

Unlocking the potential of wild rice to bring missing nutrition to elite grains

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ABSTRACT

The domestication and artificial selection of rice involved profound genetic changes that rendered wild rice more suitable for cultivation and consumption. As a result, rice has been extensively used as a caloric source to address hunger without sufficiently considering its total nutritional value. In this review, we high-light how domestication has altered starch quality and other nutritional traits in rice, including flavonoid, protein, and lipid content, as well as digestibility and texture. Precise genetic alterations through transgenic technologies hold significant promise for the reintroduction of key nutrient biosynthesis genes that have been lost in cultivated rice. Although there is currently little concrete evidence that genome editing has improved wild rice, the *de novo* domestication of wild rice enables the retention of its multi-nutritional properties while enhancing its agronomic performance and grain quality. We propose that the use of accelerated breeding techniques to introgress beneficial nutritional alleles from wild rice into elite pools could advance efforts to use wild rice to improve human health.

Key words: wild rice, domestication, starch, flavonoids, gene editing

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INTRODUCTION

Crop domestication involves the conversion of wild plants into cultivated crops through human-driven artificial selection, tailoring plant traits to fulfill human needs and preferences. Most crops were domesticated within the last 12 000 years, with rice believed to have been domesticated around 9000 years ago (Meyer and Purugganan, 2013). The domestication of rice is not only considered a model for natural evolution but has also been crucial to the advancement of human societies by providing a staple food source and supporting the caloric demands of rapid population growth. Recently, Jing et al. (2023) found that the domestication alleles for most genes trace back to wild rice from China (Figure 1A). However, a notable minority (around 20%) originate from wild rice in South and Southeast Asia, indicating distinct domestication events for Asian rice (Jing et al., 2023; Zhang et al., 2024). Concurrently, an independent domestication event within the Oryza genus is believed to have occurred in Africa, where early farmers began cultivating Oryza barthii, leading to the development of the African rice Oryza glaberrima (Fornasiero et al., 2022). Comparative analyses of domestication genes such as OsSh1

and *Sh4* in Asian rice (Figures 1B and 1C) have identified orthologous genes in African rice that also underwent lossof-function mutations (Fornasiero et al., 2022). All major cereals, including rice, experienced convergent morphological changes during domestication—collectively known as the domestication syndrome—including loss of seed dispersal, reduced seed dormancy, increased apical dominance, and larger inflorescences and grains (Alseekh et al., 2021). Previous studies have demonstrated the feasibility of using direct DNA transfer to introduce genes from *Zizania* into cultivated rice, offering an alternative method for the incorporation of desirable traits from wild rice species when specific target genes are unknown (Abedinia et al., 2000).

Although past research has primarily focused on using wild rice for traits related to abiotic and biotic resistance, relatively few studies have explored its potential to improve the nutritional value of domesticated varieties. The primary gene pool offered by the *Oryza* genus, particularly among AA genome species, remains largely underutilized in rice breeding programs. Rice domestication selected for *de novo* mutations, loss-of-function variants, and coding sequence variations (Chen et al., 2023). Some

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Figure 1. Overview of genetic, geographic, and evolutionary insights into rice domestication and nutritional traits

(A) Geographic distribution of wild Oryza species and dispersal patterns of domesticated alleles common to japonica and indica varieties.

(B) Key mutations, functional classifications, and origins of variation in domestication-related genes.

(C) Domestication and post-domestication genes that influence nutritional traits in rice.

 $(\ensuremath{\mathsf{D}}\xspace)$ Timeline of rice domestication, including inferred changes in grain quality and pigmentation.

(E) Expression levels of major starch-related genes during rice grain development.

(F) Evolutionary relationships among Wx alleles, showing four Wx^{lv} haplotypes (Wx^{lv-l} to Wx^{lv-lV}) in O. sativa.

(G) Global distribution of Wx alleles in O. sativa, with pie charts indicating allele proportions by region. Pie chart reflects sample count.

grain-related genes, such as Rc and OsMnS, which are linked to changes in the nutritional content of cultivated rice, lost their function during the domestication process (Figure 1B) (Chen et al., 2021). Furthermore, traits such as grain hardness and texture were modified during domestication to enhance processing qualities and palatability (Misra et al., 2018). Consequently, domesticated rice exhibits notably higher starch content than its wild progenitors, reflecting an evolutionary shift toward foods with more calories but fewer nutrients. The transition from smaller wild grains to larger grains in domesticated varieties (Figure 1D) has been marked by a significant increase in digestible carbohydrates with a higher glycemic index (GI), often accompanied by reductions in protein, fiber, and mineral content (Tiozon et al., 2024b). One key strategy is to examine the domestication process to identify and harness beneficial genes from wild rice species that can enhance the nutritional value of cultivated rice to optimize caloric content and nutritional density while maintaining consumer-preferred traits such as flavor, texture, and aroma.

In this review, we highlight genes affected by domestication and *de novo* domestication that could elevate the nutritional profile of rice. Drawing from recent studies on rice domestication, we discuss how these insights could shape modern crop breeding strategies for using wild rice to reintroduce nutritional traits lost in elite pools, thereby addressing the nutritional demands of an expanding population dependent on rice and cereals as dietary staples to ensure food and nutritional security. The review out-

lines two strategies, enabled by recent advances in genomics and breeding tools, to improve the nutritional quality of rice varieties by recovering traits lost during domestication and subsequent selection for yield and consumer-preferred qualities: (1) the reintroduction of genes essential for adaptation and nutritional quality from wild ancestors and landraces into elite cultivars, a concept now widely referred to as "rewilding," and (2) *de novo* domestication strategies using genome editing of domestication genes in wild rice to optimize its yield and other agronomic traits while retaining its broad nutritional composition.

STARCH-RELATED GENES ASSOCIATED WITH DOMESTICATION AND POST-DOMESTICATION INFLUENCE RICE STARCH COMPOSITION, TEXTURE, AND DIGESTIBILITY

Starch quality, granule morphology, and functional properties differ significantly between wild and domesticated rice. Wild rice starches, such as those from Australian wild rice (Tikapunya et al., 2017) and Chinese wild rice (Zhang et al., 2022a), generally exhibit higher amylose content (AC) than domesticated varieties, a trait associated with higher gelatinization temperature (GT) and greater gelatinization enthalpy. Starch in wild rice typically occurs in angular to polygonal granules, with higher crystallinity and an A-type X-ray diffraction pattern, which indicate a more ordered structure

(Henry et al., 2009). This structural difference, along with differences in chain-length distributions, results in distinct *in vitro* digestibility profiles, with wild rice starches exhibiting slower digestion rates (Zhao et al., 2022). Wild rice starches also show higher resistance to retrogradation, greater thermal stability, and different textural properties than domesticated rice (Tikapunya et al., 2017). With respect to pasting behavior, wild rice groups such as taxa A (*Oryza rufipogon*-like) and B (*O. meridionalis*-like) exhibit lower viscosities and different pasting characteristics due to poor gelatinization, suggesting that wild rice could be particularly valuable for developing products high in resistant starch (RS) or for applications requiring slowly digestible starch (Tikapunya et al., 2017).

Starch content is approximately 38% of dry weight in wild rice and has increased to 64% in domesticated rice (Hoover et al., 1996). Many indica rice varieties from South and Southeast Asia exhibit intermediate to high AC, a trait also present in the wild ancestor of rice, O. rufipogon (Yu et al., 2011). By contrast, rice with lower AC produces cohesive grains when cooked, a preferred trait in japonica varieties. The molecular evolution of starch-related genes indicates that, except for AGPL2, which regulates starch biosynthesis rates, no adaptive changes occurred in AGPS2b, SSIIa, SBEIIb, Wx, and ISA1 in wild rice (O. rufipogon), suggesting a lack of selection for starch traits in these populations (Yu et al., 2011). Interestingly, differential positive selection of specific starch biosynthesis gene combinations occurred at the subspecies level. For example, the positive selection of Wx was observed in tropical and temperate japonica rice, whereas both SBEIIb and Wx were preferentially selected in aromatic rice (Yu et al., 2011). Additionally, the Wx locus has been linked to domestication and local adaptation (Zhang et al., 2019). The SSIIa gene (also called the ALK locus and located near Wx on chromosome 6) influences GT and exhibits sequence variation between wild and domesticated rice, with ancestral haplotype 5 found exclusively in wild rice (Singh et al., 2017). This ancestral haplotype gave rise to two others: haplotype 1, found in wild, indica, and aus accessions, and haplotype 2, present in wild, japonica, and indica subspecies. In addition to the four SSIIa/ALK alleles (ALK^a, ALK^b, ALK^c, and ALK^d), nine functional polymorphic sites (FPSs) have been identified (Chen et al., 2020; Zhang et al., 2020). The ALK^a and ALK^b alleles lead to low GT, whereas ALK^{c} and ALK^{d} are associated with high GT. The high-GT ALKalleles were found to negatively affect rice milling and eating quality (Zhu et al., 2024) and may also influence digestibility. High-GT rice does not fully gelatinize, resulting in increased RS content and reduced digestibility (Pan et al., 2022; Zhu et al., 2024).

Figure 1E illustrates the starch biosynthetic pathways, highlighting key enzymes that have been targeted in rice breeding to tailor starch properties for diverse applications. The Wx gene, which encodes granule-bound starch synthase I (GBSSI), has long been a focal point of selection during both the domestication and the subsequent diversification of rice (Yamanaka et al., 2004; Gross and Zhao, 2014). Evolutionary analysis of Wx between wild rice and cultivated species has identified distinct haplotypes (e.g., Hap_55, Hap_56, Hap_57, and Hap_59) present only in wild rice, whereas haplotype 1 is abundantly represented in temperate *japonica, indica,* tropical

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japonica, and wild rice accessions (Maung et al., 2021). The Wx gene exhibits various alleles, including Wx^{lv} , Wx^{a} , Wx^{in} , Wx^{b} , Wx^{mw/la}, Wx^{op/hp}, Wx^{mp}, Wx^{mq}, Wx^{ela}, and wx, each distinguishable by eight FPSs: Int1-1, Ex2-112, Ex4-53, Ex4-77, Ex5-52, Ex6-62, Ex10-115, and Ex12-20 (Zhang et al., 2019; Zhou et al., 2021a; Huang et al., 2021; Lin et al., 2024). Although the Wxmp (Ex4-53), Wx^{mq} (Ex4-53 + Ex5-52), and Wx^{ela} (Ex12-20) alleles were identified in rice mutants derived from chemical mutagenesis (Lin et al., 2024; Yang et al., 2024), the other seven alleles originated from natural rice germplasm. These alleles have contributed to regional variations in rice AC and have consequently influenced consumer preferences (Larkin and Park, 2003; Mikami et al., 2008). The Int1-1 site (a G/T SNP), located at the 5' splice site of Wx in non-glutinous rice, disrupts normal splicing, leading to incomplete post-transcriptional processing of pre-mRNA and reduced amylose production; this SNP is the primary difference between the Wx^a (as well as $Wx^{\prime\nu}$, Wx^{in} , and $Wx^{op/hp}$) and Wx^{b} (as well as $Wx^{mw/la}$, Wx^{mp} , Wx^{mq} , and Wx^{ela}) alleles (Wang et al., 1995). A comparison of Wx genomic sequences in rice shows that the Int1-1T SNP is common in temperate japonica varieties but rare or absent in tropical japonica, indica, aus, and aromatic varieties (Olsen et al., 2006). Further sequence analysis within a 500-kb region centered on the Wx gene revealed patterns indicative of a selective sweep in temperate japonica varieties associated with this SNP. Regarding allele function, the Wx^{lv} and Wx^{a} alleles result in a high AC of approximately 25%, whereas the Wxⁱⁿ allele produces an intermediate AC of approximately 20% (Huang et al., 2021). Moreover, the substitution of Ser415 with Pro415 in Wx^{lv} increases GBSSI activity compared to Wx^{a} , which results in a higher AC (Zhang et al., 2019), Wx^{b} and $Wx^{mw/la}$ lead to low AC (around 14%–15%), and alleles such as Wx^{mp}, Wx^{mq}, Wx^{op/} hp, and Wx^{ela} are associated with very low AC (5%-12%) (Zhang et al., 2021; Sreenivasulu et al., 2022).

In a separate study, Zhang et al. (2019) identified the ancestral $Wx^{\prime\nu}$ allele, which produces short-chain amylose and significantly influences rice grain texture and starch properties, thereby affecting digestibility (Zhang et al., 2019). In-depth sequencing of this allele traces its origin to the wild rice species O. rufipogon. In cultivated rice, nearly all predominant Wx alleles originated from base-pair substitutions at the FPSs. Table 1 shows the allelic variations and their effects on AC. Figure 1F shows that the $Wx^{\prime\nu}$ allele initially evolved from the $Wx^{\prime\nu-w}$ haplotype in wild rice and subsequently diversified into distinct alleles through artificial selection or independent domestication events. Following this, the Wx^{b} and Wx^{in} alleles, both consistently present in the japonica subspecies, arose from the Wx^{lv-l} haplotype through single functional SNPs at Int1-1 and Ex6-62, respectively. A global distribution map of Wx alleles suggests that these alleles were selected in response to regional cultural preferences worldwide. The Wx^{lv} and Wxⁱⁿ alleles are predominantly found in tropical regions of South America and Southeast Asia, whereas Wx^a and Wx^{b} are more common in temperate zones. Specifically, Wx^{b} is largely present in high-latitude regions such as northern China and Japan, whereas Wx^a is more frequent in mid-latitude areas, including central and southern China, Interestingly, the $Wx^{mw/la}$ allele is thought to have originated from a spontaneous recombination event between the Wxⁱⁿ and Wx^b alleles during the later stages of rice domestication (Zhou et al., 2021a; Huang et al., 2021). For the non-functional wx allele, a 23-bp

Alleles	Int1-2	Ex2-138	Ex4-53	Ex4-77	Ex5-52	Ex6-62	Ex10-115	Ex12-20	AAC (%)	Description	Evolutionary phase
MX'^	D	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	>25	natural variation, from wild rice O. <i>rufipogon</i>	domestication
Wx ^a	D	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	<u>T</u> CT (<u>Ser</u>)	ACC (Thr)	24–26	natural variation	post-domestication
WX ⁱⁿ	g	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	T <u>C</u> T (Ser)	CCT (Pro)	ACC (Thr)	18–22	natural variation	post-domestication
qXM	t	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	14–16	natural variation	post-domestication
WX ^{mw/la}	t	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	T <u>C</u> T (<u>Ser</u>)	CCT (Pro)	ACC (Thr)	13-14	natural variation	post-domestication
$_{dq/do}XM$	g	I	CGT (Arg)	G <u>G</u> C (GI <u>V</u>)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	11-12	natural variation	post-domestication
<i>dmX</i> mp	t	I	C <u>A</u> T (<u>His</u>)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	8-11	chemical mutagenesis	post-domestication
$\mathcal{W}_{\mathcal{X}^{mq}}$	t	I	C <u>A</u> T (<u>His</u>)	GAC (Asp)	<u>C</u> AC (His)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	2–6	chemical mutagenesis	post-domestication
WX ^{ela}	t	I	CGT (Arg)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ATC (IIe)	3–5	chemical mutagenesis	I
XM	g/t	23 bp	CGT (Arg)	GAC (Asp)	TAC (Tyr)	TAT (Tyr)	CCT (Pro)	ACC (Thr)	8-0	natural variation	post-domestication
Table 1. Al	elic varia	tions and eff	ects of the fu	inctional sites	of the Wx as	ene					

the Wx^{mp} (Ex4-53) mutation site is widely selected and used in modern breeding programs to develop soft rice varieties with low amylose content in southern China (Zhang et al., 2013, 2021; Zhou et al., 2021a; Lin et al., 2024). The underline highlights specific nucleotide sequence changed. -, no Mutation sites, other than those induced by mutagenesis, are classified as domestication sites. In addition, observed changes

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insertion in exon 2 is the primary cause of the glutinous phenotype. This allele is believed to have first evolved in *japonica* rice, with later introgression into *indica* varieties in Southeast Asia (Wanchana et al., 2003; Jiang et al., 2022). It is hypothesized that populations in the mountainous regions of the Indochina Peninsula in Southeast Asia preferred waxy (glutinous) starch for cultural and ethnic reasons. This region, often referred to as the "glutinous rice zone," is characterized by the predominant cultivation and use of waxy rice cultivars in daily diets and cultural practices (Figure 1G).

Studies have found that a low methylation rate in the Wx promoter region is strongly associated with higher AC and reduced GI (Anacleto et al., 2019). Editing the CpG sites within the Wx promoter to manipulate DNA methylation may be a promising strategy for developing rice cultivars with lower GI. Posttranscriptional and post-translational modifications of Wx have been used to enhance amylose synthesis, thereby increasing RS levels (Tiozon et al., 2023). Moreover, several gene-editing approaches targeting starch biosynthesis genes have successfully increased amylose and RS levels, though their effect on GI reduction still requires thorough evaluation (Tiozon et al., 2023, 2024b). Table 2 highlights additional genes associated with increased RS content. In the b10 mutant of the indica cultivar R7954, a G-to-A mutation and a 4-bp deletion in the SSIIIa gene led to a premature stop codon, resulting in a 4-fold increase in RS content (Zhou et al., 2016). In addition, the rs4 mutant, which carries loss-offunction mutations in both SSIIIa and SSIIIb, alongside a strong Wx allele, exhibited approximately 10.8% RS in cooked rice (Wang et al., 2023). This genetic combination led to higher amylose and lipid levels, promoting RS5 formation. These findings provide a promising approach for developing healthier rice cultivars with improved RS content and dietary benefits (Tiozon et al., 2023).

POST-DOMESTICATION EFFECTS ON GRAIN MICRONUTRIENTS, PROTEINS, AND LIPIDS AT THE SUBSPECIES LEVEL

Hidden hunger is a global public health problem characterized by vitamin and mineral deficiencies in individuals who consume sufficient calories but have nutrient-poor diets. It affects the health of more than three billion people globally, particularly in rice-consuming countries (Lowe, 2021; Tiozon et al., 2021). Comparative studies of wild and cultivated rice have shown that wild rice retains superior nutritional composition and content (Ricachenevsky and Sperotto, 2016; Li et al., 2024). Crosses between O. sativa and O. rufipogon have identified multiple quantitative trait loci (QTLs) associated with the accumulation of various mineral nutrients in rice grains (Garcia-Oliveira et al., 2009; Hu et al., 2016). Notably, these studies demonstrated colocalization of QTLs associated with different minerals, which indicates that a single allele may be used to enhance multiple nutritional traits simultaneously. Similarly, a cross between Oryza longistaminata and elite cultivated rice identified 33 QTLs linked to both micronutrient accumulation and heavy metal content (Liu et al., 2020). The inheritance of target genes from O. rufipogon by cultivated rice enhances mineral accumulation and increases protein content, as previously reported (Ding et al., 2011; Li et al., 2024). Tiozon et al. (2024a) used machine learning approaches to

Gene	Phenotype	Causative sites	Causative changes	Function type	Variation origin	Selection evidence	Reference	
WX	starch and eating quality	G-to-T mutation at the 5' splice site of <i>Wx</i> intron 1	coding variation	loss of function	<i>de novo</i> mutation	yes	Wang et al. (1995)	
gif2	grain filling and starch synthesis	1	1	loss of function	<i>de novo</i> mutation	yes	Wei et al. (2017)	
RISBZ1	starch and storage protein biosynthesis	5 (4 aa) in <i>indica</i> ; 2 (2 aa) in <i>japonica</i>	regulatory change and coding variation	gain of expression	standing variation	ои	Onodera et al. (2001)	
SSIIIa	starch synthesis and resistant starch formation	G-to-A mutation at the 3' splice site of intron 5	coding variation	loss of function	1	I	Zhou et al. (2016)	
Chalk5	rice texture	3 (1 aa)	coding variation	gain of expression	standing variation	ои	Li et al. (2014)	
Rc	pericarp color and flavonoid biosynthesis	14-bp frameshift deletion	coding variation	loss of function	<i>de novo</i> mutation	yes	Sweeney et al. (2006)	
OsB2/Kala4	anthocyanins	11.0-kb insertion in the promoter	regulatory change and coding variation	gain of function	<i>de novo</i> mutation	yes	Xia et al. (2021)	

Table 2. Summary of key genes linked to starch, glycemic index, and texture.

-, not reported.

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identify subspecies-specific ionomic patterns, which revealed marked differences between *indica* and *japonica* rice.

Notably, a strong selective sweep around OsAMT1;1 (a high-affinity ammonia transporter) led to a marked reduction in nucleotide variation at this locus, which likely reflects selection pressure on nitrogen uptake efficiency in paddy soils during rice domestication (Ding et al., 2011). OsAMT1;1 underwent significant selection in the Indl subgroup, whereas three other genes-OsNRT2.3, OsNAR2.2, and OsNiR1-which belong to the high-affinity nitrate transporter family or encode key proteins involved in nitrate uptake, were under strong selection in Indll (Xu et al., 2012). Moreover, Xie et al. (2015) identified several genes critical to phosphate and potassium uptake-such as OsSPX1, LOC_Os02g39750 (an inorganic phosphate transporter), OsPHO1;1, OsPHO1;2, OsK1.1, OsK2.1, OsK2.2, OsK4.1, OsK4.2, and OsHAK12-that are under selection in either Indl or Indll. However, further research is needed to identify the specific micronutrient-related genes associated with these selective sweeps. Although it is well established that the nutritional content of cultivated rice has declined over time, the genetic factors driving these changes during domestication remain poorly understood.

Mannan is a major class of cell wall storage polysaccharides that cannot be directly digested by the human body. As a result, it is considered a valuable source of dietary fiber, contributing to improved gut health and potentially to the regulation of glucose metabolism (Voiniciuc, 2022). *OsMnS*, which encodes a β -1,4-mannan synthase, has been identified as a key mannose biosynthesis gene in rice endosperm (Yoshida et al., 2023). The functional Hap.B allele, derived from wild rice, is responsible for the high mannose content observed in certain rice cultivars. However, due to selective breeding for improved palatability, this allele was lost during the modern breeding process, resulting in reduced mannose content in domesticated rice.

Elevated protein content in rice endosperm enhances grain milling quality by improving structural integrity, which helps minimize grain breakage during processing. Compared to Oryza sativa iaponica cv. Nipponbare. O. meridionalis. O. rufipogon. and O. australiensis display higher protein content (Kasem et al., 2014). Glutelins are the most abundant storage proteins in rice, accounting for about 40%-60% of total protein content. In this context, Yang et al. (2019) identified OsGluA2, which encodes a alutelin type-A2 precursor responsible for rice grain protein content. Polymorphisms in this gene divide haplotypes into low (OsGluA2^{LET}) and high (OsGluA2^{HET}) expression types; OsGluA2^{LET} is predominantly found in japonica accessions and originated from wild rice, whereas OsGluA2^{HET} is the dominant haplotype in indica varieties and arose through mutation of OsGluA2^{LET} (Yang et al., 2019). In addition, OsSMF1 is a key regulator of grain filling and storage protein synthesis during seed development (Kawakatsu et al., 2009). As a bZIP transcription factor, it influences the final protein content in rice grains by regulating the synthesis of storage proteins (Kawakatsu et al., 2009; Tiozon et al., 2023). In domesticated rice, selection for higher yields and faster growth has likely influenced OsSMF1 expression, affecting both protein quantity and quality and ultimately shaping the nutritional profile of modern cultivars. Conversely, the gif2 mutant, which exhibits

defective grain filling, has a slower filling rate, significantly lower final grain weight, and reduced yield compared with the wild type. Notably, *gif2* shows a marked decrease in starch content but an increase in protein content, along with altered physicochemical properties. Extremely low levels of GIF2 nucleotide variability were found in wild rice and many landraces. This gene was preserved during the domestication of contemporary rice through both natural and artificial selection (Wei et al., 2017). In addition, *Chalk5* is tightly linked to two QTLs associated with grain width, weight, and yield, namely *qSW5* (*GW5*) and *GS5* (Li et al., 2014). Although *Chalk5* leads to an abnormal starch structure, it also contributes to higher protein content (Li et al., 2014). Other genes regulating protein content in cereals also exhibit reduced expression as a result of domestication (Lang et al., 2014; Luo et al., 2018).

Lipids are important macronutrients that accumulate in rice bran and endosperm (Tiozon et al., 2023). The accumulation of fatty acids differs between indica and japonica rice. Consistent with this observation, Zhou et al. (2021b) confirmed the roles of lipid-related genes such as LOC_Os06g05130 (qPAL6) and LOC_Os06g40500 (qLIN6) in determining fatty acid composition and oil quality. Specific haplotypes of these genes have been selected for in japonica rice, supporting the accumulation of fatty acids in this subspecies and potentially contributing to its cold tolerance and textural properties. In addition, Tiozon et al. (2025) identified distinct selection signals in OsGELP genes between indica and japonica, which play key roles in lipid mobilization and contribute to stress response and adaptation. However, further investigation is needed to determine whether domestication has influenced the divergence of these genes between wild and cultivated rice.

THE EFFECT OF DOMESTICATION AND SUBSEQUENT BREEDING ON GRAIN PIGMENTATION AND SECONDARY METABOLITE CONTENT

Secondary metabolites, such as flavonoids, influence rice pigmentation, which was selected out during domestication. Genes associated with pigmentation and domestication loci are often implicated in genetic hitchhiking, yet the mechanism by which a favorable domestication allele became linked to a mutant allele affecting pigmentation and nutritional traits remains unclear. A notable example is the Rc gene, which is linked not only to red pigmentation but also to traits such as seed shattering and dormancy (Sweeney et al., 2006). A 14-bp deletion in Rc, inherited from O. rufipogon, results in the loss of red pigmentation in rice grains, thereby distinguishing the whitegrained O. sativa from its wild, red-grained relatives (Sweeney et al., 2006). Brotman et al. (2021) showed that Rc, which is involved in catechin and proanthocyanidin biosynthesis, also lowers the GI of rice. This effect may result from increased catechin levels, which can form inclusion complexes that reduce rice digestibility. The early domestication of Asian rice involved selective sweeps at anthocyanin pathway genes, such as OsDFR and OsC1, and at shikimate pathway-related genes, including OsEPSPS, OsDAHPS2, and OsMYB15 (Lu et al., 2022). Consistent with this observation, the flavor and nutritional value of early Asian rice were altered by mutations in

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these enzymes or by changes in their expression. Furthermore, the *Kala4/OsB2* gene, which governs black pigmentation, contains an 11.0-kb insertion in its promoter region that induces purple pericarp (Oikawa et al., 2015). This mutation, which originated in *japonica*, was later introgressed into *indica*, supporting the hypothesis that anthocyanin biosynthesis evolved under a single-origin domestication model (Oikawa et al., 2015). Meta-analyses of randomized controlled trials have shown that increased anthocyanin content in foods can reduce digestibility (Mao et al., 2023), highlighting the potential of flavonoids to modulate the glycemic response and enhance the antioxidant and nutritional profiles of rice.

REINTRODUCTION OF THE NUTRITIONAL PROPERTIES OF WILD RICE INTO CULTIVATED RICE

Wild rice from genus Zizania has demonstrably higher RS and lower digestibility than cultivated rice due to its increased AC (Zhang et al., 2022a). In addition, comparisons of AC between wild Oryza species and rice cultivars such as O. sativa cv. Nipponbare and O. sativa cv. TeQing have shown that O. alta and O. latifolia possess higher AC levels (Kasem et al., 2014). Genebank collections should therefore be used to identify variation in nutritional components (Sreenivasulu et al., 2023). China maintains the largest wild rice collections, primarily composed of O. rulipogon, O. officinalis, and O. meyeriana, followed by IRRI, which holds a more diverse collection of wild rice species covering the AA, BB, BBCC, CC, CCDD, EE, FF, GG, HHJJ, HHKK, and KKLL genomes (Figures 2A and 2B). The use of high-throughput phenotyping to assess starch composition, GI, and textural attributes, as well as for multinutritional profiling, can provide valuable insights into the nutritional potential of wild rice.

Wild rice hybridization has long been used to incorporate beneficial traits, such as disease resistance, insect resistance, and male sterility, by transferring QTLs or genes from wild species into elite breeding materials. However, challenges such as low crossing success, sterility, and limited recombination between wild and cultivated species have posed significant obstacles, necessitating further advances to overcome these barriers (McCouch et al., 2007). Pre-breeding research plays a key role in identifying and transferring favorable alleles from wild species into elite cultivars, with modern genetic tools enabling more accurate selection and offering speed breeding strategies to advance generations rapidly. Chromosome segment substitution lines can be an efficient tool for transferring beneficial alleles associated with low GI and other nutritional properties from wild species into elite cultivars (Figure 2C). The process involves generating F1 hybrids from crosses between wild rice and elite cultivars, followed by multiple generations of backcrossing, in which progeny are repeatedly crossed with the elite parent to restore the majority of the elite genome while retaining selected wild rice chromosome segments linked to the targeted nutritional properties. Once the causal gene is identified through genetic mapping, unnecessary flanking regions that may cause undesirable phenotypes can be removed using recombinant selection. Although chromosome segment substitution lines have



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Figure 2. Wild rice distribution and breeding strategies for neo-domestication

(A) Number of wild rice accessions preserved in major gene banks.

(B) Distribution of wild rice accessions held by the International Rice Research Institute, highlighting the extensive genetic variation within the collection. (C) Diagram of a mapping population approach using chromosome segment substitution lines (CSSLs) to introgress alleles from wild rice species and acquire nutritional traits.

(D) Introduction of nutrition-related target genes into the elite pool. Genome editing targets recessive genes or overexpression of wild rice genes in elite varieties that affect nutritional content, including biosynthetic genes for micronutrients (e.g., iron, zinc, and vitamin A) and bioactive compounds (e.g., anthocyanins, flavonoids and polyphenols).

effectively facilitated the transfer of beneficial alleles from wild rice species, they are time-consuming and resource-intensive.

One promising approach involves characterizing wild rice germplasm through a multi-omics approach to identify genes influencing multi-nutritional properties, followed by the transfer of key genes from the wild rice gene pool into elite *indica* and *japonica* cultivars via transformation and genetic engineering to improve nutritional quality (Figure 2D). In the future, gene pyramiding, which involves stacking combinations of genes from the wild pool into elite cultivars, could also serve as a powerful and effective tool to integrate multi-nutritional properties into modern cultivars while simultaneously enhancing resistance to abiotic and biotic stresses.

DE NOVO DOMESTICATION VIA GENOME EDITING OF DOMESTICATION-RELATED GENES WHILE RETAINING THE NUTRITIONAL PROPERTIES OF WILD RICE

It is important to establish high-throughput nutritional phenotyping platforms to efficiently screen large collections of wild rice accessions for donors with the highest nutritional density, undertake multi-omics analyses to discover nutrition-related genes, and establish reference genomes for these accessions (Figure 3A). Genome editing holds great promise for accelerating the domestication and improvement of wild rice species by enabling precise genetic modifications through CRISPR-Cas technology (Figure 3B). Notably, Shimizu-Sato et al. (2020) successfully used Agrobacterium-mediated transformation in O. barthii, O. glumaepatula, O. rufipogon, and O. brachyantha using immature embryos, achieving stable transformation in the T₁ generation. However, analysis of advanced generations is necessary to validate trait stability, ensure long-term heritability, and assess potential off-target effects or unintended phenotypic consequences. Several challenges must be addressed to enable successful de novo domestication of diverse wild rice species. Only a limited number of wild species have been successfully transformed, including some AAgenome species (O. barthii, O. rufipogon, and O. glumaepatula), an FF-genome species (O. brachyantha), and a CCDD-genome species (O. alta) (Shimizu-Sato et al., 2020; Yu et al., 2021; Zhang et al., 2022b). Increasing transformation efficiency is critical to generating sufficient numbers of transgenic plants. One potential barrier is the high frequency of somaclonal mutations observed during wild rice transformation, as these are often associated with undesirable phenotypes (Miyao et al., 2012; Park et al., 2019). Therefore, it is necessary to minimize

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Figure 3. Neo-domestication of wild rice to enhance agronomic and yield-related traits while preserving nutritional quality. (A) Identification of domestication-linked genes in a large collection of wild rice species via genome-wide association studies and other genomics-based approaches.

(B) Targeted gene editing in wild rice accessions to modify key domestication-associated genes to enhance yield and other desirable agronomic traits. *An-1*, Awn-1; *An-2*/LABA1, Awn-2/long and barbed Awn 1; *DTH7*, days to heading 7; *Ghd7*, grain number, plant height, and heading date 7; GS3, grain size 3; *GW2*, grain width 2; *Hd1*, heading date 1; *IPA1*, ideal plant architecture 1; *OsbZIP46*, *Oryza sativa* basic leucine zipper 46; *PROG1*, prostrate growth 1; *PROG7*, prostrate growth 7; *qSD7-1/Rc*, quantitative trait locus for seed dormancy on chromosome 7/red pericarp; *qSH1*, quantitative trait locus for seed shattering 1; *RPAD*, rice plant architecture domestication; *Sdr4*, seed dormancy 4; *sd1*, semi-dwarf 1; *SH4*, shattering 4; *SPL14*, SQUAMOSA promoter-binding-like 14; *TIG1*, tillering inclined growth 1; *TN1*, tiller number 1.

somaclonal mutations while improving the efficiency of wild rice transformation.

Breakthroughs in genome editing have demonstrated the rapid enhancement of six essential agronomic traits in O. alta, a wild rice species. This strategy exemplifies the de novo domestication approach, in which wild relatives of crops are rapidly domesticated by precisely modifying domestication genes (Yu et al., 2021). For example, the *qsh1CR-1* mutant, which carries bi-allelic frameshift mutations in OagSH1-CC and heterozygous frameshift mutations in OaSH1-DD, lacks a layer of abscission cells, confirming that edits to qSH1 homologs effectively prevent seed shattering in O. alta. Similarly, targeting OaAn-1-CC (OalC04g136090) and OaAn-1-DD (OalD04g130280) generated two mutants, an-1^{CR}-1 and an-1^{CR}-2, which exhibited significantly shorter awns (1.61 and 2.63 cm, respectively) than wildtype O. alta. In addition, CRISPR-Cas9 targeting of the sd1 homologs OaSD1-CC (OalC01g172060) and OaSD1-DD (OalD01g109880) resulted in the sd1CR-1 mutant, which harbored bi-allelic frameshift mutations and exhibited a significant reduction in plant height. Notably, a highly efficient multiplex CRISPR-Cas9 approach successfully edited the homologs of key flowering time regulators, including OaGhd7-CC, OaGhd7-DD, OaDTH7-CC, and OaDTH7-DD, generating eight PPR1 mutant lines with varying heading dates. Line 1, which carried mutations in all four genes. flowered 82 days after transplantation, whereas wild-type PPR1 had not flowered after 150 days. Furthermore, targeting homologs of GS3, a key regulator of grain size, generated six independent mutants with significantly longer grains than wild-type O. alta (Yu et al., 2021). Base editing of the IPA1 homolog introduced a point mutation in OalPA1-DD (OalD08g132520) without altering OalPA1-CC (OalC08g106170). The resulting ipa1^{CR}-1 mutant exhibited significantly increased stem diameter, likely improving lodging resistance. Therefore, base substitution edits can expand gene improvement strategies in other wild rice species. CRISPR-

heading dates, demonstrating its effectiveness for de novo domestication. These genome-edited lines exhibit superior agronomic performance and enhanced yield. In the future, they can be subjected to gene pyramiding to combine the best alleles from multiple domestication-related loci, further increasing yield while retaining the advantageous nutritional quality of wild rice. These domestication-related genes can also be prioritized for editing in other wild rice species to further validate the potential of CRISPR-Cas9 in improving agronomic traits while retaining beneficial nutritional traits. Using this approach for the de novo domestication of O. coarctata-a wild rice species with a KKLL genome (Zhao et al., 2023), native to coastal regions of India and Bangladesh and traditionally consumed as a delicacy-could enable the development of crops with exceptional salt tolerance, unique grain qualities, and high nutritional value, addressing the challenge of food security in saline environments while supporting novel culinary applications. The same approach can also be applied to other wild rice species, where techniques such as base substitution editing and multiplex genome editing could retain nutritional traits without compromising yield or sensory quality.

Cas9-based mutagenesis in O. alta produced lines with shorter

awns, non-shattering seeds, reduced plant height, and altered

Precision breeding initiatives have also been applied to other crops. For instance, Zsogon et al. (2018) used CRISPR-Cas technology to domesticate wild tomatoes, achieving a remarkable 500% increase in lycopene accumulation compared to domesticated cultivars. With the increasing availability of pan-genome data for wild and cultivated rice, many loci with functional variations have been identified, including presence-absence variations (Huang et al., 2012; Zhao et al., 2018). This poses a major challenge for the direct *de novo* domestication of wild rice. Although direct evidence of wild rice improvement through genome editing remains limited (Yu et al., 2021), recent advances have enabled the

modification of domestication-related traits in *O. alta* and other crop species, indicating potential applications in wild rice. Nevertheless, it may be more feasible to use marker-assisted selection or CRISPR-Cas9-mediated gene knock-in technologies to introduce or modify key biosynthetic genes for missing nutrients from wild rice into cultivated rice.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Key genes involved in rice domestication can be identified using high-throughput resequencing resources, high-quality reference genomes of wild rice and various rice subspecies (aus, aromatic, indica, and tropical and temperate japonica), and population differentiation metrics such as nucleotide diversity (π), F_{ST}, Tajima's D, and cross-population composite likelihood ratios. Combining these selective sweep analyses across crops with methods for analyzing syntenic relationships enables the identification of conserved genes across species that have undergone longterm artificial selection to improve grain quality traits and also provides important insights into genomic nutrition signatures that were lost during selection due to a lack of precise phenotyping methods. A recent study found that orthologs encoding a WD40 protein underwent convergent selection during domestication in both maize and rice. When these genes were knocked out, yield increased in both crops without affecting other agronomic traits (Chen et al., 2021, 2022).

Future strategies should incorporate multi-omics approaches such as expression QTL mapping, metabolite-based association studies, methylome profiling, and phosphorylation- and acetylation-based proteomics analyses of wild and cultivated species to investigate the complex regulatory processes that influence domestication. Key candidate genes influencing multinutritional traits can be targeted using genetic engineering tools to modify pathways in elite indica and japonica cultivars. In addition, precise base editing and nanoparticle-mediated delivery of small interfering RNAs (Liu et al., 2024) have been demonstrated successful in improving agronomic traits, including reduced seed shattering, awn removal, improved heading, and ideal panicle architecture in wild rice species. It is necessary to evaluate whether these newly domesticated wild rice lines maintain their multi-nutritional advantages alongside climatic adaptability. Collectively, integrating these techniques can enhance the use of wild rice for sustainable production, supporting the nutritional demands of expanding populations in Asia and Africa in the 21st century.

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AUTHOR CONTRIBUTIONS

R.J.N.T. and N.S. conceived the study; R.J.N.T., S.-R.K., and N.S. wrote the original draft; R.J.N.T. generated the figures; and A.R.F., Q.L., C.Z., and N.S. reviewed and edited the manuscript. All authors have approved the final version of the manuscript.

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