm due to proteolytic degradation, with NifH-S18 identified as the key residue responsible for this susceptibility. Through site-directed mutagenesis, researchers generated NifH variants that retained Fe protein activity and exhibited resistance to proteases, thereby improving in planta stability. These findings represent a significant step toward the functional reconstitution of a complete nitrogenase pathway in rice, laying the foundation for future development of cereals capable of autonomous nitrogen fixation (Shang *et al.* 2025).

6. Synthetic biofortification of micronutrient in rice

Rice endosperm, the major edible component of milled rice, contains only trace amounts of essential vitamins and minerals. The widespread consumption of polished rice contributes to micronutrient malnutrition, often referred to as “hidden hunger,” a significant global public health issue (Majumder *et al.* 2019). Synthetic biology strategies have been successfully applied to engineer rice for enhanced micronutrient content (Table 2), offering a sustainable solution to this challenge.

6.1. Thiamin (vitamin B_1)

Thiamine is an essential cofactor in central metabolic pathways and is critical for human neurological and cardiovascular function (Makarchikov *et al.* 2025). To increase thiamine levels in rice endosperm, three key biosynthesis genes (*THIC*, *THI1*, and *TH1*) were overexpressed using strong endosperm-specific promoters. The resulting engineered lines exhibited up to a threefold

Table 2 Micronutrient biofortification in rice

Component	Manipulated gene	Reference
Thiamin (VB_1)	*THIC*, *THI1*, *TH1*	Strobbe *et al.* (2021)
Riboflavin (VB_2)	*ScRIB1–ScRIB5*	Tian *et al.* (2021)
Pyridoxine (VB_6)	*AtPDX1.1*, *AtPDX2*	Mangel *et al.* (2019)
Folates (VB_9)	*AtADCS*, *AtGTPCHI*	Storozhenko *et al.* (2007)
	AtADCS, *AtGTPCHI*, *FPGS*, *FBP*	Blancquaert *et al.* (2015)
	OsADCS, *OsGTPCHI*	Lai *et al.* (2025)
Oil	*AtDGAT1*, *AGPL2* KO, *MTSSB1* KO	Liu *et al.* (2024)
Iron	Ferritin	Oliva *et al.* (2014)
	MxIRT1	Tan *et al.* (2015)
	OsNAS2	Trijatmiko *et al.* (2016)
	aIMA	Peng *et al.* (2022)
Zinc	*SferH-1*	Trijatmiko *et al.* (2016)

increase in thiamine content in polished rice relative to wild-type controls, with retention of the vitamin after cooking (Strobbe *et al.* 2021).

6.2. Riboflavin (vitamin B_2)

Riboflavin is a central component of redox metabolism and is essential for human growth and development. Deficiency leads to oral-ocular-genital syndrome (Thakur *et al.* 2017). Six riboflavin biosynthesis genes from Saccharomyces cerevisiae (*ScRIB1–ScRIB5* and *ScRIB7*) were introduced into rice under the control of the *Glutelin 1* promoter. Transgenic lines accumulated up to 2.168 μg g^{-1} dry weight (DW) in brown rice and 1.091 μg g^{-1} DW in polished rice (Tian *et al.* 2021).

6.3. Pyridoxine (vitamin B_6)

Pyridoxine serves as a vital cofactor in amino acid metabolism and has been implicated in the prevention of inflammation-related disorders, including cardiovascular diseases and cancer (Kumrungsee *et al.* 2022). Overexpression of *AtPDX1.1* and *AtPDX2* resulted in up to 28.3- and 12-fold increases in pyridoxine content in leaves and roots, respectively. In seeds, pyridoxine levels increased by up to 3.1-fold, predominantly in embryo and seed coat tissues, with limited enhancement observed in the endosperm. However, some transgenic lines exhibited reduced grain yield (Mangel *et al.* 2019).

6.4. Folates (vitamin B_9)

Folates are essential for one-carbon metabolism and are particularly crucial during pregnancy and early fetal development. Folate deficiency is associated with neural tube defects, anemia, and chronic conditions such as cancer and Alzheimer's disease (Iyer and Tomar 2009). Overexpression of the *Arabidopsis* genes *ADCS* and *GTPCHI* led to up to a 100-fold increase in folate content in rice seeds (Storozhenko *et al.* 2007). Further co-expression of *FPGS* and *FBP* genes enhanced folate stability during storage, yielding grains with folate levels 150-fold higher than wild type (Blancquaert *et al.* 2015). More recently, co-expression of the rice orthologs *OsADCS* and *OsGTPCHI* achieved folate concentrations of 5.65 and 2.95 μg g^{-1} in brown and polished rice, respectively (Lai *et al.* 2025).

6.5. Oil

To enhance the nutritional value of rice through oil enrichment, *AtDGAT1* was introduced under an endosperm-specific promoter. CRISPR/Cas9-mediated knockout of the starch synthesis-limiting gene *AGPL2* and the repressor of aleurone layer thickness *MTSSB1*, in Nangeng 46 background, significantly increased grain oil content from 2.33 to 11.72% (Liu *et al.* 2024).

6.6. Iron and zinc

Deficiencies in iron and zinc are linked to anemia, developmental delays, immune dysfunction, and other physiological impairments (Senguttuvel *et al.* 2023). Transgenic strategies have proven effective in enhancing the accumulation of these micronutrients in grain. Expression of soybean or rice ferritin genes in IR64 rice increased iron content by 3.4-fold (Oliva *et al.* 2014). Overexpression of *MxIRT1* elevated both iron and zinc levels by approximately threefold in T_3 seeds (Tan *et al.* 2015). Co-expression of *OsNAS2* and *SferH-1* resulted in 15 μg g^{-1} iron and 45.7 μg g^{-1} zinc in polished rice (Trijatmiko *et al.* 2016). Additionally, overexpression of *aIMA*, a synthetic peptide derived from *OsPRI1*, increased iron content by ~2-fold without compromising fertility (Peng *et al.* 2022).

7. Synthetic production of micronutrient and pharmaceutical ingredient

Rice endosperm is naturally deficient in many essential micronutrients and bioactive compounds. Recent advances in synthetic biology have enabled the *de novo* synthesis of these valuable components (Table 3), offering novel strategies for nutritional enhancement and the development of functional crops.

7.1. Carotenoids

Vitamin A deficiency remains a major global health issue, leading to vision impairment, immune dysfunction, and increased mortality from infectious diseases (Gannon *et al.* 2025). β-Carotene, a provitamin A carotenoid, is not naturally synthesized in rice endosperm. The first generation of Golden Rice was developed by introducing three bacterial and plant-derived genes: *PSY* from daffodil, *CRTI* from *Erwinia uredovora*, and *LCYB* from *Narcissus pseudonarcissus*, resulting in 1.6 μg g^{-1} total carotenoids in the endosperm (Ye *et al.* 2000). A second-generation line, Golden Rice 2, utilized maize *PSY1* and bacterial *CRTI*, achieving up to 37 μg g^{-1} total carotenoids with preferential β-carotene accumulation, representing a 23-fold increase over the original line (Paine *et al.* 2005). A bicistronic *PAC* vector encoding Capsicum *PSY* and Pantoea *CRTI via* a self-cleavable 2A sequence produced an average of 1.3 μg g^{-1} total carotenoids (Ha *et al.* 2010). Codon-optimized *PAC* (*stPAC*) significantly enhanced β-carotene levels to 2.37 μg g^{-1} and total carotenoids to

Table 3 Micronutrient and pharmaceutical ingredient synthesis in rice

Compounds	Transferred gene	Reference
Provitamin A	*NpPSY*, *EuCRTI*, *NpLCYB*	Ye *et al.* (2000)
	ZmPSY1, *EuCRTI*	Paine *et al.* (2005)
	PAC	Ha *et al.* (2010)
	stPAC	Jeong *et al.* (2017)
Astaxanthin	*ZmPSY1*, *PaCRTI*, *CrBKT*	Bai *et al.* (2017)
	ZmPSY1, *PaCRTI*, synthetic *CrBKT*, *HpBHY*	Zhu *et al.* (2018)
Zeaxanthin	*CaBch-stPAC*	Ha *et al.* (2019)
Ketocarotenoids	*BAK-stPAC*	Ha *et al.* (2019)
Ketoxanthophylls	*CaBch-stPAC*, *CaCcs*	Ha *et al.* (2019)
Taxifolin, quercetin	*C1*, *R-S*	Shin *et al.* (2006)
Naringenin, kaempferol, genistein, apigenin	*OsPAL*, *OsC4H*, *Os4CL*, *OsCHS*, *OsCHI*	Ogo *et al.* (2013)
Anthocyanin	*ZmLc*, *ZmPl*, *SsCHS*, *SsCHI*, *SsF3H*, *SsF3′H*, *SsDFR*, *SsANS*	Zhu *et al.* 2017
Sakuranetin	NOMT	Zhao *et al.* (2024)
Oleanolic acid	βAS	Huang Z *et al.* (2015a)
PPD, PPT	DS	Huang Z *et al.* (2015b)
PPD, DD	*PgDDS*, *PgCYP716A47*	Han *et al.* (2019)
Resveratrol	peanut *STS*	Baek *et al.* (2013)
Betalain	*melO*, *BvDODA1*, *BvCYP76AD1*	Tian *et al.* (2020)
	sRUBY, *BvADHα*	Tan J *et al.* (2025b)
Coenzyme Q_{10}	Amino acid residues editing of *COQ1*	Xu J J *et al.* (2025)
Catechins	*OsMYBP1*, *OsRc*, *CsCHI*, *CsCHS*, *CsF3H*, *CcF3′5′H*, *CsDFR*, *CsLAR*,	Zhu J *et al.* (2025)
Theanine	*CsAlaDC*, *CsTS*, *OsAlaAT*	Chen K *et al.* (2025)

3.50 μg g^{-1}, representing 4- and 2.9-fold increases, respectively, compared to *PAC* seeds (Jeong *et al.* 2017).

Astaxanthin, a red ketocarotenoid, exhibits strong antioxidant, anti-inflammatory, and anti-aging properties, offering therapeutic benefits for UV-induced damage, immune function, and cardiovascular disease (Xie *et al.* 2025). Zeaxanthin and capsanthin are valued as natural pigments in food and cosmetics (Mares 2016). The astaxanthin biosynthesis pathway was reconstructed in rice endosperm by expressing *ZmPSY1*, *PaCRTI*, and synthetic *CrBKT*, yielding 6.42–8.78 μg g^{-1} DW of total carotenoids, although astaxanthin accounted for only 2.9% of this pool, with canthaxanthin as the dominant species (Bai *et al.* 2017). Co-expression of synthetic *HpBHY* further increased astaxanthin content to 9.8–16.2 μg g^{-1} DW, resulting in a red-pigmented rice variety named Chijingmi with enhanced antioxidant activity (Zhu *et al.* 2018). Through conventional breeding of transgenic lines harboring *stPAC*, *CaBch*, and *BAK* (a bicistronic construct using a *2A* sequence to link *CaBch* and the Haematococcus *β-carotene ketolase* gene), novel varieties with *CaBch-stPAC* and *BAK-stPAC* configurations were developed, accumulating zeaxanthin (0.8 μg g^{-1} DW) and ketocarotenoids, including astaxanthin (1.4 μg g^{-1} DW). Additionally, capsanthin (0.4 μg g^{-1} of ketoxanthophylls, including capsanthin) was produced in rice by intercrossing a *CaBch-stPAC* line with a line expressing the capsanthin-capsorubin synthase gene from Capsicum. These results demonstrate the feasibility of multi-pathway stacking for customized nutrient and pigment production (Ha *et al.* 2019).

7.2. Flavonoids and anthocyanins

Flavonoids such as anthocyanins and sakuranetin exhibit potent antioxidant activities and contribute to functional food development (Alappat *et al.* 2020; Stompor 2020; Shen *et al.* 2022).

Expression of maize regulatory genes *C1* and *R-S* in rice endosperm led to the accumulation of taxifolin and quercetin (Shin *et al.* 2006). Naringenin-enriched rice was generated by introducing *PAL* and *CHS* genes, while further introduction of genes encoding flavonol synthase, isoflavone synthase, and flavone synthase enabled the synthesis of kaempferol, genistein, and apigenin, respectively. Tricin was also produced through additional expression of hydroxylase and methyltransferase (Ogo *et al.* 2013).

A high-efficiency vector system containing eight anthocyanin-related genes — two regulatory genes from maize and six structural genes from *Coleus scutellarioides* — reconstituted the anthocyanin biosynthesis pathway in rice endosperm, producing Purple Endosperm Rice (Zijingmi), characterized by high anthocyanin content and antioxidant capacity (Zhu *et al.* 2017).

Additionally, transgenic rice expressing *NOMT* accumulated substantial levels of sakuranetin during grain filling, conferring resistance to panicle blast without compromising key nutritional parameters (Zhao *et al.* 2024).

7.3. Ginsenosides

Ginsenosides are triterpene saponins with immunomodulatory and anticancer properties (Lee *et al.* 2025). Introduction of βAS from *Panax japonicus* enabled accumulation of oleanolic acid (8.3–11.5 mg 100 g^{-1} DW) in rice grains (Huang Z *et al.* 2015a). Overexpression of the *DS* gene yielded 20(S)-protopanaxadiol (PPD, 0.35–0.59 mg g^{-1} DW) and protopanaxatriol (PPT, 0.23–0.43 mg g^{-1} DW) (Huang Z *et al.* 2015b). Further engineering with *PgDDS* and *CYP716A47* genes from *Panax ginseng* under the *α-globulin* promoter resulted in PPD and DD concentrations of 16.4 and 4.5 μg g^{-1} DW, respectively (Han *et al.* 2019). Extracts from PPD-enriched rice exhibited superior immunomodulatory activity compared to both synthetic PPD and natural ginseng in

RAW264.7 macrophage assays (Monmai *et al.* 2022, 2023).

7.4. Resveratrol

Resveratrol is associated with multiple health benefits, including anti-obesity, cardioprotective, neuroprotective, antidiabetic, and anti-aging effects (Zhang *et al.* 2021). Transgenic rice expressing the peanut *STS* gene under the maize *Ubi1* promoter accumulated up to 1.9 μg g^{-1} DW of resveratrol in grains. Feeding trials demonstrated significant amelioration of metabolic syndrome in animals on high-fat diets (Baek *et al.* 2013). Synergistic anti-inflammatory effects were observed in extracts co-enriched with resveratrol and PPD (Monmai and Baek 2024).

7.5. Betalain

Betalain is a natural pigment with strong antioxidant and therapeutic potential against hypertension, dyslipidemia, cancer, neurological disorders, and vascular stenosis (Rahimi *et al.* 2019). It does not occur naturally in cereals. By introducing three synthetic betalain biosynthetic genes (*melO* from *Aspergillus oryzae*, *BvDODA1*, and *BvCYP76AD1* from red beet (*Beta vulgaris*)) under endosperm-specific promoters, a new rice germplasm named Betanin Rice (BR) was developed, featuring betanin accumulation (169.4 μg g^{-1} DW) and enhanced antioxidant activity (Tian *et al.* 2020). Recently, a synthetic codon-optimized *RUBY* construct — comprising *BvCYP76AD1*, *BvDODA1*, and *MjDOPA5GT* from marvel-of-Peru (*Mirabilis jalapa*) organized into a single ORF linked by P2A peptide sequences — was co-expressed with a feedback-insensitive ADH from red beet (*BvADHα*) in rice. This yielded Enhanced RUBY (eRUBY) rice with the highest betalain content of 308.4 μg g^{-1} DW (Tan *et al.* 2025).

7.6. Coenzyme Q_{10}

As a vital electron carrier in the mitochondrial respiratory chain and a lipophilic antioxidant, coenzyme Q (CoQ) plays essential roles in cellular energy metabolism and oxidative stress protection (Wang Y *et al.* 2024). Among its isoforms, CoQ_{10}, characterized by a side chain of 10 isoprenoid units, is the predominant form utilized in human physiology, particularly for cardiovascular health. However, most cereal crops and common fruits/vegetables synthesize CoQ_9, which exhibits limited bioavailability in humans. Through comparative sequence analysis and machine learning-based modeling of *COQ1* gene sequences across more than 1,000 plant species, five key amino acid residues determining side-chain length specificity in the CoQ biosynthetic enzyme *COQ1* were identified. Using targeted gene editing, these residues were modified in rice and wheat, enabling the synthesis of CoQ_{10} in the grain endosperm at levels reaching 5 μg g^{-1} DW (Xu J J *et al.* 2025).

7.7. Catechins

Catechins are flavan-3-ols predominantly found in tea (*Camellia sinensis*) and are well known for their anti-inflammatory, antimicrobial, anticancer, and cardioprotective effects (Isemura 2019). To enable *de novo* catechin biosynthesis in rice endosperm, a multi-gene expression cassette was constructed, incorporating eight genes: *OsMYBP1*, *OsRc*, *CsCHI*, *CsCHS*, *CsF3H*, *CcF3′5′H*, *CsDFR*, and *CsLAR*, all driven by endosperm-specific promoters. Two transgenic rice lines exhibited total catechin contents of 401.8 and 532.2 μg g^{-1} in polished grains. Although no significant differences were observed in major agronomic traits, the grains displayed reduced size and darker pigmentation (Zhu J *et al.* 2025).

7.8. Theanine

Theanine is a unique non-proteinogenic amino acid found in tea plants, associated with neuroprotective, calming, and cognitive-enhancing properties (Li M Y *et al.* 2022). A synthetic gene construct was developed to express a fusion of *CsAlaDC* and *CsTS via* a self-cleaving F2A peptide, together with a feedback-insensitive *OsAlaAT*, in the *indica* rice variety Nanguizhan. Transgenic rice expressing this cassette, termed TheaRice, accumulated up to 100 μg g^{-1} DW of theanine in grains, accompanied by a marked increase in gamma-aminobutyric acid (GABA) levels. Dietary supplementation with TheaRice in blue-breasted quails resulted in downregulation of hepatic cholesterol biosynthesis enzymes, enhanced immune function, and modulation of gut microbiota, characterized by reduced pathogenic bacteria and increased beneficial taxa (Chen K *et al.* 2025).

8. Rice endosperm as bioreactors for recombinant protein production

Beyond its role as a staple food, rice endosperm has emerged as a promising bioreactor for the expression and delivery of recombinant proteins. Advantages include high-level protein expression, capacity for complex post-translational modifications, biosafety, ease of downstream processing, low production cost, convenient storage, and environmental sustainability (Zhu *et al.* 2022). To date, over 10 types of recombinant proteins, including therapeutic peptides, antigens/vaccines, cytokines, and antibodies, have been successfully expressed in rice endosperm (Table 4), with several retaining biological activity after oral administration.

8.1. Pharmaceutical proteins

Lysozyme (N-acetylmuramide glycanohydrolase) degrades bacterial cell walls and neutralizes viruses by binding directly to viral proteins (Jollès 1964). Transgenic rice expressing human lysozyme under the control of the *Glutelin 1* promoter accumulated up to 0.6% of brown rice weight or 45% of total soluble proteins (Huang *et al.* 2002).

Human lactoferrin (hLF) is a key immunomodulatory component of breast milk that supports infant immunity (Wang Y *et al.* 2023). A codon-optimized synthetic *hLF* gene driven by the *Glutelin 1* promoter was introduced into rice (Nandi *et al.* 2002). The recombinant protein (rhLF) reached 0.5% of brown rice weight and was stably expressed across nine generations. rhLF was efficiently extracted and purified from rice flour with a final purity of approximately 95% (Nandi *et al.* 2005).

Human granulocyte-macrophage colony-stimulating factor

Table 4 Recombinant protein/peptide production in rice endosperm

Class	Protein/Peptide	Function	Reference
Pharmaceutical proteins	Lysozyme	Anti bacteria and virus	Huang *et al.* (2002)
	hLF	Supports infant immunity	Nandi *et al.* (2002)
	hGM-CSF	Treatment of leukopenia	Sardana *et al.* (2007)
	hIGF-1	Treatment of metabolic and degenerative diseases	Xie *et al.* (2008)
	hIL-10	Anti-inflammatory cytokine	Fujiwara *et al.* (2010)
	hSA	Maintain fluid balance and blood volume	He *et al.* (2011)
	bFGF-2	Stimulate cell proliferation	An *et al.* (2013)
	hAAT	Treatment of emphysema and liver disease	Zhang *et al.* (2013)
	GRFT	Against HIV	Vamvaka *et al.* (2016a)
Pharmaceutical peptides	MAHPs	Antihypertensive	Qian *et al.* (2020)
	AMPs	Broad-spectrum antimicrobial	Dash and Bhattacharjya (2021); Song *et al.* (2023)
Antigens/vaccines	CTB	Cholera vaccine and mucosal adjuvant	Nochi *et al.* (2007); Oszvald *et al.* (2008)
	As16	Antigen for roundworm	Matsumoto *et al.* (2009)
	Aβ42	Vaccine for AD	Yoshida *et al.* (2011)
	BoHc	Vaccine for botulism	Yuki *et al.* (2012)
	rS1	Antigen for SARS-CoV-2	Song *et al.* (2025)
Therapeutic antibodies	ARP1	Anit Rotavirus infection	Tokuhara *et al.* (2013)
	2G12	Neutralize HIV	Vamvaka *et al.* (2016b)

(hGM-CSF) is clinically used to treat leukopenia and has potential applications in immunotherapy (Baron and Nagler 2017). Using a strong endosperm-specific promoter and codon optimization, transgenic rice seeds achieved hGM-CSF levels of 1.3% of total soluble protein (14 μg/seed) (Sardana *et al.* 2007). The protein exhibited biological activity by promoting TF-1 cell proliferation and effectively alleviated leukopenia via oral administration of unprocessed grains (Ning *et al.* 2008).

Human insulin-Like Growth Factor 1 (hIGF-1) plays critical roles in cell proliferation and is used therapeutically in metabolic and degenerative diseases (Spagnoli *et al.* 2005). A fusion protein of hIGF-1 and a rice luminal binding protein was expressed in rice seeds, reaching (6.8±0.5)% of total seed protein. Orally administered transgenic seeds significantly elevated plasma hIGF-1 levels and reduced blood glucose in diabetic mice (Xie *et al.* 2008).

Human interleukin-10 (hIL-10) is an anti-inflammatory cytokine with therapeutic potential for the treatment of autoimmune and inflammatory disorders (Moore *et al.* 2001). Transgenic rice seeds expressing bioactive hIL-10 were developed, with the protein being extractable at high purity and minimal endotoxin contamination (Fujiwara *et al.* 2010).

Human serum albumin (hSA) is essential for clinical and biopharmaceutical applications but faces supply constraints and a high risk of viral transmission due to its reliance on blood donations. Rice-based expression of recombinant hSA (rhSA) under an endosperm-specific promoter yielded 10.58% of total soluble protein. Large-scale production achieved >99% purity at a yield of 2.75 g kg^{-1} brown rice (He *et al.* 2011). In a multicenter, randomized, double-blind, positive-controlled trial, rice-derived rhSA demonstrated non-inferiority to plasma-derived HSA in both efficacy and safety (Niu *et al.* 2025)

Basic fibroblast growth factor (bFGF-2) is widely used in clinical applications for scald and wound healing to stimulate cell proliferation. Additionally, it is applied to inhibit stem cell differentiation in cultures (Matsumoto *et al.* 2013). Rice-expressed recombinant bFGF-2 reached 185.66 mg kg^{-1} in brown rice. A simple purification protocol yielded 8.33 mg kg^{-1} with 4.49% recovery efficiency (An *et al.* 2013).

Deficiency in Human Alpha-1-Antitrypsin (hAAT) leads to emphysema and liver disease and requires lifelong treatment with infusions of human plasma-derived AAT (Flotte *et al.* 2004). Rice-expressed recombinant hAAT showed protease inhibitory activity and was produced at 2.24 g kg^{-1} brown rice, with a final purified yield of 0.366 g kg^{-1} (Zhang *et al.* 2013).

Acquired immunodeficiency syndrome (AIDS), caused by HIV, has posed a significant global public health challenge for over four decades (Bekker *et al.* 2023). GRFT is a potent antiviral lectin with activity against HIV (Lee 2019). Expressed in rice endosperm, GRFT reached 223 μg g^{-1} DW. Both crude and purified rice-derived GRFT exhibited strong anti-HIV activity without cytotoxicity (Vamvaka *et al.* 2016a).

8.2. Pharmaceutical peptides

Food-derived antihypertensive peptides inhibit angiotensin-converting enzyme (ACE) and offer a safe alternative to conventional drugs (Aluko 2015). A multi-antihypertensive peptide (MAHPs) construct was expressed in rice endosperm at 46–76 μg g^{-1}. Oral administration of both transgenic rice flour and extracted MAHPs from transgenic rice seed significantly lowered systolic blood pressure in spontaneously hypertensive rats (Qian *et al.* 2020).

AMPs are short peptides with broad-spectrum antimicrobial activity. They are widely regarded as next-generation antimicrobials to replace conventional antibiotics (Dash and Bhattacharjya 2021). An artificial gene encoding four shrimp-derived AMPs was expressed under the *Pzein* promoter. AMP content in rice seeds ranged from 251–300 μg g^{-1}, with retained antibacterial activity even after boiling. These

peptides inhibited methicillin-resistant *Staphylococcus* aureus (Song *et al.* 2023).

8.3. Antigens and vaccines

CTB is a component of the toxin protein produced by the pathogen *Vibrio cholerae* (Sixma *et al.* 1991), and serves as a cholera vaccine and mucosal adjuvant (Oszvald *et al.* 2008). Rice-expressed CTB accumulated at 2.1% of total soluble protein and formed functional pentamers (Oszvald *et al.* 2008). In another study, CTB accumulation reached 30 μg/seed. The protein remained stable and retained immunogenicity at room temperature for over 1.5 years in rice. Oral administration of CTB rice seeds elicited CTB-specific serum IgG and mucosal IgA antibodies in mice (Nochi *et al.* 2007). A phase 1 study showed that CTB-specific IgG and IgA antibody concentrations in participants increased significantly compared to those in placebo groups (Yuki *et al.* 2021).

Antigen As16 is protective against roundworm *Ascaris suum* (Tsuji *et al.* 2003). Rice expressing an As16-CTB fusion protein (50 μg g^{-1}) elicited specific antibody responses and reduced lung worm burden from *A. suum* eggs in mice (Matsumoto *et al.* 2009).

Aβ-composed senile plaques are hallmarks of AD. Amyloid β42 (Aβ42) is a target for AD vaccines (Schenk *et al.* 1999). GFP-fused Aβ42 was expressed in rice at up to 400 μg g^{-1} brown rice (Yoshida *et al.* 2011). Oral administration of raw or boiled transgenic Aβ rice mixed with CTB to mice induced anti-Aβ antibody production (Nojima *et al.* 2011a; Yoshida *et al.* 2011) and demonstrated therapeutic efficacy in Tg2576 AD model mice (Nojima *et al.* 2011b).

Botulinum neurotoxins, produced by *Clostridium botulinum*, are the most potent bacterial toxins and cause botulism. The BoHc fragment has been identified as a promising vaccine candidate for botulism (Dong *et al.* 2019). By suppressing major rice storage proteins, transgenic rice expressing BoHc accumulated up to 100 μg/seed and conferred protective immunity in mice (Yuki *et al.* 2012).

Despite the rapid development and deployment of multiple commercial vaccines, SARS-CoV-2, the causative agent of COVID-19, continues to circulate and evolve globally. The development of safe, effective, and cost-effective vaccine strategies remains a public health priority. Transgenic rice plants expressing the SARS-CoV-2 spike protein subunit 1 (S1) to 282 μg g^{-1} DW were generated. The rice-derived S1 protein demonstrated strong binding affinity to human angiotensin-converting enzyme 2 (ACE2). Purified recombinant S1 (rS1) protein, combined with various adjuvants, was able to elicit both humoral and cellular immune responses, highlighting its potential as a candidate subunit vaccine (Song *et al.* 2025).

8.4. Therapeutic antibodies

Rotavirus infection is a leading cause of severe diarrheal disease in infants and young children worldwide (Fischer *et al.* 2007). Transgenic rice expressing a rotavirus-specific llama heavy-chain antibody fragment *Anti-rotavirus llama single-domain antibody* (*ARP1*), combined with RNAi-mediated suppression of major endogenous storage proteins, was developed. The expressed ARP1 retained full neutralizing activity after one year of storage or boiling. Oral administration of ARP1-expressing rice significantly reduced viral load in both immunocompetent and immunodeficient mouse models, even after heat treatment (Tokuhara *et al.* 2013).

Human monoclonal antibody 2G12 broadly neutralizes HIV (Trkola *et al.* 1996). This HIV-neutralizing antibody was successfully expressed in rice endosperm, reaching 46.4 μg g^{-1} DW in T_3 generation plants. The rice-derived 2G12 exhibited approximately sevenfold higher neutralizing potency against HIV than tobacco-expressed counterparts (Vamvaka *et al.* 2016b). Furthermore, a multi-protein strategy was employed to co-express 2G12, GRFT, and cyanovirin-N (CV-N) in rice endosperm. The resulting protein cocktail achieved synergistic neutralization of HIV-1 only when all three components were present (Vamvaka *et al.* 2018).

9. Conclusions and perspectives

Synthetic biology has emerged as a transformative discipline in plant science, offering innovative solutions to global challenges in agriculture, energy, and human health (Ye *et al.* 2024). In rice, synthetic biology enables not only improvements in yield and grain quality to enhance food security, but also empowers the crop to synthesize valuable micronutrients, pharmaceutical compounds, and therapeutic proteins or peptides, thereby conferring novel health-promoting and medicinal functionalities (Fig. 5). Furthermore, emerging traits such as environmental biosensing, fluorescent reporter-based monitoring for smart agriculture (Wang J *et al.* 2025a), and autoluminescence (Ge *et al.* 2024) are anticipated to expand the functional utility of rice in precision farming and beyond. Although most rice bioreactor research focuses on the endosperm, rice straw represents a highly attractive alternative due to its abundance as a major agricultural by-product. The “green biorefinery” concept involves engineering rice plants to express hydrolytic enzymes such as cellulases and xylanases in their straw at maturity. This “self-digestion” strategy simplifies biofuel production by enabling the plant to break down its own biomass during post-harvest processing.

Advances in artificial intelligence (AI) are increasingly accelerating progress in synthetic biology (Bradbury *et al.* 2025). AlphaFold 3 now enables highly accurate predictions of protein structures and interactions involving nucleic acids, small molecules, ions, and post-translationally modified residues (Abramson *et al.* 2024). AlphaGenome can directly predict biological function and variation effects from DNA sequences at single-base resolution (https://storage.googleapis.com/deepmind-media/papers/alphagenome.pdf). Meanwhile, LucaOne, a foundational biological model that effectively captures the principles of molecular biology’s central dogma, has been developed (He *et al.* 2025). ChatNT, a multimodal conversational agent with advanced comprehension of biological sequences, was proposed as an interface between biological foundation models and conversational agents (de Almeida *et al.* 2025). Additionally,

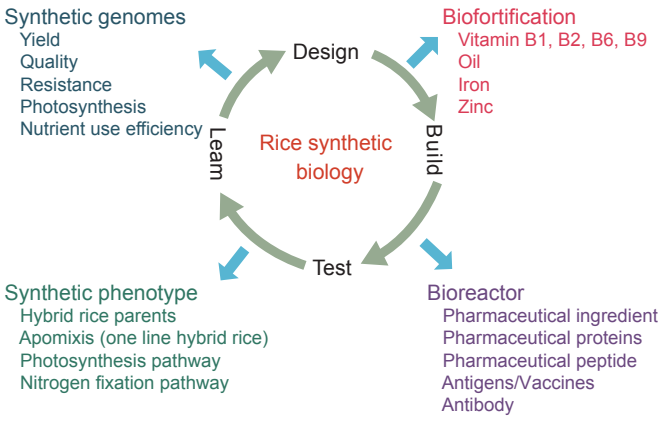


Fig. 5 Main applications of rice synthetic biology.

SYMPLEX, a universal gene-part mining platform powered by large language models (LLMs), provides a scalable framework for synthetic biology design (Wang T *et al.* 2025). The emergence of PlantGPT, a virtual expert in *Arabidopsis* genotype-phenotype relationships, demonstrates the potential of LLMs in crop functional genomics (Zhang *et al.* 2025). Similarly, SeedLLM·Rice offers a powerful tool for rice research, enabling novel insights into genetic improvement and climate adaptation through advanced data integration and reasoning (Yang F *et al.* 2025). Furthermore, AlphaFold-guided precise editing of *GmSWEET10a* has successfully enhanced oil content in field-grown soybean, demonstrating the practical impact of AI-assisted genome engineering (Wang J *et al.* 2025b).

Despite these advances, public acceptance remains a key challenge in rice synthetic biology. Concerns regarding the integration of selectable marker genes (SMGs) into plant genomes, particularly their potential impacts on human health, biodiversity, and the environment, continue to hinder its widespread adoption. Various strategies have been employed to generate SMG-free transgenic or genome-edited rice lines, and researchers are encouraged to adopt innovative approaches to eliminate SMGs from transgenic backgrounds (Singh *et al.* 2022). Recently, efficient methods for generating transgene-free edited progeny were demonstrated in *Arabidopsis* and rice (Cheng *et al.* 2025; Zhu M *et al.* 2025). Additional limitations of rice as a synthetic chassis include its long growth cycle, which prolongs research timelines, and the time- and labor-intensive nature of genetic transformation, which inherently limits throughput. The low protein yield and absence of complete mammalian-like post-translational modifications are key constraints when using the rice endosperm as a protein production bioreactor. Despite successes in producing various compounds, the endosperm remains an unsuitable chassis for synthesizing volatile or labile metabolites. Rice synthetic biology also faces critical challenges. One major challenge involves metabolic burden and resource allocation constraints, specifically competition between introduced pathways and endogenous growth processes for limited energy and precursor metabolites. Additional challenges include imprecise spatiotemporal regulation of multigene expression networks, transgenerational instability of complex gene circuits, discrepancies in performance between controlled laboratory environments and real-world field applications, and the risk of gene flow to non-transgenic crops and food chain contamination during production. Furthermore, current tools for multi-gene stacking (Zhu *et al.* 2017; Hathwaik *et al.* 2021) and MGE (Huang *et al.* 2021; Tan *et al.* 2024; Zhong *et al.* 2024) remain inadequate for achieving the sophisticated regulation of multi-gene networks required to engineer complex agronomic traits.

In the future, rice synthetic biology will prioritize modular design and construction of complex traits through data-driven and AI-powered approaches, develop more advanced tools for multi-gene stacking and genome editing, and enable seamless integration of synthetic biology workflows with automated, field-based high-throughput phenotyping platforms. By integrating interdisciplinary expertise and engineered systems, this field will transition from “reading nature” to “designing and writing life,” heralding a new era in breeding technology. This progress will create unprecedented opportunities for sustainable agriculture and next-generation bioproduction.

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Declarations of competing interest

The authors declare that they have no conflicts of interest.

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

During the preparation of this manuscript, the authors used DeepSeek, Qwen, and Doubao to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

References

Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard A J, Bambrick J, Bodenstein S W, Evans D A, Hung C C, O'neill M, Reiman D, Tunyasuvunakool K, Wu Z, Žemgulytė A, Arvaniti E, Beattie C, *et al.* 2024. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*, **630**, 493–500.

Alappat B, Alappat J. 2020. Anthocyanin pigments: Beyond aesthetics. *Molecules*, **25**, 5500.

Albertsen M C, Fox T W, Hershey H P. 2006. *Nucleotide Sequences Mediating Plant Male Fertility and Method of Using Same*. U.S. Patent, Patent No. WO2007002267, Patent and Trademark Office, Washington, D.C., USA.

De Almeida B P, Richard G, Dalla-Torre H, Blum C, Hexemer L, Pandey P, Laurent S, Rajesh C, Lopez M, Laterre A, Lang M, Şahin U, Beguir K, Pierrot T. 2025. A multimodal conversational agent for DNA, RNA and protein tasks. *Nature Machine Intelligence*, **7**, 928–941.

Aluko R E. 2015. Antihypertensive peptides from food proteins. *Annual Review of Food Science and Technology*, **6**, 235–262.

An N, Ou J, Jiang D, Zhang L, Liu J, Fu K, Dai Y, Yang D. 2013.

Expression of a functional recombinant human basic fibroblast growth factor from transgenic rice seeds. *International Journal of Molecular Sciences*, **14**, 3556–3567.
An X, Dong Z, Tian Y, Xie K, Wu S, Zhu T, Zhang D, Zhou Y, Niu C, Ma B, Hou Q, Bao J, Zhang S, Li Z, Wang Y, Yan T, Sun X, Zhang Y, Li J, Wan X. 2019. *ZmMs30* encoding a novel GDSL lipase is essential for male fertility and valuable for hybrid breeding in maize. *Molecular Plant*, **12**, 343–359.
An X, Ma B, Duan M, Dong Z, Liu R, Yuan D, Hou Q, Wu S, Zhang D, Liu D, Yu D, Zhang Y, Xie K, Zhu T, Li Z, Zhang S, Tian Y, Liu C, Li J, Yuan L, *et al*. 2020. Molecular regulation of *ZmMs7* required for maize male fertility and development of a dominant male-sterility system in multiple species. *Proceedings of the National Academy of Sciences of the United States of America*, **117**, 23499–23509.
Anzalone A V, Koblan L W, Liu D R. 2020. Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnology*, **38**, 824–844.
Baek S H, Shin W C, Ryu H S, Lee D W, Moon E, Seo C S, Hwang E, Lee H S, Ahn M H, Jeon Y, Kang H J, Lee S W, Kim S Y, D'souza R, Kim H J, Hong S T, Jeon J S. 2013. Creation of resveratrol-enriched rice for the treatment of metabolic syndrome and related diseases. *PLoS ONE*, **8**, e57930.
Bai C, Berman J, Farre G, Capell T, Sandmann G, Christou P, Zhu C. 2017. Reconstruction of the astaxanthin biosynthesis pathway in rice endosperm reveals a metabolic bottleneck at the level of endogenous β-carotene hydroxylase activity. *Transgenic Research*, **26**, 13–23.
Bao J, Zhou X, Xu F, He Q, Park Y J. 2017. Genome-wide association study of the resistant starch content in rice grains. *Starch-Stärke*, **69**, 1600343.
Baron F, Nagler A. 2017. Novel strategies for improving hematopoietic reconstruction after allogeneic hematopoietic stem cell transplantation or intensive chemotherapy. *Expert Opinion on Biological Therapy*, **17**, 163–174.
Bekker L G, Beyrer C, Mgodi N, Lewin S R, Delany-Moretlwe S, Taiwo B, Masters M C, Lazarus J V. 2023. HIV infection. *Nature Reviews of Disease Primers*, **9**, 42.
Blancquaert D, Van Daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H, Gellynck X, Lambert W, Stove C, Van Der Straeten D. 2015. Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering. *Nature Biotechnology*, **33**, 1076–1078.
Bradbury A, Clapp O, Biacsi A S, Kuo P, Gaju O, Hayta S, Zhu J K, Lambing C. 2025. Integrating genome editing with omics, artificial intelligence and advanced farming technologies to increase crop productivity. *Plant Communications*, **6**, 101386.
Calzada J P V, Crane C F, Stelly D M. 1996. Apomixis-the asexual revolution. *Science*, **274**, 1322–1323.
Chai N, Xu J, Zhang R, Li G, Wen J, Su L, Xue Y, Li T, Liu J, Zeng D, Tan J, Huang J, Chen L, Liu Y G, Zhu Q. 2025. Synthetic metabolic engineering of functional crops: Boosting nutrition and human health. *The Crop Journal*, doi: 10.1016/j.cj.2025.06.003.
Chang Z, Chen Z, Wang N, Xie G, Lu J, Yan W, Zhou J, Tang X, Deng X W. 2016. Construction of a male sterility system for hybrid rice breeding and seed production using a nuclear male sterility gene. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 14145–14150.
Chen G, Li Y, Jin K, Gao J, Wu S, Cui X, Mao C, Yin X, Lu T, Zhang Z. 2025. Synthetic photorespiratory bypass improves rice productivity by enhancing photosynthesis and nitrogen uptake. *The Plant Cell*, **37**, koaf015.
Chen J H, Chen S T, He N Y, Wang Q L, Zhao Y, Gao W, Guo F Q. 2020. Nuclear-encoded synthesis of the D1 subunit of photosystem II increases photosynthetic efficiency and crop yield. *Nature Plants*, **6**, 570–580.
Chen K, Sun K, Ye C, Liu Y, Hu Y, Jia B, Jiang S, Zou Y, Cao R, Guo J, Chen D, Zhou X, Liu J, Qian Q, Peng X, Shen M, Zhou D, Cheng S, Mi J, Zhu Q, *et al*. 2025. Metabolic engineering of theanine biosynthesis in rice endosperm to develop biofortified theaRice for benefiting human health. *Plant Communications*, **6**, 101411.
Chen R, Deng Y, Ding Y, Guo J, Qiu J, Wang B, Wang C, Xie Y, Zhang Z, Chen J, Chen L, Chu C, He G, He Z, Huang X, Xing Y, Yang S, Xie D, Liu Y, Li J. 2022. Rice functional genomics: Decades' efforts and roads ahead. *Science China Life Sciences*, **65**, 33–92.
Chen X, Li G, He H, Xie W, Cui L, Zhang Z, Peng X, Zhu G. 2025. A synthetic glycolate metabolism bypass in rice chloroplasts increases photosynthesis and yield. *The Crop Journal*, doi: 10.1016/j.cj.2025.03.001.
Chen Z, Du H, Tao Y, Xu Y, Wang F, Li B, Zhu Q H, Niu H, Yang J. 2022. Efficient breeding of low glutelin content rice germplasm by simultaneous editing multiple glutelin genes *via* CRISPR/Cas9. *Plant Science*, **324**, 111449.
Chen Z, Wu J, Deng X W, Tang X. 2023. Establishment and advances of third-generation hybrid rice technology: A review. *Rice*, **16**, 56.
Cheng T, Sun M X. 2025. A highly efficient and environmentally friendly strategy to obtain transgene-free genome-edited progeny in flowering plants. *Molecular Plant*, **18**, 911–914.
Dan J, Xia Y, Wang Y, Zhan Y, Tian J, Tang N, Deng H, Cao M. 2024. One-line hybrid rice with high-efficiency synthetic apomixis and near-normal fertility. *Plant Cell Reports*, **43**, 79.
Dash R, Bhattacharjya S. 2021. Thanatin: An emerging host defense antimicrobial peptide with multiple modes of action. *International Journal of Molecular Sciences*, **22**, 1522.
Deng X, Wang H, Tang X, Zhou J, Chen H, He G, Chen L, Xu Z. 2013. Hybrid rice breeding welcomes a new era of molecular crop design. *Scientia Sinica Vitae*, **43**, 864–868. (in Chinese)
D'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R. 2009. Turning meiosis into mitosis. *PLoS Biology*, **7**, e1000124.
Dong M, Masuyer G, Stenmark P. 2019. Botulinum and tetanus neurotoxins. *Annual Review of Biochemistry*, **88**, 811–837.
Dong X X, Jin H L, Wang H B. 2016. Research progress on adaptive mechanisms of photosystem to highlight in plants. *Plant Physiology Journal*, **52**, 1725–1732. (in Chinese)
Du S, Wang Z, Chen Y, Tan Y, Li X, Zhu W, He G, Lei K, Guo L, Zhang Y. 2022. Coleoptile purple line regulated by A-P gene system is a valuable marker trait for seed purity identification in hybrid rice. *Rice Science*, **29**, 451–461.
Fischer T K, Viboud C, Parashar U, Malek M, Steiner C, Glass R, Simonsen L. 2007. Hospitalizations and deaths from diarrhea and rotavirus among children <5 years of age in the United States, 1993–2003. *The Journal of Infectious Diseases*, **195**, 1117–1125.
Flotte T R, Brantly M L, Spencer L T, Byrne B J, Spencer C T, Baker D J, Humphries M. 2004. Phase I trial of intramuscular injection of a recombinant adeno-associated virus alpha 1-antitrypsin (rAAV2-CB-hAAT) gene vector to AAT-deficient adults. *Human Gene Therapy*, **15**, 93–128.
Fujiwara Y, Aiki Y, Yang L, Takaiwa F, Kosaka A, Tsuji N M, Shiraki K, Sekikawa K. 2010. Extraction and purification of human interleukin–10 from transgenic rice seeds. *Protein Expression and Purification*, **72**, 125–130.
Gannon B M, Huey S L, Mehta N H, Shrestha N, Lopez-Perez L, Martinez R X, Rogers L M, Garcia-Casal M N, Mehta S. 2025. Selected laboratory-based biomarkers for assessing vitamin A deficiency in at risk individuals. *Cochrane Database of Systematic Reviews*, **5**, CD013742.
Ge J, Lang X, Ji J, Qu C, Qiao H, Zhong J, Luo D, Hu J, Chen H, Wang S, Wang T, Li S, Li W, Zheng P, Xu J, Du H. 2024. Integration of biological and information technologies to enhance plant autoluminescence. *The Plant Cell*, **36**, 4703–4715.
Guo K, Yang J, Yu N, Luo L, Wang E. 2023. Biological nitrogen fixation in cereal crops: Progress, strategies, and perspectives. *Plant Communications*, **4**, 100499.
Ha S H, Kim J K, Jeong Y S, You M K, Lim S H, Kim J K. 2019. Stepwise pathway engineering to the biosynthesis of zeaxanthin, astaxanthin and capsanthin in rice endosperm. *Metabolic Engineering*, **52**, 178–189.
Ha S H, Liang Y S, Jung H, Ahn M J, Suh S C, Kweon S J, Kim D H, Kim Y M, Kim J K. 2010. Application of two bicistronic systems involving 2A and IRES sequences to the biosynthesis of carotenoids in rice endosperm. *Plant Biotechnology Journal*, **8**, 928–938.
Han J Y, Baek S H, Jo H J, Yun D W, Choi Y E. 2019. Genetically modified rice produces ginsenoside aglycone (protopanaxadiol). *Planta*, **250**, 1103–1110.
Hao Y, Xu B, Zong W, Li S, Du D, Chen M, Xiao D, Song Y, Guo X, Li W, Wu Z, Zhang K, Liao N, Hu D, Liu Y G, Guo J. 2025. Directional improvement of agronomic traits in salt-tolerant rice by multiplex-genome-editing. *Journal of Integrative Plant Biology*, **67**, 2480–2490.

Hathwaik L T, Horstman J, Thomson J G, Thilmony R. 2021. Efficient gene stacking in rice using the GAANTRY system. *Rice*, **14**, 17.
He W, Wang L, Lin Q, Yu F. 2021. Rice seed storage proteins: Biosynthetic pathways and the effects of environmental factors. *Journal of Integrative Plant Biology*, **63**, 1999–2019.
He Y, Fang P, Shan Y, Pan Y, Wei Y, Chen Y, Chen Y, Liu Y, Zeng Z, Zhou Z, Zhu F, Holmes E C, Ye J, Li J, Shu Y, Shi M, Li Z. 2025. Generalized biological foundation model with unified nucleic acid and protein language. *Nature Machine Intelligence*, **7**, 942–953.
He Y, Ning T, Xie T, Qiu Q, Zhang L, Sun Y, Jiang D, Fu K, Yin F, Zhang W, Shen L, Wang H, Li J, Lin Q, Sun Y, Li H, Zhu Y, Yang D. 2011. Large-scale production of functional human serum albumin from transgenic rice seeds. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19078–19083.
Hu F, Liu C, Jin X, Sun T, Hong L, Rao Y, Qian Q, Wang K. 2025. *OsPLDα2*-dependent synthetic apomixis enables normal seed setting in hybrid rice *via* genome editing. *Science Bulletin*, **70**, 3957–3959.
Hu S, Zhou L, Wang J, Mawia A M, Hui S, Xu B, Jiao G, Sheng Z, Shao G, Wei X, Wang L, Xie L, Zhao F, Tang S, Hu P. 2024. Production of grains with ultra-low heavy metal accumulation by pyramiding novel alleles of *OsNramp5* and *OsLsi2* in two-line hybrid rice. *Plant Biotechnology Journal*, **22**, 2921–2931.
Huang J, Nandi S, Wu L, Yalda D, Bartley G, Rodriguez R, Lonnerdal B, Huang N. 2002. Expression of natural antimicrobial human lysozyme in rice grains. *Molecular Breeding*, **10**, 83–94.
Huang Y, Dong H, Shang M, Wang K. 2021. CRISPR/Cas systems: The link between functional genes and genetic improvement. *The Crop Journal*, **9**, 678–687.
Huang Y, Meng X, Rao Y, Xie Y, Sun T, Chen W, Wei X, Xiong J, Yu H, Li J, Wang K. 2025. OsWUS-driven synthetic apomixis in hybrid rice. *Plant Communications*, **6**, 101136.
Huang Z, Lin J, Cheng Z, Xu M, Guo M, Huang X, Yang Z, Zheng J. 2015a. Production of oleanane-type sapogenin in transgenic rice *via* expression of β-amyrin synthase gene from *Panax japonicus* C. A. Mey. *BMC Biotechnology*, **15**, 45.
Huang Z, Lin J, Cheng Z, Xu M, Huang X, Yang Z, Zheng J. 2015b. Production of dammarane-type sapogenins in rice by expressing the dammarenediol-II synthase gene from *Panax ginseng* C.A. Mey. *Plant Science*, **239**, 106–114.
Hong W J, Kim Y J, Chandran A K N, Jung K H. 2019. Infrastructures of systems biology that facilitate functional genomic study in rice. *Rice*, **12**, 15.
Isemura M. 2019. Catechin in human health and disease. *Molecules*, **24**, 528.
Iyer R, Tomar S K. 2009. Folate: A functional food constituent. *Journal of Food Science*, **74**, R114–R122.
Jeong Y S, Ku H K, Kim J K, You M K, Lim S H, Kim J K, Ha S H. 2017. Effect of codon optimization on the enhancement of the β-carotene contents in rice endosperm. *Plant Biotechnology Reports*, **11**, 171–179.
Jollès P. 1964. Recent developments in the study of lysozymes. *Angewandte Chemie International Edition in English*, **3**, 28–36.
Khanday I, Skinner D, Yang B, Mercier R, Sundaresan V. 2019. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*, **565**, 91–95.
Koltunow A M, Grossniklaus U. 2003. Apomixis: A developmental perspective. *Annual Review of Plant Biology*, **54**, 547–574.
Kumrungsee T, Peipei zhang, Yanaka N, Suda T, Kato N. 2022. Emerging cardioprotective mechanisms of vitamin B6: A narrative review. *European Journal of Nutrition*, **61**, 605–613.
Lai C, Hu S, Jiao G, Wang L, Shao G, Zhao F, Xie L, Wei X, Lyu Y, Sheng Z, Tang S, Hu P. 2025. Enhancing folate content in japonica rice through co-expression of *OsADCS* and *OsGTPCHI indica* alleles. *Rice Science*, **32**, 353–366.
Lee C. 2019. Griffithsin, a highly potent broad-spectrum antiviral lectin from red algae: From discovery to clinical application. *Marine Drugs*, **17**, 567.
Lee D S, Chen L J, Li C Y, Liu Y, Tan X L, Lu B R, Li J, Gan S X, Kang S G, Suh H S, Zhu Y. 2013. The bsister MADS gene *FST* determines ovule patterning and development of the zygotic embryo and endosperm. *PLoS ONE*, **8**, e58748.
Lei D, Tang W, Xie Z Liu H, Chen LY. 2013. How to tackle insecurity problems in seed production of two-line hybrid rice. *Acta Agronomica Sinica*, **39**, 1569–1575. (in Chinese)
Lee D Y, Noren Hooten N, O'connell J F, Lee B Y, Kim Y. 2025. The role of ginseng and its bioactive compounds in aging: Cells and animal studies. *Annual Review of Food Science and Technology*, **16**, 333–354.
Leister D. 2023. Enhancing the light reactions of photosynthesis: Strategies, controversies, and perspectives. *Molecular Plant*, **16**, 4–22.
Li H, Pinot F, Sauveplane V, Werck-Reichhart D, Diehl P, Schreiber L, Franke R, Zhang P, Chen L, Gao Y, Liang W, Zhang D. 2010. Cytochrome P450 family member CYP704B2 catalyzes the ω-hydroxylation of fatty acids and is required for anther cutin biosynthesis and pollen exine formation in rice. *The Plant Cell*, **22**, 173–190.
Li M Y, Liu H Y, Wu D T, Kenaan A, Geng F, Li H B, Gunaratne A, Li H, Gan R Y. 2022. L-theanine: A unique functional amino acid in tea (*Camellia sinensis* L.) with multiple health benefits and food applications. *Frontiers in Nutrition*, **9**, 853846.
Li S, Li W, Huang B, Cao X, Zhou X, Ye S, Li C, Gao F, Zou T, Xie K, Ren Y, Ai P, Tang Y, Li X, Deng Q, Wang S, Zheng A, Zhu J, Liu H, Wang L, *et al.* 2013. Natural variation in PTB1 regulates rice seed setting rate by controlling pollen tube growth. *Nature Communications*, **4**, 2793.
Li W, Guo X, Wu W, Yu W, Li S, Luo D, Wang T, Zhu Q, Chen L, Lee D. 2022. Construction of a novel female sterility system for hybrid rice. *Frontiers in Plant Science*, **12**, 815401.
Li Y L, Tang H Y, Huang Q C, Li X Q. 2024. Research and application of the third-generation hybrid rice. *Molecular Plant Breeding*, **22**, 5124–5131. (in Chinese)
Liao C, Yan W, Chen Z, Xie G, Deng X W, Tang X. 2021. Innovation and development of the third-generation hybrid rice technology. *The Crop Journal*, **9**, 693–701.
Liu C, He Z, Zhang Y, Hu F, Li M, Liu Q, Huang Y, Wang J, Zhang W, Wang C, Wang K. 2023. Synthetic apomixis enables stable transgenerational transmission of heterotic phenotypes in hybrid rice. *Plant Communications*, **4**, 100470.
Liu J, Zhang R, Chai N, Su L, Zheng Z, Liu T, Guo Z, Ma Y, Xie Y, Xie X, Lin Q, Chen L, Liu Y G, Zhu Q. 2025. Programmable genome engineering and gene modifications for plant biodesign. *Plant Communications*, **6**, 101427.
Liu P, He L, Mei L, Zhai W, Chen X, Ma B. 2022. Rapid and directional improvement of elite rice variety *via* combination of genomics and multiplex genome editing. *Journal of Agricultural and Food Chemistry*, **70**, 6156–6167.
Liu X, Li Z, Ying J, Shu Y, Liu W, Li G, Chen L, Luo J, Wang S, Wang Y, Tong X, Huang J, Du H, Zhang J. 2024. Multi-gene engineering boosts oil content in rice grains. *Plant Communications*, **5**, 100736.
Lu H P, Liu S M, Xu S L, Chen W Y, Zhou X, Tan Y Y, Huang J Z, Shu Q Y. 2017. CRISPR-S: An active interference element for a rapid and inexpensive selection of genome-edited, transgene-free rice plants. *Plant Biotechnology Journal*, **15**, 1371–1373.
Majumder S, Datta K, Datta S K. 2019. Rice biofortification: High iron, zinc, and vitamin-A to fight against "Hidden Hunger". *Agronomy*, **9**, 803.
Makarchikov A F, Wins P, Bettendorff L. 2025. Biochemical and medical aspects of vitamin B1 research. *Neurochemistry International*, **185**, 105962.
Mangel N, Fudge J B, Li K T, Wu T Y, Tohge T, Fernie A R, Szurek B, Fitzpatrick T B, Gruissem W, Vanderschuren H. 2019. Enhancement of vitamin B6 levels in rice expressing *Arabidopsis* vitamin B6 biosynthesis de novo genes. *The Plant Journal*, **99**, 1047–1065.
Mares J. 2016. Lutein and zeaxanthin isomers in eye health and disease. *Annual Review of Nutrition*, **36**, 571–602.
Matsumoto S, Tanaka R, Okada K, Arita K, Hyakusoku H, Miyamoto M, Tabata Y, Mizuno H. 2013. The effect of control-released basic fibroblast growth factor in wound healing: Histological analyses and clinical application. *Plastic and Reconstructive Surgery — Global Open*, **1**, e44.
Matsumoto Y, Suzuki S, Nozoye T, Yamakawa T, Takashima Y, Arakawa T, Tsuji N, Takaiwa F, Hayashi Y. 2009. Oral immunogenicity and protective efficacy in mice of transgenic rice plants producing a vaccine candidate antigen (As16) of *Ascaris suum* fused with cholera toxin B subunit. *Transgenic Research*, **18**, 185–192.
Miao C, Xiao L, Hua K, Zou C, Zhao Y, Bressan R A, Zhu J K. 2018.

Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, 6058–6063.
Mieulet D, Jolivet S, Rivard M, Cromer L, Vernet A, Mayonove P, Pereira L, Droc G, Courtois B, Guiderdoni E, Mercier R. 2016. Turning rice meiosis into mitosis. *Cell Research*, **26**, 1242–1254.
Mo B, Chen X, Yang J, Chen L, Guo W, Wu S, Peng X, Zhang Z. 2025. Engineering of photorespiration-dependent glycine betaine biosynthesis improves photosynthetic carbon fixation and panicle architecture in rice. *Journal of Integrative Plant Biology*, **67**, 979–992.
Monmai C, Baek S H. 2024. Anti-inflammatory effects of the combined treatment of resveratrol- and protopanaxadiol-enriched rice seed extract on lipopolysaccharide-stimulated RAW264.7 cells. *Molecules*, **29**, 4343.
Monmai C, Kim J S, Baek S H. 2022. Transgenic rice seed extracts exert immunomodulatory effects by modulating immune-related biomarkers in RAW264.7 macrophage cells. *Nutrients*, **14**, 4143.
Monmai C, Kim J S, Baek S H. 2023. Effect of ginseng sapogenin protopanaxadiol-enriched rice (DJ-PPD) on immunomodulation. *Plants*, **12**, 767.
Moore K W, De Waal Malefyt R, Coffman R L, Apos, Garra A. 2001. Interleukin–10 and the interleukin–10 receptor. *Annual Review of Immunology*, **19**, 683–765.
Nandi S, Suzuki Y A, Huang J, Yalda D, Pham P, Wu L, Bartley G, Huang N, Lönnerdal B. 2002. Expression of human lactoferrin in transgenic rice grains for the application in infant formula. *Plant Science*, **163**, 713–722.
Nandi S, Yalda D, Lu S, Nikolov Z, Misaki R, Fujiyama K, Huang N. 2005. Process development and economic evaluation of recombinant human lactoferrin expressed in rice grain. *Transgenic Research*, **14**, 237–249.
Ning T, Xie T, Qiu Q, Yang W, Zhou S, Zhou L, Zheng C, Zhu Y, Yang D. 2008. Oral administration of recombinant human granulocyte-macrophage colony stimulating factor expressed in rice endosperm can increase leukocytes in mice. *Biotechnology Letters*, **30**, 1679–1686.
Niu J, Gao Y, Wang G, Qin Z, Wu C, Yu Z, Wang L, Hu Z, Li X, Zhang Z, Chen Y, Yao L, Yang J, Li G M, Yang Y, Lu X, Gu Y, Wu X, Mao X, Zhou Z, *et al.* 2025. Rice-derived recombinant human serum albumin as an alternative to human plasma for patients with decompensated liver cirrhosis: A randomised, double-blind, positive-controlled and non-inferiority trial. *Gut*, **74**, 1476–1485.
Nochi T, Takagi H, Yuki Y, Yang L, Masumura T, Mejima M, Nakanishi U, Matsumura A, Uozumi A, Hiroi T, Morita S, Tanaka K, Takaiwa F, Kiyono H. 2007. Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 10986–10991.
Nojima J, Ishii-Katsuno R, Futai E, Sasagawa N, Watanabe Y, Yoshida T, Ishiura S. 2011a. Production of anti-amyloid β antibodies in mice fed rice expressing amyloid β. *Bioscience*, *Biotechnology*, *and Biochemistry*, **75**, 396–400.
Nojima J, Maeda A, Aoki S, Suo S, Yanagihara D, Watanabe Y, Yoshida T, Ishiura S. 2011b. Effect of rice-expressed amyloid β in the Tg2576 Alzheimer's disease transgenic mouse model. *Vaccine*, **29**, 6252–6258.
Ogo Y, Ozawa K, Ishimaru T, Murayama T, Takaiwa F. 2013. Transgenic rice seed synthesizing diverse flavonoids at high levels: A new platform for flavonoid production with associated health benefits. *Plant Biotechnology Journal*, **11**, 734–746.
Oliva N, Chadha-Mohanty P, Poletti S, Abrigo E, Atienza G, Torrizo L, Garcia R, Dueñas C, Poncio M A, Balindong J, Manzanilla M, Montecillo F, Zaidem M, Barry G, Hervé P, Shou H, Slamet-Loedin I H. 2014. Large-scale production and evaluation of marker-free *indica* rice IR64 expressing phytoferritin genes. *Molecular Breeding*, **33**, 23–37.
Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia J C, Perez-Quintero A, Li T, Eom J S, Li C, Nguyen H, Liu B, Auguy F, Sciallano C, Luu V T, Dossa G S, Cunnac S, Schmidt S M, Slamet-Loedin I H, Vera Cruz C, Szurek B, Frommer W B, *et al.* 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nature Biotechnology*, **37**, 1344–1350.
Oszvald M, Kang T J, Tomoskozi S, Jenes B, Kim T G, Cha Y S, Tamas L, Yang M S. 2008. Expression of cholera toxin B subunit in transgenic rice endosperm. *Molecular Biotechnology*, **40**, 261–268.
Ozias-Akins P, Van Dijk P J. 2007. Mendelian genetics of apomixis in plants. *Annual Review of Genetics*, **41**, 509–537.
Paine J A, Shipton C A, Chaggar S, Howells R M, Kennedy M J, Vernon G, Wright S Y, Hinchliffe E, Adams J L, Silverstone A L, Drake R. 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology*, **23**, 482–487.
Papikian A, Liu W, Gallego-Bartolomé J, Jacobsen S E. 2019. Site-specific manipulation of *Arabidopsis* loci using CRISPR-Cas9 SunTag systems. *Nature Communications*, **10**, 729.
Patel-Tupper D, Kelikian A, Leipertz A, Maryn N, Tjahjadi M, Karavolias N G, Cho M J, Niyogi K K. 2024. Multiplexed CRISPR-Cas9 mutagenesis of rice *PSBS1* noncoding sequences for transgene-free overexpression. *Science Advances*, **10**, eadm7452.
Peng F, Li C, Lu C, Li Y, Xu P, Liang G. 2022. IRONMAN peptide interacts with OsHRZ1 and OsHRZ2 to maintain Fe homeostasis in rice. *Journal of Experimental Botany*, **73**, 6463–6474.
Perez-Prat E, Van Lookeren Campagne M M. 2002. Hybrid seed production and the challenge of propagating male-sterile plants. *Trends in Plant Science*, **7**, 199–203.
Prodhan Z H, Shu Q Y. 2020. Rice aroma: A natural gift comes with price and the way forward. *Rice Science*, **27**, 86–100.
Qian D, Qiu B, Zhou N, Takaiwa F, Yong W, Qu L Q. 2020. Hypotensive activity of transgenic rice seed accumulating multiple antihypertensive peptides. *Journal of Agricultural and Food Chemistry*, **68**, 7162–7168.
Rahimi P, Saeed A, Alireza M N S, Ostadrahimi A. 2019. Betalains, the nature-inspired pigments, in health and diseases. *Critical Reviews in Food Science and Nutrition*, **59**, 2949–2978.
Ren H, Shankle K, Cho M J, Tjahjadi M, Khanday I, Sundaresan V. 2024. Synergistic induction of fertilization-independent embryogenesis in rice egg cells by paternal-genome-expressed transcription factors. *Nature Plants*, **10**, 1892–1899.
Rezaei E E, Webber H, Asseng S, Boote K, Durand J L, Ewert F, Martre P, Maccarthy D S. 2023. Climate change impacts on crop yields. *Nature Reviews Earth & Environment*, **4**, 831–846.
Saeed P A, Ghorbani A, Rostami M, Ashrafi-Dehkordi E. 2025. Integrating multi-omic data to identify key genes and pathways involved in rice bacterial leaf streak disease. *Scientific Reports*, **15**, 21025.
Sang S, Sun X, Ma T, Zhang Y, Yao G, Wang X, Tan X, Feng L, Li J, Ji S, Cheng H. 2025. Efficient promoter editing of the SBEIIb gene enables fine-tuning of the resistant starch content in rice. *International Journal of Biological Macromolecules*, **290**, 138904.
Sardana R, Dudani A K, Tackaberry E, Alli Z, Porter S, Rowlandson K, Ganz P, Altosaar I. 2007. Biologically active human GM-CSF produced in the seeds of transgenic rice plants. *Transgenic Research*, **16**, 713–721.
Sasaki T, International Rice Genome Sequencing P. 2005. The map-based sequence of the rice genome. *Nature*, **436**, 793–800.
Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, *et al.* 1999. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, **400**, 173–177.
Senguttuvel P, Padmavathi G, Jasmine C, Sanjeeva Rao D, Neeraja C, Jaldhani V, Beulah P, Gobinath R, Aravind Kumar J, Sai Prasad S, Subba Rao L, Hariprasad A, Sruthi K, Shivani D, Sundaram R, Govindaraj M. 2023. Rice biofortification: Breeding and genomic approaches for genetic enhancement of grain zinc and iron contents. *Frontiers in Plant Science*, **14**, 1138408.
Shang Y, Shi H, Liu M, Lan P, Li D, Liu X, Wang M, Zhang Z, Chen S. 2025. Using synthetic biology to express nitrogenase biosynthesis pathway in rice and to overcome barriers of nitrogenase instability in plant cytosol. *Trends in Biotechnology*, **43**, 946–968.
Shen B R, Wang L M, Lin X L, Yao Z, Xu H W, Zhu C H, Teng H Y, Cui L L, Liu E E, Zhang J J, He Z H, Peng X X. 2019. Engineering a new chloroplastic photorespiratory bypass to increase photosynthetic efficiency and productivity in rice. *Molecular Plant*, **12**, 199–214.
Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity.

Food Chemistry, **383**, 132531.
Shin Y M, Park H J, Yim S D, Baek N I, Lee C H, An G, Woo Y M. 2006. Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. *Plant Biotechnology Journal*, **4**, 303–315.
Singh R, Kaur N, Praba U P, Kaur G, Tanin M J, Kumar P, Neelam K, Sandhu J S, Vikal Y. 2022. A prospective review on selectable marker-free genome engineered rice: Past, present and future scientific realm. *Frontiers in Genetics*, **13**, 882836.
Sixma T K, Pronk S E, Kalk K H, Wartna E S, Van Zanten B A M, Witholt B, Hoi W G J. 1991. Crystal structure of a cholera toxin-related heat-labile enterotoxin from *E. coli*. *Nature*, **351**, 371–377.
Song L, Wen Y, Zhou Y, Zhang H, Tian Y, Wang J, Cui Y, Tan R, Xiong D, Meng C, Zhou Y, Li Q, Pan Z, Liu Q, Jiao X. 2025. Rice-derived SARS-CoV–2 glycoprotein S1 subunit vaccine elicits humoral and cellular immune responses. *Plant Biotechnology Journal*, **23**, 2570–2582.
Song M, Li F, Chen Z, Hou H, Wang Y, Liu H, Liu D, Li J, Peng T, Zhao Y, Du Y, Sun H, Du C, Zhang J, Zhao Q, Miao C. 2024a. Engineering high-frequency apomixis with normal seed production in hybrid rice. *iScience*, **27**, 111479.
Song M, Wang W, Ji C, Li S, Liu W, Hu X, Feng A, Ruan S, Du S, Wang H, Dai K, Guo L, Qian Q, Si H, Hu X. 2024b. Simultaneous production of high-frequency synthetic apomixis with high fertility and improved agronomic traits in hybrid rice. *Molecular Plant*, **17**, 4–7.
Song S, Wang T, Li Y, Hu J, Kan R, Qiu M, Deng Y, Liu P, Zhang L, Dong H, Li C, Yu D, Li X, Yuan D, Yuan L, Li L. 2021. A novel strategy for creating a new system of third-generation hybrid rice technology using a cytoplasmic sterility gene and a genic male-sterile gene. *Plant Biotechnology Journal*, **19**, 251–260.
Song X, Qiao Y, Ma J, Zhang X, Liu J, Xin W, Xing S, Wang Y. 2023. Co-expression of four penaeidins in transgenic rice seeds: An alternative strategy for substitute antibiotic agricultural products. *Transgenic Research*, **32**, 463–473.
Spagnoli A, Longobardi L, O'rear L. 2005. Cartilage disorders: Potential therapeutic use of mesenchymal stem cells. *Endocrine Development*, **9**, 17–30.
Stompor M. 2020. A review on sources and pharmacological aspects of sakuranetin. *Nutrients*, **12**, 513.
Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang G F, Lambert W, Van Der Straeten D. 2007. Folate fortification of rice by metabolic engineering. *Nature Biotechnology*, **25**, 1277–1279.
Strobbe S, Verstraete J, Stove C, Van Der Straeten D. 2021. Metabolic engineering of rice endosperm towards higher vitamin B1 accumulation. *Plant Biotechnology Journal*, **19**, 1253–1267.
Susaki D, Suzuki T, Maruyama D, Ueda M, Higashiyama T, Kurihara D. 2021. Dynamics of the cell fate specifications during female gametophyte development in *Arabidopsis*. *PLoS Biology*, **19**, e3001123.
Tan J, Sun K, Qian Q, Chai N, Peng X, Shen M, Zhou D, Liu Y G, Zhu Q, Liu Q. 2025a. Development of PAM-flexible multiplex genome editors for enhancing cold-tolerance in *indica* rice. *The Crop Journal*, **13**, 991–995.
Tan J, Wang Y, Lin Z, Chai N, Xue Y, Chen L, Liu Y G, Zhu Q. 2025b eRUBY rice: Co-expression of a feedback-insensitive TyrA arogenate dehydrogenase with RUBY enhances endosperm betalain levels. *Plant Physiology*, **199**, kiaf416.
Tan S, Han R, Li P, Yang G, Li S, Zhang P, Wang W B, Zhao W Z, Yin L P. 2015. Over-expression of the MxIRT1 gene increases iron and zinc content in rice seeds. *Transgenic Research*, **24**, 109–122.
Tan W, Liang S, Gu X, Yi C, Liang G, Zhang C, Zhou Y. 2024. Application and prospects of genome-editing technology for improvement of grain yield and quality in rice. *Chinese Science Bulletin*, **70**, 2468–2482.
Tanenbaum M E, Gilbert L A, Qi L S, Weissman J S, Vale R D. 2014. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. *Cell*, **159**, 635–646.
Tao H, Xiao N, Wang R, He F, Cai Y, Jiang S, Wang M, Wang D, Chen H, You X, Li A, Wang G L, Ning Y. 2025. Development of elite rice with broad-spectrum resistance through pyramiding of key resistance gene and simultaneously editing multiple susceptibility genes. *Journal of Integrative Plant Biology*, **67**, 1691–1693.
Tao Y, Chen Z, Xu Y, Wang F, Jiang Y, Fan F, Li W, Zhu J, Li X, Wang J, Zhu Q H, Xu G, Yang J. 2025. CRISPR-mediated targeted mutagenesis for improving nitrogen use efficiency in *japonica* rice. *Plant Communications*, **6**, 101189.
Teng B, Chen Z, Ying Z, Shiyun D, Min X, Fengshun S, Jinlong N, Zhixiang L, And Ni D. 2018. Effects of different Wx alleles on amylopectin molecular structure and enzymatic hydrolysis properties of rice starch. *International Journal of Food Properties*, **21**, 2772–2784.
Thakur K, Kumar T S, Kumar S A, Surajit M, And Arora S. 2017. Riboflavin and health: A review of recent human research. *Critical Reviews in Food Science and Nutrition*, **57**, 3650–3660.
Tian H, Li Y, Lu Y, Zhang Q, Wang Z, Li S, Zhou Y, Zhang Q, Xiao J. 2024. A cooking and eating quality evaluating system for whole grain black rice. *Molecular Breeding*, **45**, 7.
Tian Y S, Fu X Y, Yang Z Q, Wang B, Gao J J, Wang M Q, Xu J, Han H J, Li Z J, Yao Q H, Peng R H. 2020. Metabolic engineering of rice endosperm for betanin biosynthesis. *New Phytologist*, **225**, 1915–1922.
Tian Y S, Xu J, Wang B, Fu X Y, Gao J J, Han H J, Li Z J, Wang L J, Zhang F J, Zhang W H, Deng Y D, Wang Y, Peng R H, Yao Q H. 2021. Riboflavin fortification of rice endosperm by metabolic engineering. *Plant Biotechnology Journal*, **19**, 1483–1485.
Tokuhara D, Álvarez B, Mejima M, Hiroiwa T, Takahashi Y, Kurokawa S, Kuroda M, Oyama M, Kozuka-Hata H, Nochi T, Sagara H, Aladin F, Marcotte H, Frenken L G J, Iturriza-Gómara M, Kiyono H, Hammarström L, Yuki Y. 2013. Rice-based oral antibody fragment prophylaxis and therapy against rotavirus infection. *The Journal of Clinical Investigation*, **123**, 3829–3838.
Trijatmiko K R, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines F M, Adeva C, Balindong J, Oliva N, Sapasap M V, Borrero J, Rey J, Francisco P, Nelson A, Nakanishi H, Lombi E, Tako E, Glahn R P, Stangoulis J, Chadha-Mohanty P, Johnson A A T, *et al.* 2016. Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Scientific Reports*, **6**, 19792.
Trkola A, Purtscher M, Muster T, Ballaun C, Buchacher A, Sullivan N, Srinivasan K, Sodroski J, Moore J P, Katinger H. 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. *Journal of Virology*, **70**, 1100–1108.
Tsuji N, Suzuki K, Kasuga-Aoki H, Isobe T, Arakawa T, Matsumoto Y. 2003. Mice intranasally immunized with a recombinant 16-kilodalton antigen from roundworm *Ascaris* parasites are protected against larval migration of *Ascaris suum*. *Infection and Immunity*, **71**, 5314–5323.
Vamvaka E, Arcalis E, Ramessar K, Evans A, O'keefe B R, Shattock R J, Medina V, Stöger E, Christou P, Capell T. 2016a. Rice endosperm is cost-effective for the production of recombinant griffithsin with potent activity against HIV. *Plant Biotechnology Journal*, **14**, 1427–1437.
Vamvaka E, Farré G, Molinos-Albert L M, Evans A, Canela-Xandri A, Twyman R M, Carrillo J, Ordóñez R A, Shattock R J, O'keefe B R, Clotet B, Blanco J, Khush G S, Christou P, Capell T. 2018. Unexpected synergistic HIV neutralization by a triple microbicide produced in rice endosperm. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E7854–E7862.
Vamvaka E, Twyman R M, Murad A M, Melnik S, Teh A Y H, Arcalis E, Altmann F, Stoger E, Rech E, Ma J K C, Christou P, Capell T. 2016b. Rice endosperm produces an underglycosylated and potent form of the HIV-neutralizing monoclonal antibody 2G12. *Plant Biotechnology Journal*, **14**, 97–108.
Vernet A, Meynard D, Lian Q, Mieulet D, Gibert O, Bissah M, Rivallan R, Autran D, Leblanc O, Meunier A C, Frouin J, Taillebois J, Shankle K, Khanday I, Mercier R, Sundaresan V, Guiderdoni E. 2022. High-frequency synthetic apomixis in hybrid rice. *Nature Communications*, **13**, 7963.
Wakasa Y, Kawakatsu T, Ishimaru K, Ozawa K. 2024. Generation of major glutelin-deficient (GluA, GluB, and GluC) semi-dwarf Koshihikari rice line. *Plant Cell Reports*, **43**, 51.
Wang A, Cheng Q, Li W, Kan M, Zhang Y, Meng X, Guo H, Jing Y, Chen M, Liu G, Wu D, Li J, Yu H. 2025. Creation of high-resistant starch rice through systematic editing of amylopectin biosynthetic genes in rs4. *Plant Biotechnology Journal*, **23**, 480–488.
Wang A, Jing Y, Cheng Q, Zhou H, Wang L, Gong W, Kou L, Liu G, Meng X, Chen M, Ma H, Shu X, Yu H, Wu D, Li J. 2023. Loss of

function of *SSIIIa* and *SSIIIb* coordinately confers high RS content in cooked rice. *Proceedings of the National Academy of Sciences of the United States of America*, **120**, e2220622120.
Wang C, Liu Q, Shen Y, Hua Y, Wang J, Lin J, Wu M, Sun T, Cheng Z, Mercier R, Wang K. 2019. Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nature Biotechnology*, **37**, 283–286.
Wang C, Wang K, Kou Y. 2024. Genome editing creates disease-resistant crops without yield penalties. *Trends in Plant Science*, **29**, 114–116.
Wang J, Yang X, Fan L, Ye H, Hao G, Wang P. 2025a. The rational design of a Rhodamine fluorescent probe enables the selective detection and bioimaging of salicylic acid in plants under abiotic stress. *Plant Biotechnology Journal*, **23**, 1736–1750.
Wang J, Zhang L, Wang S, Wang X, Li S, Gong P, Bai M, Paul A, Tvedt N, Ren H, Yang M, Zhang Z, Zhou S, Sun J, Wu X, Kuang H, Du Z, Dong Y, Shi X, Li M, *et al.* 2025b. AlphaFold-guided bespoke gene editing enhances field-grown soybean oil contents. *Advanced Science*, **12**, 2500290.
Wang L M, Shen B R, Li B D, Zhang C L, Lin M, Tong P P, Cui L L, Zhang Z S, Peng X X. 2020. A synthetic photorespiratory shortcut enhances photosynthesis to boost biomass and grain yield in rice. *Molecular Plant*, **13**, 1802–1815.
Wang T, Qin B R, Li S, Wang Z, Li X, Jiang Y, Qin C, Ouyang Q, Lou C, Qian L. 2025. Discovery of diverse and high-quality mRNA capping enzymes through a language model-enabled platform. *Science Advances*, **11**, eadt0402.
Wang W, Mauleon R, Hu Z, Chebotarov D, Tai S, Wu Z, Li M, Zheng T, Fuentes R R, Zhang F, Mansueto L, Copetti D, Sanciangco M, Palis K C, Xu J, Sun C, Fu B, Zhang H, Gao Y, Zhao X, *et al.* 2018. Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature*, **557**, 43–49.
Wang Y, Florez I D, Morgan R L, Foroutan F, Chang Y, Crandon H N, Zeraatkar D, Bala M M, Mao R Q, Tao B, Shahid S, Wang X, Beyene J, Offringa M, Sherman P M, El Gouhary E, Guyatt G H, Sadeghirad B. 2023. Probiotics, prebiotics, lactoferrin, and combination products for prevention of mortality and morbidity in preterm infants: A systematic review and network meta-analysis. *JAMA Pediatrics*, **177**, 1158–1167.
Wang Y, Lilienfeldt N, Hekimi S. 2024. Understanding coenzyme Q. *Physiological Reviews*, **104**, 1533–1610.
Wei X, Liu C, Chen X, Lu H, Wang J, Yang S, Wang K. 2023. Synthetic apomixis with normal hybrid rice seed production. *Molecular Plant*, **16**, 489–492.
Wei Y, Zhang H, Fan J, Cai Q, Zhang Z, Wang J, Zhang M, Yan F, Jiang J, Xie H, Luo X, Wei L, Lin Y, He W, Qu M, Zhang X, Zhu Y, Xie H, Zhang J. 2024. Multiplex-genome-editing based rapid directional improvement of complex traits in rice. *Plant Biotechnology Journal*, **22**, 2624–2628.
Williams M, Leemans J. 1993. *Maintenance of Male-Sterile Plants*. U.S. Patent, PCT Pub. No. WO93/ 25695. Patent and Trademark Office, Washington, D.C., USA.
Wu J, Qiu S, Wang M, Xu C, Deng X W, Tang X. 2021. Construction of a weight-based seed sorting system for the third-generation hybrid rice. *Rice*, **14**, 66.
Wu S, Jin K, Li H, Chen G, Zhang L, Yang J, Zhai S, Li Y, Sun X, Cui X, Sun J, Lu T, Zhang Z. 2025. A synthetic facultative CAM-like shuttle in C_3 rice plants. *Advanced Science*, **12**, e2500418.
Wu W, Miao R, Li Z, Fang Z. 2025. CRISPR/Cas9-mediated editing of *BADH2* and *Wx* genes for the development of novel aromatic and soft-textured black and red rice. *Physiologia Plantarum*, **177**, e70194.
Wu Y, Fox T W, Trimnell M R, Wang L, Xu R J, Cigan A M, Huffman G A, Garnaat C W, Hershey H, Albertsen M C. 2016. Development of a novel recessive genetic male sterility system for hybrid seed production in maize and other cross-pollinating crops. *Plant Biotechnology Journal*, **14**, 1046–1054.
Xia Y, Tang N, Hu Y, Li D, Li S, Bu X, Yu M, Qi S, Yang Y, Zhu H, Cao C, Li P, Yuan L, Cao M. 2019. A method for mechanized hybrid rice seed production using female sterile rice. *Rice*, **12**, 39.
Xie E, Li Y, Tang D, Lv Y, Shen Y, Cheng Z. 2019. A strategy for generating rice apomixis by gene editing. *Journal of Integrative Plant Biology*, **61**, 911–916.
Xie T, Qiu Q, Zhang W, Ning T, Yang W, Zheng C, Wang C, Zhu Y, Yang D. 2008. A biologically active rhIGF-1 fusion accumulated in transgenic rice seeds can reduce blood glucose in diabetic mice via oral delivery. *Peptides*, **29**, 1862–1870.
Xie X, Moyu Z, Xinxin H, Xinrui Y, Nasser M, Ussemane M C, Ren M. 2025. Astaxanthin biosynthesis for functional food development and space missions. *Critical Reviews in Biotechnology*, **45**, 923–937.
Xiong J, Ji Y, Yang S, Huang Y, Qiu X, Qian Q, Underwood C J, Wang K. 2025. Extending Mendel's legacy: The application of hawkweed PpPAR for inducing synthetic apomixis in hybrid rice. *Plant Communications*, **6**, 101387.
Xu H, Wang H, Zhang Y, Yang X, Lv S, Hou D, Mo C, Wassie M, Yu B, Hu T. 2023. A synthetic light-inducible photorespiratory bypass enhances photosynthesis to improve rice growth and grain yield. *Plant Communications*, **4**, 100641.
Xu J J, Lei Y, Zhang X F, Li J X, Lin Q, Wu X D, Jiang Y G, Zhang W, Qian R, Xiong S Y, Tan K, Jia Y, Zhou Q, Jiang Y, Fan H, Huang Y B, Wang L J, Liu J Y, Kong Y, Zhao Q, *et al.* 2025. Design of CoQ10 crops based on evolutionary history. *Cell*, **188**, 1941–1954.
Xu N, Yu D, Zhang L, Wang T, Hu Z, Lei Q, Qiu Y, Yuan Z, Song S, Li L. 2025. Establishment of a novel engineering breeding technology for cytoplasmic male sterility. *The Crop Journal*, **13**, 1305–1311.
Xu Z, Xu X, Gong Q, Li Z, Li Y, Wang S, Yang Y, Ma W, Liu L, Zhu B, Zou L, Chen G. 2019. Engineering broad-spectrum bacterial blight resistance by simultaneously disrupting variable TALE-binding elements of multiple susceptibility genes in rice. *Molecular Plant*, **12**, 1434–1446.
Yang F, Kong H, Ying J, Chen Z, Luo T, Jiang W, Yuan Z, Wang Z, Ma Z, Wang S, Ma W, Wang X, Li X, Hu Z, Ma X, Liu M, Wang X Q, Chen F, Dong N. 2025. SeedLLM·Rice: A large language model integrated with rice biological knowledge graph. *Molecular Plant*, **18**, 1118–1129.
Yang Q, Tang X, Wu Y, Zhu W, Zhang T, Zhang Y. 2024. CRISPR-based modulation of uORFs in DEP1 and GIF1 for enhanced rice yield traits. *Rice*, **17**, 67.
Yang R, Bai J, Fang J, Wang Y, Lee G, Piao Z. 2016. A single amino acid mutation of *OsSBEIIb* contributes to resistant starch accumulation in rice. *Breeding Science*, **66**, 481–489.
Yang X, Wu D, Shi J, He Y, Pinot F, Grausem B, Yin C, Zhu L, Chen M, Luo Z, Liang W, Zhang D. 2014. Rice CYP703A3, a cytochrome P450 hydroxylase, is essential for development of anther cuticle and pollen exine. *Journal of Integrative Plant Biology*, **56**, 979–994.
Yang Y, Shen Z, Li Y, Xu C, Xia H, Zhuang H, Sun S, Guo M, Yan C. 2022. Rapid improvement of rice eating and cooking quality through gene editing toward glutelin as target. *Journal of Integrative Plant Biology*, **64**, 1860–1865.
Yang Z, Li D, Wei R, Zhao X, Lian X, Ren C, Xiao J, Li Y, Chen X, Wing R A, Zhang Q, Chen H. 2025. Whole grain black rice: A paradigm shift from mainly calories to a nutritious food that benefits human health. *Science China Life Sciences*, **68**, 2173–2176.
Yao L, Zhang Y, Liu C, Liu Y, Wang Y, Liang D, Liu J, Sahoo G, Kelliher T. 2018. OsMATL mutation induces haploid seed formation in *indica* rice. *Nature Plants*, **4**, 530–533.
Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I. 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, **287**, 303–305.
Ye X, Qin K, Fernie A R, Zhang Y. 2024. Prospects for synthetic biology in 21st century agriculture. *Journal of Genetics and Genomics*, **52**, 967–986.
Yi X, Li C. 2022. Main controllers for improving the resistant starch content in cooked white rice. *Food Hydrocolloids*, **122**, 107083.
Yin T, Tai Y, Sun Y, Cheng Z, Wu C, Sui Y. 2025. Precise tiller angle control by manipulating TAC1 expression in rice. *Journal of Integrative Plant Biology*, **67**, 1444–1446.
Yoshida T, Kimura E, Koike S, Nojima J, Futai E, Sasagawa N, Watanabe Y, Ishiura S. 2011. Transgenic rice expressing amyloid β-peptide for oral immunization. *International Journal of Biological Sciences*, **7**, 301–307.
Yuan L P. 1987. Strategic conception of hybrid rice breeding. *Hybrid Rice*, **1**, 1–3. (in Chinese)
Yuki Y, Mejima M, Kurokawa S, Hiroiwa T, Kong I G, Kuroda M, Takahashi Y, Nochi T, Tokuhara D, Kohda T, Kozaki S, Kiyono H. 2012. RNAi suppression of rice endogenous storage proteins enhances the production of rice-based *Botulinum neutrotoxin* type A vaccine. *Vaccine*, **30**, 4160–4166.

Yuki Y, Nojima M, Hosono O, Tanaka H, Kimura Y, Satoh T, Imoto S, Uematsu S, Kurokawa S, Kashima K, Mejima M, Nakahashi-Ouchida R, Uchida Y, Marui T, Yoshikawa N, Nagamura F, Fujihashi K, Kiyono H. 2021. Oral MucoRice-CTB vaccine for safety and microbiota-dependent immunogenicity in humans: A phase 1 randomised trial. *The Lancet Microbe*, **2**, e429–e440.

Zhan Y, Xia Y, Wang Y, Liu S, Zhang X, Xiong S, Lv Q, Cao M. 2025. Efficient clonal seeds sorting for apomictic hybrid rice using a pollen-specific gene switch system. *Plant Biotechnology Journal*, **23**, 2266–2275.

Zhang L, Shi J, Jiang D, Stupak J, Ou J, Qiu Q, An N, Li J, Yang D. 2013. Expression and characterization of recombinant human alpha-antitrypsin in transgenic rice seed. *Journal of Biotechnology*, **164**, 300–308.

Zhang L X, Li C X, Kakar M U, Khan M S, Wu P F, Amir R M, Dai D F, Naveed M, Li Q Y, Saeed M, Shen J Q, Rajput S A, Li J H. 2021. Resveratrol (RV): A pharmacological review and call for further research. *Biomedicine & Pharmacotherapy*, **143**, 112164.

Zhang R, Wang Y, Yang W, Wen J, Liu W, Zhi S, Li G, Chai N, Huang J, Xie Y, Xie X, Chen L, Gu M, Liu Y G, Zhu Q. 2025. PlantGPT: An *Arabidopsis*-based intelligent agent that answers questions about plant functional genomics. *Advanced Science*, **12**, e03926.

Zhang X. 2019. Synthetic biology in China: Review and prospects. *Scientia Sinica* (Vitae), **49**, 1543–1572. (in Chinese)

Zhao G P. 2018. Synthetic biology: Unsealing the convergence era of life science research. *Bulletin of Chinese Academy of Sciences*, **33**, 1135–1149. (in Chinese)

Zhao Y, Hu J, Zhou Z, Li L, Zhang X, He Y, Zhang C, Wang J, Hong G. 2024. Biofortified rice provides rich sakuranetin in endosperm. *Rice*, **17**, 19.

Zheng X, Wei F, Cheng C, Qian Q. 2024. A historical review of hybrid rice breeding. *Journal of Integrative Plant Biology*, **66**, 532–545.

Zhong Z, Fan T, He Y, Liu S, Zheng X, Xu Y, Ren J, Yuan H, Xu Z, Zhang Y. 2024. An improved plant prime editor for efficient generation of multiple-nucleotide variations and structural variations in rice. *Plant Communications*, **5**, 100976.

Zhou H, Wang L, Liu G, Meng X, Jing Y, Shu X, Kong X, Sun J, Yu H, Smith S M, Wu D, Li J. 2016. Critical roles of soluble starch synthase SSIIIa and granule-bound starch synthase Waxy in synthesizing resistant starch in rice. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 12844–12849.

Zhou S, Cai L, Wu H, Wang B, Gu B, Cui S, Huang X, Xu Z, Hao B, Hou H, Hu Y, Li C, Tian Y, Liu X, Chen L, Liu S, Jiang L, Wan J. 2024. Fine-tuning rice heading date through multiplex editing of the regulatory regions of key genes by CRISPR-Cas9. *Plant Biotechnology Journal*, **22**, 751–758.

Zhu J, Zhou S, Wang W, Lv Y, Yang C, Wang Q, Zhang R, Shen S, Luo J. 2025. Development of “Tea Rice” by engineering catechin biosynthesis in rice endosperm. *Plant Biotechnology Journal*, **23**, 2364–2366.

Zhu M, Yan L, Zhan Z, Chen H, Wang D, Xu M, Zheng Z, Zhang Y, Yang N, Wu J, Zhan H, Tian Y, Xiong L, He Y. 2025. Transgene-Killer-CRISPR version 2 (TKC2) eliminates occasional transgene escape by coupling with a RUBY reporter. *Plant Biotechnology Journal*, **23**, 4621–4632.

Zhu Q, Tan J, Liu Y G. 2022. Molecular farming using transgenic rice endosperm. *Trends in Biotechnology*, **40**, 1248–1260.

Zhu Q, Yu S, Zeng D, Liu H, Wang H, Yang Z, Xie X, Shen R, Tan J, Li H, Zhao X, Zhang Q, Chen Y, Guo J, Chen L, Liu Y G. 2017. Development of “Purple Endosperm Rice” by engineering anthocyanin biosynthesis in the endosperm with a high-efficiency transgene stacking system. *Molecular Plant*, **10**, 918–929.

Zhu Q, Zeng D, Yu S, Cui C, Li J, Li H, Chen J, Zhang R, Zhao X, Chen L, Liu Y G. 2018. From golden rice to aSTARice: Bioengineering astaxanthin biosynthesis in rice endosperm. *Molecular Plant*, **11**, 1440–1448.

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