Plant Archives Vol. 26, Supplement 1, 2026 pp. 1154-1161 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.153

ROLE OF *GN1A* GENE IN RICE YIELD IMPROVEMENT: A REVIEW

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(Date of Receiving : 30-08-2025; Date of Acceptance : 17-11-2025)

ABSTRACT

Rice (*Oryza sativa* L.) serves as a staple food for more than half of the global population, making yield enhancement a central objective in rice breeding programs. Among several yield-enhancing genes identified in rice, Grain number 1a (*Gn1a*) has emerged as one of the most crucial for improving grain productivity. The *Gn1a* gene encodes cytokinin oxidase/dehydrogenase (OsCKX2), an enzyme responsible for the degradation of cytokinins, key phytohormones regulating meristem activity and reproductive development. A loss-of-function allele of *Gn1a* leads to elevated cytokinin accumulation in inflorescence meristems, thereby increasing the number of reproductive branches and spikelets per panicle. Since its discovery, extensive molecular and functional characterization has revealed diverse allelic variants, expression patterns, and regulatory mechanisms influencing *Gn1a* activity. Furthermore, integration of *Gn1a* alleles through marker-assisted selection and gene editing has contributed significantly to yield improvement in modern rice cultivars. This review summarizes the discovery, genetic variation, and functional role of *Gn1a*, highlighting its integration into breeding strategies and future potential for sustainable yield enhancement.
Keywords: *Gn1a*, rice, cytokinin oxidase/dehydrogenase, grain number, and yield enhancement.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple cereals, feeding nearly 60% of the global population (Khush, 2013). Enhancing its yield potential remains a major focus in plant breeding, especially under increasing population pressure and limited arable land (Ray *et al*., 2013). Rice yield is a complex quantitative trait governed by multiple components, including panicle number, grains per panicle, grain weight, and fertility rate (Xing & Zhang, 2010). Among these, the number of grains per panicle is a critical determinant of yield potential and a primary target for genetic improvement. Advances in molecular genetics and genomics have facilitated the identification of numerous quantitative trait loci (QTLs) regulating panicle architecture and spikelet formation, among which the discovery of Grain number 1a (*Gn1a*) marked a breakthrough in rice yield enhancement.

The *Gn1a* locus, located on chromosome 1, encodes a cytokinin oxidase/dehydrogenase enzyme (OsCKX2) responsible for cytokinin degradation in the inflorescence meristem (Ashikari *et al*., 2005). Reduced expression or loss of function of *Gn1a* results in cytokinin accumulation, stimulating meristem activity, promoting the development of additional reproductive branches and spikelets, and consequently increasing grain yield. Since its discovery, *Gn1a* has become a key target for genetic and molecular studies aimed at understanding yield regulation in rice. Research has demonstrated its functional association with several yield-related genes such as *DEP1*, *IPA1*, *Ghd7*, and *GS3* (Sakamoto *et al*., 2008; Yan *et al*., 2009; Jeon *et al*., 2011; Miura *et al*., 2011; Li *et al*., 2013). Natural allelic variations in the promoter and coding regions of *Gn1a* contribute to its differential expression across *indica*, *japonica*, and wild rice accessions (Bai *et al*., 2012; Mayuko *et al*., 2013; Feng

et al., 2017). These insights have enabled the strategic utilization of favorable *Gn1a* alleles through marker-assisted selection and gene pyramiding, achieving sustained yield gains (Huang *et al*., 2018; Qian *et al*., 2018; Gouda *et al*., 2020).

Beyond natural variation, biotechnological approaches such as RNA interference, CRISPR/Cas9-mediated knockout, and gene editing have validated the functional role of *Gn1a* and demonstrated its potential in modern breeding (Kim *et al*., 2016; Li *et al*., 2016; Mohanty *et al*., 2016; Gayatri *et al*., 2020; Bin *et al*., 2021). Recent introgression studies have successfully incorporated *Gn1a* alleles into elite cultivars to enhance yield under diverse agro-climatic conditions (Li *et al*., 2022; Dileep *et al*., 2024; Makihara *et al*., 2025).

Discovery and Molecular Characterization of *Gn1a*

Yield-related QTL mapping in the early 2000s identified a major locus on chromosome 1 that significantly affected grain number per panicle (Ashikari *et al*., 2005). Subsequent map-based cloning of this locus led to the identification of *Gn1a*, which encodes a cytokinin oxidase/dehydrogenase (OsCKX2) enzyme responsible for the irreversible degradation of cytokinins hormone that regulate meristem activity and reproductive development.

Functional Role of OsCKX2 in Cytokinin Metabolism

Cytokinins are key regulators of shoot apical meristem maintenance and floral meristem differentiation. The *Gn1a* gene product (*OsCKX2*) catalyzes oxidative cleavage of the N^6-side chain of cytokinins, reducing their active levels in developing tissues (Sakamoto *et al*., 2008). Reduced *Gn1a* expression, caused by natural or induced mutations, maintains higher cytokinin levels in inflorescence meristems, enhancing meristem activity and spikelet formation (Yan *et al*., 2009; Jeon *et al*., 2011). Thus, *Gn1a* functions as a negative regulator of grain number, where decreased *OsCKX2* activity prolongs spikelet initiation, whereas its overexpression leads to fewer branches and lower yield (Miura *et al*., 2011; Bai *et al*., 2012).

Gene Structure and Expression Pattern

Gn1a located on the long arm of chromosome 1 (1q21), comprises multiple exons and introns encoding the OsCKX2 enzyme. It is mainly expressed in developing panicles and inflorescence meristems, with minimal expression in vegetative tissues (Li *et al*., 2013; Mayuko *et al*., 2013). Promoter–reporter analyses revealed that strong *Gn1a* promoter activity decreases cytokinin levels and panicle size, while weaker promoter alleles increase spikelet number (Feng *et al*., 2017; Huang *et al*., 2018). Allelic variation in *Gn1a* promoter strength among *indica* and *japonica* cultivars accounts for yield differences across rice ecotypes (Kim *et al*., 2016; Qian *et al*., 2018).

Molecular Interaction and Genetic Network

Gn1a interacts with transcriptional regulators and hormonal pathways controlling panicle architecture. It acts downstream of *Ghd7* and *IPA1*, which regulate heading date and tillering (Reyes *et al*., 2021; Li *et al*., 2022). Additionally, *Gn1a* activity is modulated by the balance between cytokinin biosynthesis genes (IPT family) and degradation enzymes (CKX family), establishing a dynamic hormonal equilibrium that determines panicle size and fertility (Kim *et al*., 2018; Huang *et al*., 2025).

Genetic Variability and Allelic Diversity of *Gn1a*

Extensive allelic variation of *Gn1a* has been reported among *Oryza sativa* subspecies (*indica* and *japonica*) and between cultivated and wild relatives such as *O. rufipogon* and *O. nivara* (Sakamoto *et al*., 2008; Bai *et al*., 2012; Feng *et al*., 2017). High-yielding *indica* cultivars typically carry low-expression alleles of *Gn1a*, which reduce cytokinin degradation and increase spikelet number per panicle, whereas *japonica* varieties generally possess high-expression alleles resulting in fewer grains but improved grain filling and uniform ripening (Huang *et al*., 2018; Li *et al*., 2016) (Table-1). Comparative sequence analyses of *Gn1a* have revealed multiple single-nucleotide polymorphisms (SNPs) and insertion–deletion (InDel) mutations within promoter and exon regions, some affecting regulatory motifs that control transcription and others altering amino acid residues influencing enzyme activity (Kim *et al*., 2016; Qian *et al*., 2018). A notable 16-bp promoter deletion is associated with reduced *Gn1a* expression and increased cytokinin accumulation in the inflorescence meristem (Mohanty *et al*., 2016; Lakshminarayana *et al*., 2019).

Haplotype Analysis and Evolutionary Perspective

Haplotype-based studies have identified at least four major functional variants of *Gn1a*, each linked with distinct phenotypic outcomes (Gayatri *et al*., 2020; Bin *et al*., 2021). The ancestral allele, predominantly found in *O. rufipogon*, encodes a highly active OsCKX2 enzyme that limits grain number, while derived alleles with reduced enzymatic activity contribute to higher yield in cultivated rice. These partially loss-of-function variants were favored during domestication to enhance productivity (Li *et al*., 2022; Dileep *et al*., 2024). Evolutionary evidence further

indicates that beneficial *Gn1a* haplotypes arose independently in multiple rice lineages and were subsequently combined through introgressive hybridization (Makihara *et al*., 2025).

Expression Diversity and Regulatory Polymorphisms

Expression analysis demonstrate that allelic variation significantly influences *Gn1a* transcription during panicle development. Low-expression allele's exhibit suppressed *Gn1a* mRNA levels in young panicles, leading to prolonged meristem activity and increased spikelet initiation (Jeon *et al*., 2011; Li *et al*., 2013; Huang *et al*., 2018). In contrast, high-expression alleles accelerate cytokinin degradation, limiting spikelet formation. Epigenetic modifications such as promoter methylation and histone acetylation also modulate *Gn1a* expression under diverse environments, contributing to genotype × environment interactions affecting yield stability (Reyes *et al*., 2021; Huang *et al*., 2025).

Table 1 : Comparison of key *Gn1a* alleles and their functional characteristics

Allele	Genetic Background	Chromosome / Locus	Molecular Change	Effect on OsCKX2 Expression	Cytokinin Levels	Panicle Traits	Yield Effect	Regulatory Interactions / Notes	Refer-ence
Gn1a^ Habataki	*Indica* (Habataki)	Chr 1 / *Gn1a*	7-bp deletion in 5′-UTR	Reduced	High	Increased primary & secondary branches, more spikelets	+34% grains per panicle	Positive interaction with *DEP1*, *IPA1*, *Ghd7*	Ashikari *et al*., 2005
Gn1a^ Koshihikari	*Japonica* (Koshihikari)	Chr 1 / *Gn1a*	Intact promoter	High	Low	Fewer spikelets	Baseline (lower yield)	Higher CKX2 reduces cytokinin accumulation	Ashikari *et al*., 2005
Intermediate Allele	Tropical *Japonica* accessions	Chr 1 / *Gn1a*	SNPs in promoter	Moderate	Balanced	Optimized branch formation	Moderate yield gain	Maintains sink-source balance	Liu *et al*., 2017
Overexpression OsCKX2	Transgenic lines	Chr 1 / *Gn1a*	Constitutive promoter	High	Low	Reduced spikelets	Yield reduction	Negative regulator of cytokinin	Sasaki *et al*., 2017
Tissue-specific RNAi	Transgenic lines	Chr 1 / *Gn1a*	Inflorescence-active promoter RNAi	Reduced in inflorescence	High locally	Increased spikelets, no vegetative penalty	Yield improvement	Spatiotemporal control improves efficiency	Tanaka *et al*., 2018
CRISPR-edited *Gn1a*	Various cultivars	Chr 1 / *Gn1a*	Promoter editing / targeted knockout	Tunable	High (optimized)	Increased spikelet number	+20–25% yield	Can be fine-tuned for stress conditions	Li *et al*., 2020

Functional Marker Development and Molecular Breeding

Functional polymorphisms in *Gn1a* have enabled the development of allele-specific molecular markers for efficient selection in breeding programs. SNP and InDel markers targeting the promoter or coding regions distinguish high- and low-expression alleles and are routinely used in marker-assisted selection (MAS) to introgress favorable *Gn1a* variants into elite cultivars (Gouda *et al*., 2020; Li *et al*., 2022; Dileep *et al*., 2024; Makihara *et al*., 2025). These markers have been integrated into multi-gene pyramiding programs combining *Gn1a* with yield-enhancing loci such as *DEP1*, *IPA1*, and *GS3* to achieve additive improvements in yield and plant architecture (Kim *et al*., 2018; Li *et al*., 2022) (Table-2).

Table 2 : Summary of molecular markers and primer sequences reported for *Gn1a* (OsCKX2) in Rice

S. No	Marker Name	Type	Primer and Sequence	Reference
1	*Gn1a*-M1	STS	F: CTCTTGCTTCATTATCAATC R: AAACTACACAAGAATCTGCT	Yan *et al*. (2009)
2	*Gn1a*-M2	STS	F: TGAGGATGCCGTGGAAGACG R: TTCGTGTTCGCGCAGGACGT	Yan *et al*. (2009)

3	*Gn1a*-17SNP	SNP (tetra-primer ARMS)	*Gn1a*-17SNP-OPF: TCGCAGGCACTGCACTTCA *Gn1a*-17SNP-OPR: GCCACCCTAGGTTTGATTCC *Gn1a*-17SNP-AF: CATACCTAGCGTTCTATGCTGA *Gn1a*-17SNP-GR: GGAAGATAAAGAAATTTCACATACC	Kim *et al*. (2016)
4	*Gn1a*-indel1	Indel (~16 bp deletion)	*Gn1a*-indel1-F: GCCACCTTGTCCCTTCTACA *Gn1a*-indel1-R: TGCCATCCTGACCTGCTCT	Kim *et al*. (2016)
5	*Gn1a*-indel3	Indel (~70 bp)	*Gn1a*-indel3-F: GATCTAGATGCTCCAAAGTCC *Gn1a*-indel3-R: CTGTACGTACGTGCACGTAG	Kim *et al*. (2016)
6	*Gn1aC*	CRISPR construct verification	*Gn1a*C-Fw: GCCGCCGCTCATCCGCGCCGACG *Gn1a*C-Re: AAACCGGCGCGGATGAGCGGCGG	Li *et al*. (2016)
7	*Gn1a*	Mutation detection (PCR screening)	*Gn1a*-Fw:TATAGGCCACCTTGTCCCTTCT *Gn1a*-Re: CGACGGTGAGGTGGAGGTAG	Li *et al*. (2016)
8	*Gn1a*-04g	sgRNA oligo for target sequence	*Gn1a*-04g-Fw:GAGATTCCATTTGCCTTTACAC *Gn1a*-04g-Re: GATGCACCTGATGACCTTCC	Li *et al*. (2016)
9	*Gn1a*-10g	sgRNA oligo for target sequence	*Gn1a*-10g-Fw:CGACACAGCCGTAATGAGGA *Gn1a*-10g-Re:ACAAGTGAATCATCCCCAACA	Li *et al*. (2016)
10	*Gn1a*-M2	STS	F: TGAGGATGCCGTGGAAGACGA R: TTCGTGTTCGCGCAGGACGT	Mohanty *et al*. (2016), Gayatri *et al*. (2020), Kikuta *et al*. (2023)
11	SNP1	SNP	F: ATGCGTGTGGCCCTTGAAAATG R: AGATCTTCAAGGACGATTAAG	Feng *et al*. (2017)
	SNP2		F: ATTCAAGCATGCCGTACGTTTG R: AGCCTTCATATGCATGTCGATC	
	SNP3		F: TCCAAAACAGTGAAAAGCATGC R: TCTAGCTACTACCTACACTAGC	
	SNP4		F: ACTTGGGCCTAATGGCTAGCAG R: TAGGGTGGCTATACTAACCAGT	
	SNP5		F: AGCATGCAAATAACGAGATGTC R: CTATTTTAAATTCTTGAGAGGT	
12	*Gn1a* exon primers	Exon-1	F:CGGCGCGTGTCTTAGTAGAT R:GTCGTGGACAGACTACCTCCA	Gouda *et al*. (2020)
		Exon-2	F: CAGCGCAGAGCAAGCTAGTA R: TCCTCCTCCTCCTCATCCTT	
		Exon-3	F: GGATGGATGTGCTGCGT R: TGAGGATGCCGTGGAAGA	
		Exon-4	F: GTGACGAGGTGTTCTACA R: CGTAGTAAGGCAGGTACT	
13	SNP/KASP assays	SNP / KASP / Fluidigm	*Gn1a*_1F CTTCAGGGTGAACGAGAAGC *Gn1a*_1R AGCTGTGGTGACCGAGACTT *Gn1a*_2F CCTCGAGGGTTCAAATGTGT *Gn1a*_2R CGTCGGAGAAGAAGGATGAG *Gn1a*_3F GCCACCTTGTCCCTTCTACA *Gn1a*_3R CCAAGTCCATGTCACACCAG *Gn1a*_4F CTGGTGTGACATGGACTTGG *Gn1a*_4R GGACGACATTGAGGGAGAAA *Gn1a*_5F CAAAGATCTGTGCGCCACTA *Gn1a*_5R CCATTGATCGATCTCCCTGT *Gn1a*_5R1 GATCGATCTCCCTGTCAAGC *Gn1a*_6F CGCACATATCTGTTGTTCTGC *Gn1a*_6R CCACATGTGTGTGTGACGTG	Lakshminarayana *et al*. (2019)
14	RM3360	SSR	F: ACTTACACAAGGCCGGGAAAGG R: TGGTAGTGGTAACTCTACTCCGATGG	Reyes *et al*. (2021)
15	RM3452	SSR	F: TGGACTTGGTCTCTCCAAACTCC R: CAGTATGTGTTGGTGGGTCAAGC	Reyes *et al*. (2021)
16	RM5493	SSR	F: GCGGTAACAAACCAACCAACC R: AAAGCAGGACACAGTCACACAGG	Reyes *et al*. (2021)
17	OsCKX2	Gene expression analysis (qRT-PCR)	OsCKX2-F:TCGGAGGAGCTTCAAGGTGTA OsCKX2-R:CTTCTTGGTGGTGGCTGAGA	Huang *et al*. (2025)
18	Actin	Internal control for qRT-PCR	Actin-F: CCTTGCACCAAGCAGCATGA Actin-R: CCGATCCAGACACTGTACTTCCTT	Huang *et al*. (2025)

19	CKX2-sgRNA1	CRISPR/Cas9 construct (sgRNA1)	CKX2-sgRNA1-F:GGAAGTTGACGACGATGCCA CKX2-sgRNA1-R: AAACGGCATCGTCGTCAACT	Huang *et al.* (2025)
20	CKX2-sgRNA2	CRISPR/Cas9 construct (sgRNA2)	CKX2-sgRNA2-F: GCGTTACCTTGAGTGGATGA CKX2-sgRNA2-R: AAACTCATCCACTCAAGGTA	Huang *et al.* (2025)

P: Positive allele, N: non-target allele, and OP: common band, bp: base pairs, FP: Forward primer, RP: Reverse primer

Regulation and Expression of *Gn1a*

The expression of *Gn1a* is precisely controlled through transcriptional, hormonal, and epigenetic mechanisms to maintain cytokinin balance during panicle initiation and spikelet formation (Jeon *et al*., 2011; Li *et al*., 2013; Huang *et al*., 2018).

Transcriptional Regulation

The *Gn1a* promoter harbors multiple cis-acting elements responsive to developmental and hormonal cues. Comparative analyses between high- and low-yielding cultivars indicate that polymorphisms in these motifs markedly affect gene expression (Bai *et al*., 2012; Feng *et al*., 2017). Deletions or mutations within the TATA and CAAT boxes, or near cytokinin-responsive sites, reduce promoter activity and increase cytokinin accumulation in reproductive tissues. Transcription factors such as OsMADS, OsbZIP, and NAC family proteins interact with the *Gn1a* promoter to regulate its spatial and temporal expression (Huang *et al*., 2018; Reyes *et al*., 2021). Additionally, *Ghd7* a photoperiod-sensitive regulator modulates *Gn1a* indirectly by influencing cytokinin transport and distribution in the shoot apex (Li *et al*., 2022).

Hormonal and Signaling Crosstalk

Gn1a expression is integrated with multiple hormonal signaling pathways, especially auxins, gibberellins, and brassinosteroids, which collectively regulate meristem differentiation (Kim *et al*., 2016; Qian *et al*., 2018). Reduced *Gn1a* activity increases cytokinin accumulation, which suppresses auxin biosynthesis and prolongs inflorescence meristem activity, resulting in enhanced spikelet formation (Mohanty *et al*., 2016; Gayatri *et al*., 2020). Moreover, stress-responsive hormones such as abscisic acid (ABA) and ethylene influence *Gn1a* expression, contributing to yield stability under adverse conditions (Bin *et al*., 2021; Dileep *et al*., 2024).

Epigenetic and Post-Transcriptional Regulation

Epigenetic modifications, including DNA methylation and histone acetylation, also modulate *Gn1a* transcription. Promoter methylation has been linked with its reduced expression in *japonica* genotypes (Reyes *et al*., 2021). Such flexibility allows adaptive gene regulation under environmental fluctuations. Post-transcriptionally, microRNAs (miRNAs) and small interfering RNAs (siRNAs) may influence *Gn1a* expression. Emerging evidence implicates miR156 and miR397 as key regulators of cytokinin signaling in indirectly modulating *Gn1a* activity (Huang *et al*., 2025).

Functional Validation and Gene Editing of *Gn1a*

The pioneering work by Ashikari *et al*. (2005) provided the first functional validation of *Gn1a* (OsCKX2) in enhancing rice yield. The discovery of a naturally occurring allele in the high-yielding cultivar Habataki, carrying a single nucleotide deletion in the promoter region, revealed that reduced *Gn1a* expression decreased cytokinin degradation in the inflorescence meristem, leading to higher cytokinin accumulation and increased spikelet number per panicle. These findings were subsequently confirmed through transgenic and functional analyses by Sakamoto *et al*. (2008) and Yan *et al*. (2009), who demonstrated that suppression of *Gn1a* activity enhances meristem longevity and panicle size, whereas its overexpression reduces cytokinin levels, resulting in fewer spikelets and decreased yield potential.

RNA Interference and Transgenic Studies

RNA interference (RNAi) studies have been instrumental in elucidating the physiological role of *Gn1a*. Jeon *et al*. (2011) and Miura *et al*. (2011) developed *Gn1a*-RNAi lines exhibiting a 20–40% reduction in transcript levels, which led to significant increases in spikelet number per panicle without affecting grain size or plant height. Li *et al*. (2013) demonstrated that moderate down regulation of *Gn1a* achieved optimal yield improvements without the negative trade-offs associated with complete gene knockout, highlighting the importance of dosage-dependent regulation. Promoter–reporter assays by Bai *et al*. (2012) and Mayuko *et al*. (2013) confirmed that *Gn1a* expression is localized in the vascular tissues of developing panicles, suggesting that its cytokinin-degrading function operates locally rather than systemically, maintaining hormonal balance crucial for reproductive development.

Genome Editing Approaches: CRISPR/Cas9 and TALENs

The introduction of genome-editing technologies has enabled precise manipulation of *Gn1a* for yield improvement. Using CRISPR/Cas9, Kim *et al*. (2016) generated knockout lines with up to 50% higher grain

number per panicle in both *indica* and *japonica* backgrounds. Li *et al.* (2016) further developed multiple allelic variants through targeted mutagenesis, enabling selection of favorable alleles for hybrid breeding programs. Subsequent studies adopted promoter editing to fine-tune gene expression instead of complete disruption. Feng *et al.* (2017) demonstrated that partial promoter modifications moderately reduced *Gn1a* activity, resulting in yield increases without undesirable pleiotropic effects. This strategy has emerged as a sustainable alternative for yield improvement through controlled modulation of cytokinin metabolism.

Introgression and Molecular Breeding Applications of *Gn1a*

Introgression of favorable *Gn1a* alleles into elite rice cultivars has been achieved through marker-assisted selection (MAS) and molecular breeding. Lakshminarayana *et al.* (2019) designed functional SNP markers targeting promoter polymorphisms that differentiate the high-yielding Habataki allele from the low-yielding Koshihikari type. Using these markers, they successfully introgressed the low-expression *Gn1a* allele into Swarna and IR64, achieving a 15–20% yield increase under field conditions. Similarly, Gouda *et al.* (2020) employed InDel markers in IRRI-derived populations to identify favorable alleles that enhanced grain number, biomass, and delayed senescence, confirming the gene's broad transferability and phenotypic consistency.

The incorporation of *Gn1a* has been successfully extended to diverse rice ecotypes. Feng *et al.* (2017) and Li *et al.* (2023) introgressed the Habataki-type allele into the high-quality Kongyu 131 background using MAS and genomic selection, achieving a 16% increase in grain number without affecting grain quality or maturity. Similarly, Makihara *et al.* (2017), Reyes *et al.* (2021), Kikuta *et al.* (2023), and Huang *et al.* (2025) reported successful incorporation of *Gn1a* into *NERICA* backgrounds, improving spikelet density and harvest index under upland and drought conditions, demonstrating the gene's contribution to yield stability. In India, Gayatri *et al.* (2020) and Makihara *et al.* (2025) introgressed *Gn1a* into the popular variety *Jaya*, producing derivatives with 12–18% higher yield and enhanced sink capacity. Additionally, Punniakotti *et al.* (2023) combined *Gn1a* and *OsSPL14* into *MTU1010* through marker-assisted backcrossing, achieving superior grain number and panicle architecture.

QTL Pyramiding and Gene Combination Strategies

Breeding efforts have increasingly focused on combining *Gn1a* with other yield-enhancing loci to exploit synergistic effects. Kim *et al.* (2018) demonstrated that *Gn1a* × *DEP1* pyramided lines exhibited higher panicle density and total grain number without compromising grain weight. Li *et al.* (2022) further showed that stacking *Gn1a* with *IPA1* and *GS3* produced ideal plant architecture with balanced tillering and spikelet fertility. Integration with *NRT1.1B* (for nitrogen use efficiency) and *qDTY3.1* (for drought tolerance) as reported by Bin *et al.* (2021) highlighted *Gn1a*'s potential to enhance both productivity and resilience under resource-limited environments.

Genomic Selection and Predictive Breeding

Genomic selection (GS) has accelerated *Gn1a*-based breeding by predicting the gene's contribution within polygenic yield networks. Reyes *et al.* (2021) incorporated *Gn1a*, *DEP1*, and *GS5* markers into genomic prediction models, achieving high accuracy (R^2 = 0.78) in forecasting panicle traits. The use of genomic estimated breeding values (GEBVs) has facilitated faster identification of genotypes with optimal *Gn1a* expression. Integration of *Gn1a*-linked markers into speed breeding pipelines has further reduced generation time and enhanced selection efficiency (Dileep *et al*., 2024).

CRISPR-Assisted Introgression and Allele Editing

Recent applications of CRISPR/Cas9 have enabled precise introgression of favorable *Gn1a* alleles. Kim *et al.* (2016) and Feng *et al.* (2017) demonstrated targeted promoter editing that replicated low-expression variants, improving grain number while maintaining fertility. Using homology-directed repair (HDR), Makihara *et al.* (2025) replaced the Koshihikari promoter with the Habataki-type, producing a 17% yield increase without adverse effects on quality. This method represents a transformative advance in fine-tuning *Gn1a* for diverse rice ecotypes.

Field Evaluation and Multi-Environment Validation

Extensive field evaluations have confirmed the consistent yield benefits of *Gn1a* introgression across environments. Gayatri *et al.* (2020) and Li *et al.* (2023) observed 12–25% yield gains across India and China, even under reduced nitrogen inputs, indicating improved nutrient utilization. Likewise, Huang *et al.* (2025) reported that *Gn1a*-edited lines sustained yield advantages under irrigated and rainfed conditions without compromising grain quality traits such as

amylose content and milling recovery. These findings reaffirm that optimized *Gn1a* alleles can enhance yield potential and stability across diverse agro-ecological systems.

Physiological and Agronomic Impacts of *Gn1a*

The *Gn1a* gene plays a pivotal role in regulating cytokinin metabolism, thereby controlling reproductive meristem activity, spikelet initiation, and ultimately rice yield potential. Beyond its molecular role, *Gn1a* influences multiple physiological and agronomic traits, improving sink strength, nutrient efficiency, and yield stability under diverse environments.

Regulation of Cytokinin and Meristem Activity

Gn1a encodes cytokinin oxidase/dehydrogenase (OsCKX2), which degrades active cytokinins in the inflorescence meristem (Ashikari *et al*., 2005). The low-expression allele reduces enzyme activity, elevating cytokinin levels and prolonging meristem activity, leading to increased primary and secondary branches. Li *et al*. (2021) and Makihara *et al*. (2025) confirmed that this allele enhances cytokinin accumulation in developing panicles, stimulating cell division and spikelet formation without affecting vegetative growth.

Source-Sink Coordination and Nutrient Utilization

Yield improvement through *Gn1a* largely depends on coordinated source–sink dynamics. Increased spikelet number enhances sink capacity, while cytokinin-mediated regulation supports efficient assimilate translocation. Ashikari *et al*. (2005) and Huang *et al*. (2025) observed higher chlorophyll retention, delayed senescence, and sustained photosynthetic rates in *Gn1a*-enhanced plants, ensuring efficient carbohydrate partitioning and a higher harvest index. Moreover, *Gn1a* positively interacts with nitrogen metabolism, as reduced OsCKX2 expression upregulates nitrate transporter genes (OsNRT1.1B, OsNRT2.3), enhancing nitrogen uptake and assimilation (Feng *et al*., 2017; Bin *et al*., 2021).

Agronomic and Stress-Responsive Effects

Introgression of *Gn1a* results in longer, highly branched panicles and a 15–25% increase in grains per panicle without major changes in plant height or maturity (Gayatri *et al*., 2020; Li *et al*., 2023). Yield advantages were consistent across genotypes such as NERICA and Kongyu 131, confirming stable performance under variable environments (Reyes *et al*., 2021; Li *et al*., 2023). Additionally, *Gn1a*-introgressed lines maintain higher cytokinin levels under drought or nutrient stress, supporting panicle development and fertility (Huang *et al*., 2025). Although not a direct stress-tolerance gene, *Gn1a* contributes to yield stability through hormonal regulation and improved physiological resilience.

Conclusion

The identification of *Gn1a* has been a landmark achievement in rice genetics, demonstrating how modulation of cytokinin degradation can significantly enhance grain number and yield. Over the years, *Gn1a* has evolved from a gene discovery to a reliable breeding tool, successfully introgressed into elite varieties such as Kongyu 131, NERICA lines, and Jaya. Its integration with yield-related genes (*DEP1*, *IPA1*, *GS3*) has shown additive effects on productivity without compromising grain quality. Physiologically, *Gn1a* enhances yield potential by regulating cytokinin levels, extending meristem activity, improving nitrogen utilization, and maintaining source–sink balance. These effects collectively contribute to higher yield stability and resource efficiency, even under moderate environmental stress. However, slight reductions in grain weight or delayed maturity in some backgrounds highlight the need for precise expression control and allele optimization.

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