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REVIEW ARTICLE

Targeted editing of rice genome for enhanced yield: Progress and prospects

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Abstract

Rice is a staple food grain and its yield has undergone two major leaps, first during the 1960's through improving the harvest index by introducing semi-dwarf trait and secondly through the introduction of hybrids during the 1980's. However, yields have plateaued in the past decade, even as the growing population necessitates doubling rice production by 2050. Hence, genetic enhancement of yield potential in rice has become mandatory in rice-breeding programs, which can be achieved by mining novel yield genes from wild species, manipulating photosynthetic traits (e.g., C_4 rice), or creating novel alleles through targeted mutagenesis. The available genome of the Nipponbare rice genome and the 3K Rice Genome Project have identified beneficial alleles and valuable accessions for breeding. Progress towards C_4 rice highlights the need for photosynthetic trait manipulation to improve yields. However, the labor and time required stimulate breeders towards new technologies like genome editing. The CRISPR system offers a simpler, faster method to alter desired traits, with numerous candidate genes for grain yield. Rice, with its relatively small genome and strong synteny with other cereals, serves as an ideal model for the development of novel gene editing technologies. This review unveils an up-to-date investigation of rice genome editing for yield-related traits, with a focus on recent advancements, emerging trends and future directions to address the key challenges and opportunities in enhancing rice productivity. Future advancements in CRISPR-based multiplex editing, epigenome engineering and AI-driven predictive breeding will accelerate rice yield improvements, ensuring sustainable production to meet global food security demands by 2050.

Keywords

CRISPR/Cas9; grain yield; rice; yield genes

Introduction

Rice is a lifeline for nearly half of the global humanity. Global rice production rose from 450 million tonnes in 2011 to 490 million tonnes in 2020, with the current worldwide production of 776.46 million tonnes (1). India's rice production for the 2024-2025 agricultural year is projected to be approximately 145 million tons (2). By 2050, the world will be confronted with the task of feeding a population of 9.7 billion, requiring a projected 70 % increase in agricultural production to meet the rising demand for food. Due to the rising global population and diminishing cultivable area, maximizing grain yield

emerged as a major concern. Over the past decade, rice yields have plateaued, prompting the improvement of grain yield as the primary focus of rice-breeding initiatives (3). Higher yield needs to be achieved by developing rice varieties with greater yield potential, wider adaptability to diverse environments, reduced requirements for water, nutrients, pesticides and fungicides and maintaining high quality. Recent advances in genomics enabled accelerated identification, utilization and modification of rice genes involved in enhancing yield and stress adaptation. Yield can be improved by either increasing the crop's yield potential or by narrowing the gap between potential yield and actual yield. The productivity potential of crops can be improved by exploring the novel genes from wild cultivars or germplasm through allele mining, enhancing the photosynthetic efficiency *via* C_4 rice development, or inducing newer alleles through precise genetic modifications (4).

Rice yield, a complex quantitative polygenic trait is influenced by multiple genes, each contributing a small cumulative effect and exhibiting low heritability. Grain yield in rice is determined by several yield contributing characters *viz.,* number of grains per panicle, number of panicles per plant and the weight of the grains. Amongst these, grain number per panicle exhibits high variability and is influenced by factors such as the number of primary and secondary branches, panicle length and filled grains percentage. Panicle branching is controlled by inflorescence meristem activity which depends on the relative concentrations of auxins and cytokinins. Grain weight is governed by grain size and the extent of grain filling (3). Despite numerous studies on yield improvement in rice, the effects of alleles impacting yield traits have not been extensively examined, underscoring the importance of exploring allele diversity within rice germplasm. Few research groups have conducted allele mining for yield-associated traits, focusing on genes like *Gn1a, GS3, DEP1, Ghd7* and *sd1.* Novel alleles of *Gn1a, GS3 and DEP1* enhance rice yield by regulating grain number, grain size and panicle architecture, respectively. *Ghd7*, a key flowering regulator gene, when disrupted, can potentially improve rice yield. Additionally, targeted editing of the *sd1* gene has shown promising effects in improving lodging resistance and yield (5).

As grain yield is regulated by quantitative trait loci (QTLs), the prevalent approach in plant breeding involves generating a large array of QTL combinations and subsequently selecting the superior-performing variants. Many QTLs contributing to yield in various crops have been identified. However, due to the multitude of QTLs in the genetic material, the introgression of specific QTLs between varieties remains a complex and challenging process. Even when QTLs are tightly linked, traditional breeding methods find it impossible to effectively utilize a particular QTL of interest due to linkage drag. Recently, numerous genes associated with rice grain yield have been characterized. Dozens of yield-related genes have been cloned and most of them were negative regulators of yield. Farmers and breeders preferentially select and stabilize beneficial mutations. Several key genes, including *Gn1a, An1, SP1, DEP1, APO1, DEP3, EP3, GS3, PROG1, OsSPL14, GW5 and LRK1* play direct or indirect roles in regulating grain number per panicle (4). So, exploring these genes might help in understanding the genetic mechanisms that regulate spikelets per panicle, thereby offering valuable insights for improving rice yield through targeted genome editing and breeding strategies.

In recent days, targeted mutagenesis *via* genome editing is expeditiously evolving as a groundbreaking tool for the precise alterations of the targeted genes for enhancement of yield, resistance to biotic and abiotic stresses and enhancing the nutritional value of crop species. The precise introduction of targeted sequence variations serves as a transformative resource for improving agricultural crops. The genome editing system comprises zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENS) and clustered regularly inter-spaced short palindromic repeats (CRISPR/Cas9) (6). These techniques overcome the disadvantages exhibited by the already existing RNAi and antisense RNA technology that resulted in incomplete depletion of the target genes (7). This review highlights the genetic enhancement of yield potential in rice through genome editing.

Genome editing for precision mutagenesis

Rice functional genomics aims at exploring genes relevant to significant agronomic traits and their deployment in varietal advancement programs. More than 2250 functional genes have been identified in rice (5). The genomic position of key functionally characterized genes that govern rice grain yield and quality-related traits were positioned in Fig. 1. The different genes contributing directly or indirectly to yield improvement were tabulated (Table 1). In the recent past, genome editing technologies have revolutionized targeted mutagenesis (editing) in various plant species by utilizing tools such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR associated protein 9 (CRISPR/Cas9) systems. CRISPR/Cas9 offers several advantages among the three systems *viz*., enhanced targeting efficiency, multiplex genome editing, ease of design and implementation and reduced costs. The key step involved in genome editing is the transport of editing components into the plant cell and the subsequent generation of targeted edits (Fig. 2). CRISPR-mediated gene editing can be employed for plant functional studies and improvement of grain yield, grain quality and tolerance to various stresses. This approach addresses the challenges of time consumption and the extensive land requirements associated with traditional breeding techniques (32). Thus, a novel technology aids in fulfilling the requirements of the rising population. Due to its simplicity and enhanced efficiency, the tool is extensively used in innovative genome manipulation and functional genomic exploration of various crops as illustrated in Fig. 3. An interesting application of CRISPR/Cas9 in rice lies in the generation of genome-wide mutant library for identification of gene activities and for genetic advancement. CRISPR/Cas9 system is successfully employed to impart herbicide tolerance and biotic and abiotic stress tolerance, enhancing grain yield and improving grain quality in rice (33).

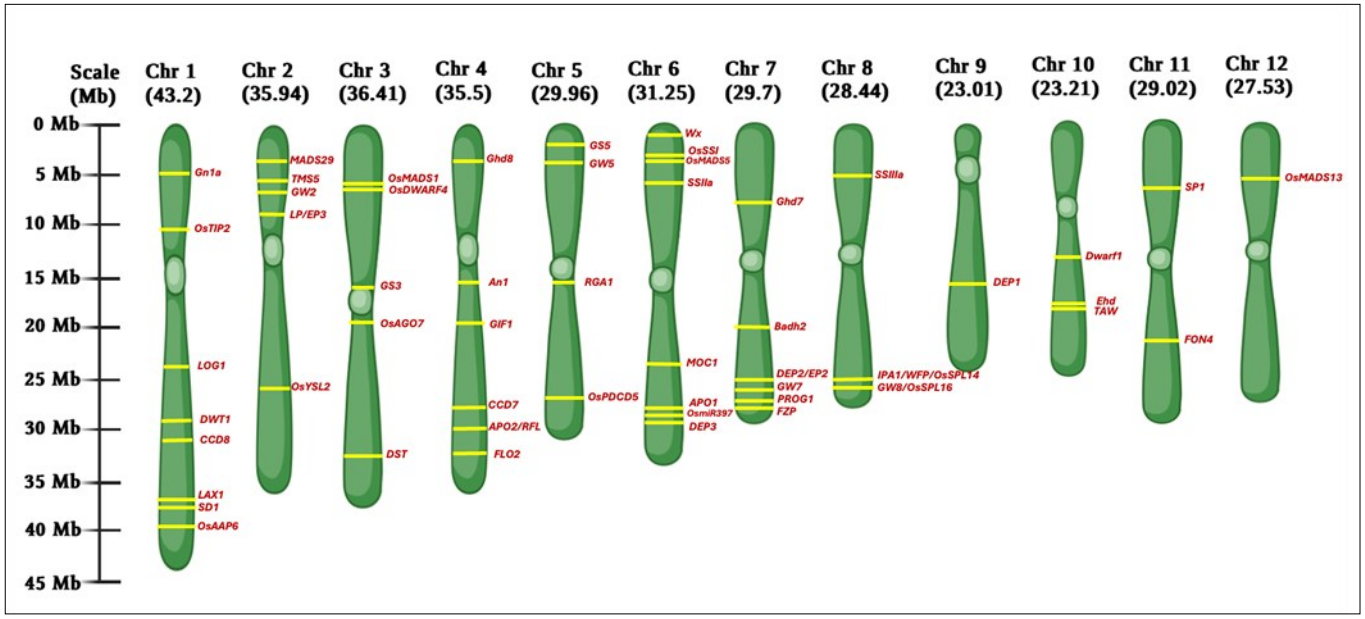

Fig. 1. Major functionally characterized genes governing grain yield and quality related traits in rice.

Table 1. Genes of agronomically important traits in rice contributing directly or indirectly to yield improvement

Gene	Functional description	Key trait (s)	Controlling trait	References
OsGA20ox2	Gibberellin 20-oxidase	Gibberellin synthesis-plant height		(8)
Ghd7	CCT (CO, CO-LIKE and TIMING OF CAB1) domain protein	Heading date		(9)
DEP1	phoshatidylethanolamine-binding protein domain	Panicle architecture		(10)
DEP2	Plant specific protein without recognized functional domain	Panicle architecture		(11)
SCM2/APO1	Arabidopsis LEAFY Ortholog	Panicle architecture, spikelet number, culm strength		(12)
GS5	Encodes serine carboxypeptidase	Grain size		(13)
OsMADS57	MADS-box gene	Tillering	Grain yield	(14)
TGW6	Encodes for IAA-glucose hydrolase activity	Grain weight		(15)
An-1	Basic helix loop like helix protein	Spikelet number		(16)
Chalk5	Vacuolar H^+-translocating pyrophosphatase	Head rice yield		(17)
GL2/OsGRF4	GRF (Growth-regulating factor)	Grain size		(18)
HOX12	Homeodomain-leucine zipper transcription factor	Panicle exsertion		(19)
OsSPL13	Encodes SPL family protein	Grain size		(20)
OsSPL16	Encodes SPL family protein	Grain size		(21)
OsPho1	α-Glucan plastidial phosphorylase	Starch synthesis and it's structure in the endosperm	Grain quality	(22)
BPH14	CC-NB-LRR protein	Brown plant hopper resistance		(23)
Xa10	TAL effector-dependent resistant gene	Bacterial Leaf Blight disease	Biotic stress	(24)
Rac1-RbohB/H	Respiratory burst oxidase-related protein	Chitin-induced immunity (blast resistance)		(25)
SNORKEL1, SNORKEL2	Ethylene response factor	Submergence		(26)
MODD	Mediator of OsbZIP46 deactivation and degradation	Drought Resistance and Regulate ABA signaling	Abiotic stress	(27)
PYL9	Pyrabactin resistance 1-like ABA receptor	drought resistance and leaf senescence		(28)
STAR1, STAR2	Bacterial ABC transporter	Aluminum tolerance		(29)
OsPTR9	Peptide transporter	nitrogen utilization efficiency, grain yield and growth	Nitrogen use efficiency	(30)
OsNRT2.3b	High-affinity nitrate transporter	long-distance nitrate transport		(31)

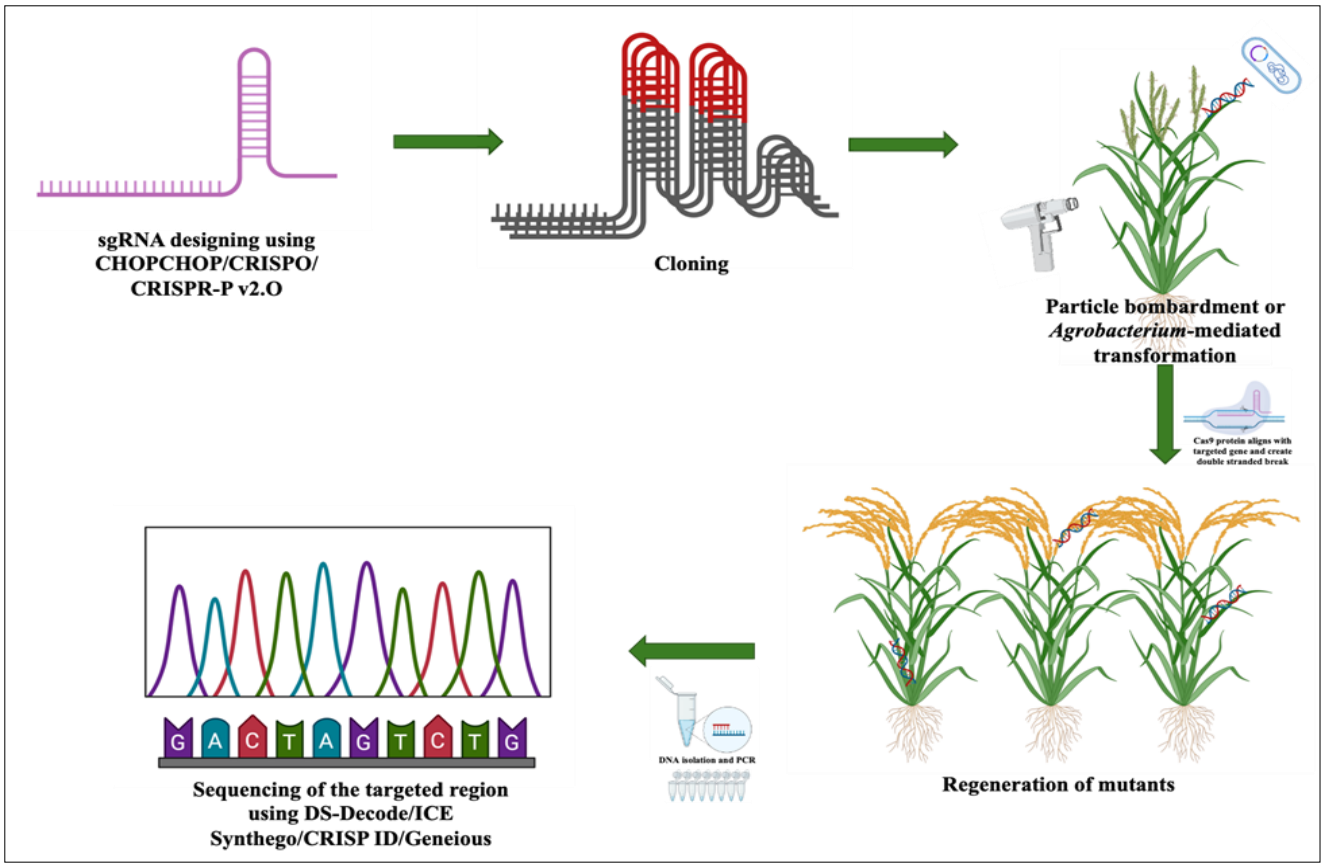

Fig. 2. CRISPR/Cas9 based gene editing in rice.

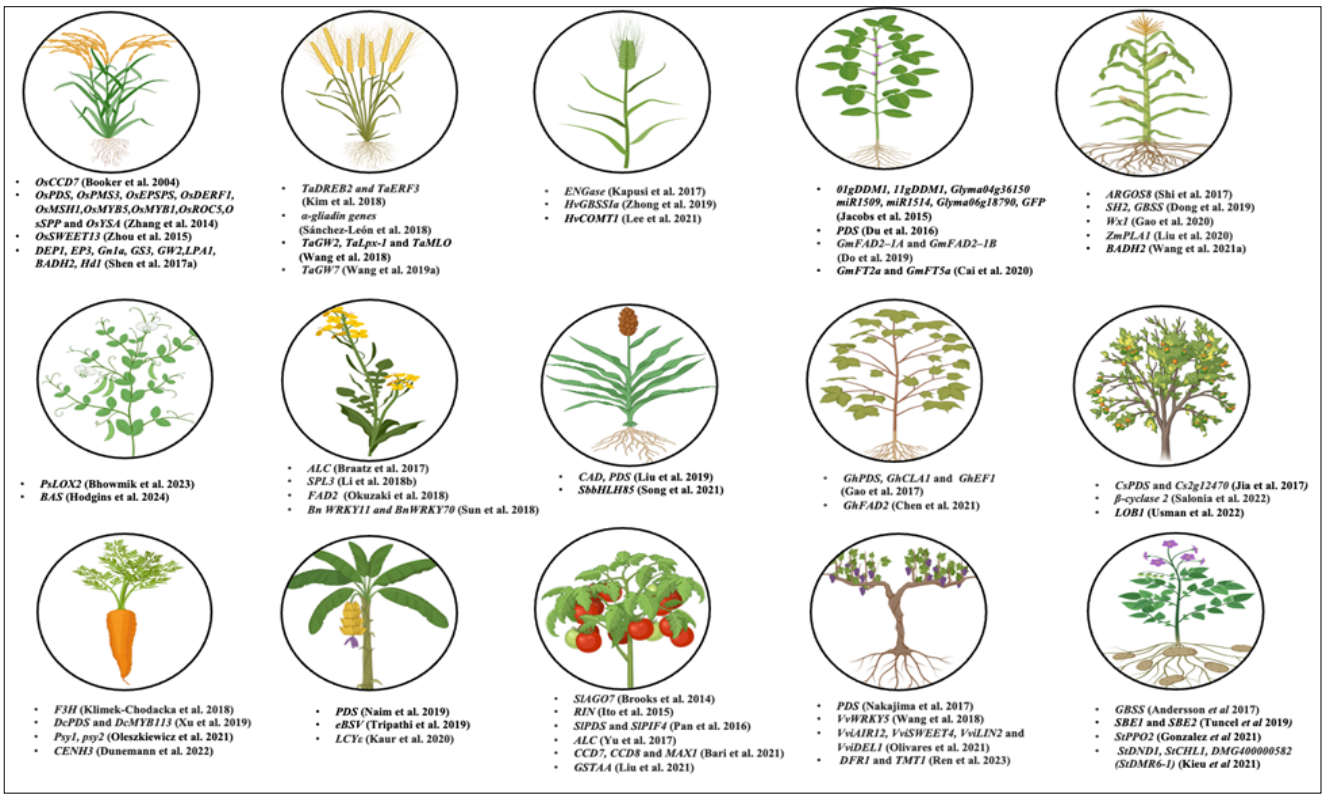

Fig. 3. CRISPR: A versatile tool for editing genes across crop species.

CRISPR/Cas9 system has enormous potential application in the model monocot, diploid crop. CRISPR is a potential tool for the mutant library construction in rice and accelerates the breeding by characterizing the functionality of unknown genes. It has its application in inducing apomixis for fixing heterosis, haploid induction, stacking beneficial alleles, etc. The marker gene elimination in transgenic plants by CRISPR is another remarkable landmark. Various candidate genes edited using the CRISPR/Cas9 system were tabulated (Table 2). Apart from the above-mentioned genes, various genes have been targeted by the researchers for yield enhancement in rice. A list of cloned yield-related genes and CRISPR-edited yield genes is illustrated in Fig. 4A. A brief description of the agronomically superior genes targeted for yield improvement in rice is discussed (Fig. 4B).

Table 2. Targeted genes of CRISPR/Cas9 for the desired traits in rice

S.No	Application	Target genes	Strategy	Transformation method/explant	Reference
1	Yield improvement	*OsMAPK5-OsWRKY72*	NHEJ	*Agrobacterium*	(34)
2	Yield improvement	*OsPUB33*	NHEJ	*Agrobacterium*	(35)
3	Yield improvement	*uORFs in DEP1 and GIF1*	NHEJ	*Agrobacterium*	(36)
4	Yield improvement	*OsSPL16*	NHEJ	*Agrobacterium*	(37)
5	Yield improvement	*OsSPL4*	NHEJ	*Agrobacterium*	(38)
6	Yield improvement	*GW2, GW5, TGW6*	NHEJ	*Agrobacterium*	(39)
7	Yield improvement	*GS3, Gn1a, GW2*	NHEJ	*Agrobacterium*	(40)
8	Altering photosynthetic traits	*OsEPF1*	NHEJ	*Agrobacterium*	(41)
9	Aroma	*OsBadh2*	NHEJ	*Agrobacterium*	(42)
10	Regulates stomatal density	*OsEPFL9*	NHEJ	*Agrobacterium*	(43)
11	Flowering time	*Hd4, Hd2, Hd5*	NHEJ	*Agrobacterium*	(44)
12	Rice bacterial blight resistance	*OsWRKY26*	NHEJ	*Agrobacterium*	(45)
13	Rice bacterial blight and blast resistance	*OsMPK15*	NHEJ	*Agrobacterium*	(46)
14	Rice blast resistance	*SEC3A*	NHEJ	*Agrobacterium*	(47)
15	Rice Tungro virus	*eIF4G*	NHEJ	*Agrobacterium*	(48)
16	Rice brown plant hopper resistance	*OsJAZ10*	NHEJ	*Agrobacterium*	(49)
17	Herbicide tolerance	*ALS*	Base editing	*Agrobacterium*	(50)
18	Herbicide tolerance	*EPSPS*	Exon replacement by NHEJ	Biobalistic	(51)
19	Salinity tolerance	*OsERS1*	NHEJ	*Agrobacterium*	(52)
20	Fragrant thermosensitive genic male sterile	*TMS5, FGR*	NHEJ	*Agrobacterium*	(53)
21	Cold stress response	*ANN3*	NHEJ	*Agrobacterium*	(54)
22	Fatty acid metabolism	*FAD2-1*	NHEJ	*Agrobacterium*	(55)
23	Nitrogen efficiency use	*NRT1.1B*	Allele replacement by HR	Biobalistic	(56)
24	Embryo formation from fertilized egg	*BBM1*	NHEJ	*Agrobacterium*	(57)

NHEJ- Non-homologous end-joining; HR- Homologous recombination.

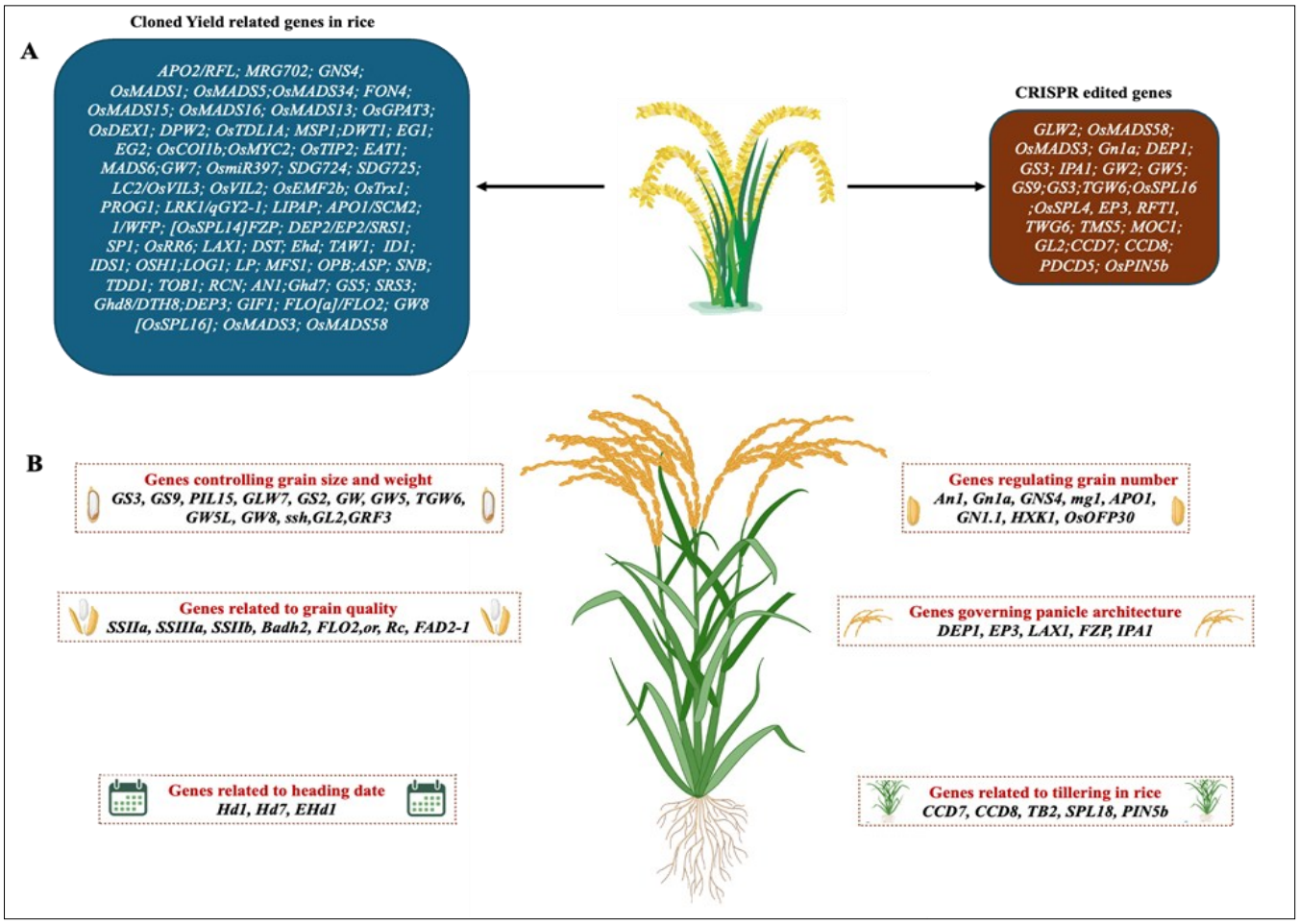

Fig. 4. List of cloned yield-related genes and CRISPR edited genes in rice (A); Genes targeted for yield enhancement in rice (B).

Genes regulating the number of grains / spikelets in rice

The number of grains per panicle, a key determinant of rice yield potential, is primarily influenced by panicle architecture and branch differentiation, which are intricately regulated by phytohormone pathways and vascular differentiation (58). The transgenic investigation has revealed that *An-1* (awn gene) plays a positive role in regulating lemma extension (awn elongation) while exerting a negative effect on the number of spikelets per panicle in rice. Efforts to enhance the spikelets per panicle by suppressing the *An-1* gene using RNA silencing have resulted in an increased grain number of approximately 13.6 - 38.4 %. Consequently, suppressing this gene results in the upregulation of the *LOG* gene, elevating cytokinin levels and enhancing meristematic activity. These series of events ultimately lead to an enhanced grain number per panicle, thereby boosting grain yield (16). A gene *Gn1a* encodes for *OsCKX*2 (cytokinin oxidase/dehydrogenase enzyme) whose reduced expression leads to cytokinin accretion in the inflorescence meristems with a surge in the reproductive structures, contributing to a higher yield. Approximately, 21 % enhancement in grain number has been observed (59). An uplift in grain yield was achieved by a rise in the tiller number and enhanced grain weight by the downregulation of *OsCKX*2 *via* the RNAi approach (60). The gene *OsGIF1* has pleiotropic effects on organ size regulation in rice. Through overexpression and functional knockout using the CRISPR/Cas9 strategy, it has been shown to not only positively regulate the size of rice stems, leaves and grains but also affect reproductive processes. Hence, *OsGIF1* played important roles in vegetative as well as reproductive developmental processes (61). A *GNS*$_4$(*GRAIN NUMBER AND SIZE*) gene, an allele of *DWARF11* positively modulates panicle size, grain size, 1000-grain weight and hence enhanced yield. RNAi lines exhibited shortened plant height, reduced number of panicles, shortened panicle length and low 1000-grain weight, whereas overexpression of this gene resulted in higher plants, longer panicles and larger and increased grains. Mutating *GNS*$_4$ yielded a *gns4* mutant with diminished organ size, smaller grains and fewer grains per panicle (62).

The moderate expression of the *APO1* (*ABERRANT PANICLE ORGANIZATION*) gene results in larger panicles and increased grain number by inhibiting the transition of inflorescence meristem to spikelet meristem. Molecular studies have also confirmed that *APO*1 encodes a protein belonging to the F-box family, that positively modulates floral homeotic genes of class-C (*OsMADS3*) (63). Targeted editing of *OsHXK1,* a hexokinase gene resulted in mutants with high light saturation points, light tolerance, photosynthetic products, stomatal conductance and high yield (*indica* rice varieties-Meixiangzhan, Huanghuazhan and Wushansimiao) (64). Recently, a QTL, *GRAIN NUMBER 1.1* (*GN1.1*), was identified as a negative modulator of spikelet number, which codes for Flowering Locus T-like1 (FT-L1) protein (65).

In summary, grain number per panicle in rice is regulated by a complex interplay of genetic and hormonal factors, including cytokinin metabolism, inflorescence meristem activity and floral homeotic gene modulation. Several genes, such as *An-1, Gn1a, OsGIF1, GNS4, APO1*, *OsHXK1* and *GN1.1*, have been identified as key regulators of spikelet number and yield potential. Targeted mutagenesis and genome editing approaches, particularly CRISPR/Cas9, offer promising strategies to fine-tune these genes for optimizing grain production. Future research should focus on integrating these genetic modifications with advanced breeding techniques to develop high-yielding, climate-resilient rice varieties.

Genes controlling grain size and weight in rice

A grain-size negative regulator gene, *GS3,* encodes for the PEBP domain. The edited lines of *GS3* have enhanced grain length (31.39 %) and 1000-seed weight (27.15 %) owing to the regulation of cysteine proteinase inhibitor and Ubiquitin -related proteins, which promoted the development of long grain type high-yielding genotypes (66). Targeted editing of *OsPIL15* lines resulted in increased grain size and grain weight by targeting a purine permease gene *OsPUP7* directly, which in turn led to enhanced yield (67). A QTL *GLW7,* which encodes for a plant-specific TF *OsSPL13* positively modulates the cell size in grain hull, promoting grain length and yield. g*w2* mutant (gene-edited) lines show increased grain productivity and lodging resistance behavior, attributed to its pleiotropic nature (68). *GW5L* knockout mutants conferred short and wider grains since *GW5L* is a negative modulator of grain size and salinity tolerance in rice (69). CRISPR/Cas9 disruption of miR396 target site derepress *OsGRF4* and *OsGRF8,* resulting in enlarged grain size and increased brown plant hopper (BPH) resistance (70). Henceforth, CRISPR technology can be employed to target these genes, regulating grain size and weight for precise genetic modifications (66-69).

Genes governing panicle architecture in rice

A pleiotropic gene *DEP1* (*DENSE AND ERECT PANICLE*) encoding for phosphatidylethanolamine-binding protein is down regulator of grain number and panicle architecture and hence the mutant *DEP1* allele (*dep1)* augments the meristematic activity with decreased inflorescence internodal length, finally resulting in a higher spikelet count per panicle and thus yield. Hence the pleiotropic gene codes for more than one phenotypic trait *viz*., erect panicle, dense panicle and elevated grain number per panicle. The gene-edited *dep1* homozygous mutants (T_0) exhibited a diminished plant height with dense, erect panicles (71). A mutation in the *EP3* (*ERECT PANICLE*) gene resulted in the erect and (or) upright panicle in rice with increased parenchyma thickness in the peduncle and increased vascular bundles, hence affecting panicle arrangement in rice which profoundly influences yield (72). The genes *LAX1* and *FRIZZY PANICLE 2* determine the panicle architecture in rice. The *LAX1* gene is essential for the formation of rachis-branch meristem (RBM) from primary inflorescence meristem (PIM) and for the development of lateral meristem from rachis-branch meristem (RBM). The rachis-branch meristem is also converted into terminal spikelet meristem by *FZP2*. Hence, the *LAX1* gene suppresses the indeterminate growth of RBM and the *FZP2* gene is responsible for spikelet identity (73). Plants which has a mutation in the *IPA1* gene

(miR156's cleavage site), have shown the ideal plant architecture (IPA) *viz*., thick and sturdy stems, reduced tiller number, reduced unproductive tillers, more number of spikelets per panicle, substantially augmenting the grain yield (74). Therefore, these genes can be targeted to modify panicle architecture in rice.

Genes related to tillering and grain quality in rice

Rice tillering, a multigenic trait influences grain yield. The targeted disruption of two key genes, *CCD7* and *CCD8* increases the tillering by altering the biosynthesis of strigolactone (75). The *GA20ox-2* gene encoding for Gibberellin 2-oxidases impacted plant development by eliminating internal gibberellins. Targeted mutagenesis of this gene yielded a semi-dwarf rice, due to some defects in gibberellin's signaling pathway. It is a pleiotropic gene controlling plant height, tillering and root architecture in rice (76). Targeted mutagenesis of *OsSPL18* leads to enhanced tiller number with reduced grain width, thickness, grain number and panicle length (77). Reduced expression of *OsPIN5b,* an auxin efflux carrier-like gene, resulted in lengthier panicles, vigorous root system, higher tiller number and hence enhanced yield (78). Similarly, knockout mutants of *OsTB1* showed enhanced tillers (79). Consequently, these genes can be harnessed for boosting tiller numbers in rice through genome editing techniques.

Grain quality, another critical trait in rice improvement, is largely influenced by starch composition and protein content. Resistant starch is instrumental in the prevention of diabetes. A soluble starch synthase gene (*SSIIIa*), the key to producing Resistant Starch depends upon the *Waxy*^{o}(*Wx*^{o}) allele expression. RNAi studies on *SSIIa and SSIIIa* revealed that they had strong effects on grain formation and starch synthesis by displaying a chalky appearance on the kernel with enhanced amylose levels and decreased viscosities. Knockout mutants of the *waxy* (*Wx*) gene led to the creation of sticky/glutinous rice varieties with little or no amylose content (80). Amylose-rich rice varieties have been created by targeted mutagenesis of *SBEI* and *SBEIIb* (starch branching enzyme) by (81). A team of researchers spotted no noticeable variation amidst the *sbeI* mutant and the parental type, however, the *sbeII* mutants exhibited a marked increase in amylose level and resistant starch content of about 25 % and 9.8 %, respectively hence altering the nutritional properties of starch. *SLG7* promoter-edited mutants showed higher amylose content, longer grains, reduced chalkiness and decreased gel consistency with no significant yield difference in comparison with the parental type (82).

The gene *FLO2* (*FLOURY ENDOSPERM 2*) plays a key role in enlarging the grain size by its overexpression and aids in heat stress tolerance during the formation of seed. A mutation in the *flo2* gene suppressed the expression of genes contributing to storage protein and starch formation in the endosperm, highlighting *FLO2*'s significance in modulating grain size and starch composition (83). The Grain Protein Content (GPC) in rice has been controlled by a QTL, *qPC1* that governs the formation and deposition of globulins, prolamins, glutelins, starch and albumins. It was reported that *qPC1* encodes an amino acid transporter *OsAAP6,* which shows a positive correlation with GPC. Higher GPC negatively impacts the eating and cooking quality (ECQ) of rice. Hence knockout mutants of *OsAAP6* and *OsAAP10* yielded a decreased GPC with reduced glutelin content and in turn improved ECQ (84). As β carotene accumulation is another important trait to combat Vitamin A deficiency, *Osor* gene knockout using CRISPR/Cas9 showed enhanced β carotene concentration in rice callus (85). A frame-shift deletion in the *Rc* gene was reversed, turning the rice pericarp color from white to red by producing an in-frame mutation. The mutant, red-colored grains displayed increased concentrations of proanthocyanidins and anthocyanins, without significant variation in agronomic traits when compared to the parental type (86). Thus, precise genome editing of these genes holds immense potential for improving both tillering and grain quality in rice without compromising yield, ultimately contributing to the development of nutritionally enriched and high-yielding rice varieties.

Genes associated with heading date

In rice, heading or inflorescence time is directly associated with grain yield. The flowering time genes exert pleiotropic functions affecting yield characteristics. There is an increase in yield with delayed days to heading, which then becomes a plateau at maximum and then decreases with an increase in biomass. The different combinations of *Heading date 1* (*Hd 1*) and *Early Heading date 1* (*EHd 1*) displayed a great diversity in the number of floral branches in panicles (87). Another study reveals that there is a decline in panicle number in the *se13* mutant, a lower grain-filling percentage in *hd1* plants and a reduced grain count per panicle in the *ghd7* mutant (88). Promoter-edited mutants of *RFT1* (Rice Flowering Locus T) displayed delayed heading by 1.4 to 9.2 days, along with decreased *RFT1* and *Heading date 3a* (*Hd3a*) genes' expression. The promoter regions of *Ghd7, DTH8* and *Hd1* genes were precisely targeted using the High-Efficiency Multiplex Promoter-Targeting (HMP) system, generating new cis-regulatory alleles (89). As a result, the precise edits of genes facilitating delayed days to heading, on disruption, can be used for enhancing yield in rice.

Genes governing biotic and abiotic stress tolerance for yield enhancement

Rice yield is influenced by diverse stress conditions. Biotic stresses like pathogens, pests, insects and weeds and abiotic stresses like salinity, drought, high temperature and cold pose a detrimental impact on rice crop growth and development. Both stresses result in yield penalty, leading to about a 70 % reduction in yield from abiotic stresses alone, which adversely affects the survival rate of the crop (90). Hence breeding rice varieties with stress tolerance enhances the yield potential of the crop.

A unique CRISPR/Cas9 mutant *dst* $^{\Delta 184-305}$(drought and salt tolerance) results in broader leaves with decreased stomatal density due to the suppression of various genes involved in stomatal development *viz*., *MUTE, ICE1 and SPCH1.* The mutant also expressed moderate to high levels of ability to withstand osmotic

stress and salinity stress in seedlings, respectively (91). By targeted disruption of the *OsRR22* gene, salinity-tolerant rice lines have been developed (92). Hence amidst the prevalence of abiotic stresses, by targeting the desired gene for the desired trait or by the introgression of donor alleles to the released varieties and land races, declining yield can be sustained.

A gene *OsSWEET14* (*Os11N3*), encoding a sugar transporter gene is a key susceptibility factor for bacterial blight in rice triggered by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The disruption of coding regions of *OsSWEET14* led to protein production with a loss of sugar transportation ability and the mutant alleles resulted in increased plant height without reduction in yield (93). There is an improvement of rice blast (*Magneporthe grisea*) resistance by targeted disruption of the ERF Transcription Factor gene *OsERF922* by CRISPR/Cas9 (94). Similarly, the targeted CRISPR/Cas9- genome editing resulted in the development of tungro disease (*eif4g*) resistant viral-free rice plants (95). Rice lines were developed for combined defense against plant hoppers and stem borers by disruption of *OsCYP71A1*, which blocked serotonin biosynthesis (96). In view of this, the strategic disruption of susceptible and executor genes associated with diseases or pests promotes the development of broad-spectrum resistance in rice. Importantly, these genetic modifications not only enhance stress tolerance but also play a critical role in ensuring yield stability under stress-prone conditions, thereby sustaining rice production.

Genes targeted for hybrid breeding in rice

Hybrid breeding, a vital tactic to augment rice yield, involving male-sterile lines, is a key to successful hybridization. High-yielding glutinous CMS (Cytoplasmic Male Sterile) rice lines were developed by targeted disruption of *Wx* and *TGW6* genes, which confers 1000 grain weight (GW) and amylose concentration (97). The pollen sterility in CMS lines was due to the absence of mitochondrial NADP+ -dependent malic enzyme (insufficient energy). On the other hand, the upregulation of acetyl-CoA synthetase and Isoamylase in both maintainer and CMS lines has a strong link with CMS and amylose content. The mutants exhibited increased grain width (GW) and gel consistency, reduced amylose content and gelatinization temperature, an opaque grain appearance and unchanged starch content. To evade the potential risks of maintaining three lines in three-line breeding, there arises a two-line breeding system of hybrid seed production in rice, “turning three-line into two-line”. Tremendous achievements in hybrid breeding have been made by CRISPR/Cas9, which yielded male sterile lines, including thermosensitive male-sterile *tms5* lines in rice and photosensitive genic male-sterile *csa* rice. Commercial TGMS (Thermo-Sensitive Genic Male Sterility) rice production by inducing specific mutations in *TMS5* (sterility-fertility transition) has been cited as well (98). A team of researchers edited the second and fourth exons of *OsOPR7* through the CRISPR/Cas9 genome editing technique, which resulted in the production of male-sterile plants that were restored to fertility by exogenous application of methyl jasmonate. Hence paved the way for the creation of two-line hybrid rice (99). rPGMS (reverse photoperiod-sensitive genic male sterility) lines were generated by focused editing on the *CSA* (*carbon starved anther*) gene in *japonica* rice (100). Considering this, the development of high-yielding, superior hybrid seeds is achievable through the innovative application of gene editing techniques.

Global progress in gene editing and regulatory landscape

The rapid advancements in plant pathway engineering and synthetic biology provide optimism for the future of gene-edited crops. A new breeding technique that revolutionizes and precisely inserts the desired traits is the breakthrough of CRISPR-based gene editing technology. The advancements of gene editing *viz*., base editing, prime editing and multiplex -based editing offer potential applications in enhancing yield components in rice. However, most of the plants generated through gene editing did not progress to the field owing to the biosafety concerns and regulatory issues adopted in each country. India is conducting field trials of gene-edited rice plants following the relaxation of regulations for SDN-1 (site-directed nuclease 1) and SDN-2 (site-directed nuclease 2) gene-edited crops. The drought and saline tolerant (*dst)* rice lines developed at the Indian Agricultural Research Institute, New Delhi seem to boost grain yield under challenging environments and are expected to be commercially available by 2026. On the other hand, China made strides with fungal-resistant wheat, the Philippines had seen the introduction of non-browning bananas, Canada approved non-browning apples and the United States has given non-browning potato varieties the go-ahead. These developments highlight the growing global significance of leveraging gene editing technologies to enhance food security achieve zero hunger goals and promote sustainable agriculture.

Conclusion

The development of climate-smart rice lines is imperative in the coming decades, as extreme climatic conditions pose serious threats to global food production. To mitigate these challenges, breeders must harness the existing genetic diversity and fully exploit its potential by channeling beneficial alleles into genetic populations for targeted breeding. Recently, genome editing has emerged as a transformative approach for precisely modifying key traits that contribute to higher productivity. Advances in targeted mutagenesis have enabled the optimization of spikelet number, panicle architecture, grain size, weight and tillering ability, all of which directly influence yield potential. Through precise modifications, genome editing has facilitated improvements in grain formation, panicle branching and biomass distribution, ultimately enhancing overall grain production. In addition to refining plant architecture, genome editing has played a pivotal role in optimizing tiller number, a key determinant of yield and improving hybrid breeding efficiency by modifying fertility-related traits. These innovations offer a promising route for the development of high-yielding hybrid rice varieties with improved seed

production efficiency. Despite these advancements, large-scale implementation of genome-edited rice faces challenges, including regulatory hurdles and the need for extensive field validation. However, with recent policy changes easing restrictions on certain categories of gene-edited crops, field trials have begun, paving the way for potential commercialization. The successful deployment of these technologies will depend on efficient regulatory approvals, large-scale field evaluations and widespread adoption by farmers. Moving forward, integrating genome editing with functional genomics and precision breeding will further accelerate the development of climate-resilient, high-yielding rice varieties. By leveraging these innovations, rice improvement efforts can achieve greater precision and efficiency, ensuring stable yields and contributing to global food security in a sustainable manner.

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Authors' contributions

SPR: Data curation, Writing an original draft, Visualization, Formal analysis, RM, GS, KT, MS: Conceptualization, Visualization, Formal analysis, Supervision, SAR, SM, GC, BN, DJ: editing, resources. All the authors have read and approved the final manuscript.

Compliance with ethical standards

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