

Molecular dissection of high-yield, high-quality, and heat-tolerance traits in Gong 887S and its hybrid rice combinations

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Abstract

Modern rice breeding urgently requires a paradigm shift from single-trait improvement to the integrated optimization of complex traits, including yield potential, grain quality, heat tolerance, and multi-resistance, aiming to establish a resource-efficient and environmentally sustainable production system. This study systematically investigated the molecular mechanisms underlying high yield, superior quality, and heat tolerance in the thermo-sensitive genic male sterile line Gong 887S, its restorer lines (Yuenongsimiao, Jingguizhan, YR592), and their hybrid combinations through an integrated approach combining whole-genome sequencing, functional gene analysis, and phenotypic characterization. High-quality genome assembly of Gong 887S demonstrated exceptional contiguity ($LAI = 26.52$) and completeness ($BUSCO = 98\%$), with 40,564 annotated genes. Notably, a 6.38% japonica introgression was identified, significantly higher than conventional indica varieties. Critical genes (such as *OsLG3*, *Gn1a*, *TT3.1*) located on Chrs 1, 3, and 7 were found to coordinately regulate grain number per panicle, grain length, and thermotolerance. The hybrid combinations (Gongliangyou Yuenongsimiao, Gongliangyou Jingguizhan, and Gong 8 Liangyou 892) pyramided 56–83 favorable loci, including *LAX1*, *GS3*, *TT1*, and *Chalk5*. Multi-location trials showed that these hybrids achieved average yields of 9,714–9,841 kg/ha, representing a 2.6–8.1% increase over controls. Heat tolerance evaluations revealed sustained seed-setting rates of 56.80–66.01% under 40°C greenhouse stress and over 85% under field conditions, significantly outperforming controls. Grain quality parameters, including head rice rate (68.7–72.0%), chalkiness (1.0–1.9%), and amylose content (15–17%), all met premium standards. Genomic structural variation analysis identified a Chr7 inversion and Chr11 deletion in Gong 887S that modulate *OsAAP6* and *Chalk5* expression, enhancing protein content while reducing chalkiness. By elucidating the genetic networks of Gong 887S and its hybrids, this study highlights the substantial contributions of indica-japonica introgression, multi-gene synergy, and structural variations to trait improvement, providing a robust theoretical foundation for molecular design breeding in rice.

1. Introduction

Rice (*Oryza sativa* L.), serving as a major staple for nearly half of the global population, occupies an indispensable strategic role in ensuring global food security and societal stability. The Food and Agriculture Organization (FAO, 2017) projects that the world's population will reach 9.7 billion by 2050, necessitating a 60% increase in food production compared to present levels. This escalation imposes substantial demands on rice production systems. Concurrently, shifting consumer preferences are diversifying the objectives of rice breeding programs. On one hand, increased market focus on eating and cooking quality, nutritional components, and environmentally sustainable production has made the development of high-quality varieties crucial for enhancing agricultural value (Huang et al. 2017). On the other hand, the rise in extreme heat events caused by climate change results in pollen sterility and diminished grain filling in rice, leading to yield losses of 20–40% alongside degraded grain appearance and cooking quality (Jagadish et al. 2007). Additionally, the excessive use of fertilizers and pesticides in traditional high-input cultivation systems has precipitated ecological issues such as soil acidification and water eutrophication (Zhou et al. 2016). In light of these challenges, modern rice breeding must transcend single-trait improvement, integrating complex traits such as high yield potential, quality optimization, heat tolerance, and multi-resistance to both biotic and abiotic stresses. This approach aims to establish resource-efficient and environmentally sustainable production systems capable of addressing the challenges posed by population growth, climate change, and ecological security.

93 – 11 (R9311) is extensively utilized as a paternal parent in two-line hybrid rice breeding and has been pivotal in developing restorer lines for three-line Honglian-type hybrid rice, as well as enhancing both male sterile and restorer lines (Wang et al. 2021). Quan 9311A, an improved line derived from 93 – 11, has emerged as the most widely

adopted three-line male sterile line in China, with 176 derived varieties successfully passing national or provincial trials(Li et al. 2023). Furthermore, female parental lines such as 1892S, Xiangling628S, and Guangzhan63-4S have facilitated the creation of numerous male sterile lines and hybrid combinations, collectively contributing to 135, 67, and 44 certified at national or provincial levels(Li et al. 2023; Zhang et al. 2025). The widespread adoption of these varieties has substantially enhanced the yield potential, grain quality, and stress tolerance of Chinese hybrid rice, affirming their critical contribution to national food security. To further enhance desirable traits from 93 – 11, 1892S, Xiangling628S, and Guangzhan63-4S, breeders developed the indica photo-thermo-sensitive genic male sterile (PTGMS) line Gong887S through ten generations of systematic selection, using 1892S as the female parent and 8-8S/93 – 11, where 8-8S is derived from Xiangling628S/Guangzhan63-4S, as the male parent. Gong887S integrates superior grain quality, high-yield potential, heat tolerance, and disease resistance, demonstrating significant breeding value.

The rapid advancement and maturation of Whole Genome Sequencing (WGS) technologies have significantly reduced sequencing costs and achieved continuous technological breakthroughs. The swift progress of sequencing platforms, combined with a robust array of bioinformatics tools, has rendered whole-genome sequencing of rice both economically feasible and technically accessible, offering diverse strategic options for genome assembly(Li et al. 2024; F et al. 2015; Li et al. 2023; Zhang et al. 2025; Zhang et al. 2019). These genomic advancements have further revolutionized hybrid rice breeding. Researchers have increasingly recognized the importance of comprehensive genomic and phenotypic evaluations of parental lines, thereby utilizing whole-genome sequencing for detailed gene and phenotype assessments. For instance, Li (Li et al. 2023) executed whole-genome sequencing on Quan 9311A, a widely used male sterile line, applying the RiceNavi technology to dissect functional genes related to agronomic traits, grain quality, pest and disease resistance, and other advantageous characteristics. Zhang(Zhang et al. 2025) employed telomere-to-telomere (T2T) assembly to construct a high-quality, gap-free genome of 1892S, revealing unique allelic variations in several known lodging-resistance genes—such as *SD1*, *Sdt97*, *SBI*, *OsFBA2*, *AP01* and *OsTB1*—through comparative genomic analysis, thereby elucidating the genetic basis for its superior lodging resistance. Jiang (Jiang et al. 2023)utilized Nanopore sequencing and Hi-C scaffolding to create a high-precision, chromosome-level genome assembly of Qigeng 10 (QG10), an early-maturing aromatic japonica variety from Northeast China. In QG10, sequence variations in 63 cloned genes associated with important traits such as grain yield, grain size, disease resistance, lodging resistance, and aroma were identified. Li(Li et al. 2024) used PacBio HiFi, Hi-C, and Oxford Nanopore Technologies (ONT) to complete a gap-free genome assembly of the indica restorer line Zhonghui8015 (ZH8015), leveraging this genome alongside transcriptomic and metabolomic analyses to systematically elucidate ZH8015's response mechanisms to brown planthopper (BPH) infestation and its underlying molecular regulatory network. Furthermore, wild rice species harbor extensive genetic variation, serving as a valuable reservoir of genes for rice breeding. Huang(Huang et al. 2024) reported the gap-free genome assembly of the wild rice haplotype Y476, developing two chromosome segment substitution line (CSSL) sets and identifying 254 quantitative trait loci (QTLs) associated with agronomic traits and responses to biotic and abiotic stresses. These studies have propelled rice breeding technology forward and provide invaluable genetic resources for future agricultural production.

Recently, utilizing Gong887S as the female parent, more than ten hybrid rice varieties—including Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, Gong 8 Liangyou 892, Gong 8 Liangyou Hesimiao, Gong 8 Liangyou 17, and Gong 8 Liangyou 382—have been certified through national or provincial trials. Among these, Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, and Gongliangyou 892, combining high yield, superior quality, and heat tolerance during the heading stage, have emerged as prominent varieties within their respective regions. As of 2024, Gongliangyou Yuenuosimiao's cumulative planting area as a single-season or ratooning rice has surpassed 2 million

mu (approximately 133,333 hectares), providing substantial support for regional grain yield enhancement and farmer income growth. In this study, we performed T2T whole-genome assembly of the elite male sterile line Gong887S and its corresponding restorer line Yuenuosimiao, conducting a systematic analysis of their genetic backgrounds and functional genes to investigate Gong887S and Yuenuosimiao's breeding advantages at genetic and genomic levels. Concurrently, we executed functional gene analyses via whole-genome sequencing on the parental lines (Gong887S, Jingguizhan, and R592) of hybrid combinations Gongliangyou Jingguizhan and Gong 8 Liangyou 892. Building upon this foundation, we comprehensively evaluated the yield traits, grain quality, and heat tolerance of the three hybrid combinations: Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, and Gongliangyou 892. This study aims to elucidate the molecular mechanisms underlying the high yield, superior grain quality, and thermotolerance traits of Gong 887S and its hybrid derivatives through whole-genome sequencing and functional gene analysis, thereby providing a theoretical foundation for molecular design breeding.

2. Materials and methods

2.1 Plant materials

The hybrid rice combinations Gongliangyou Yuenuosimiao (Gong887S/Yuenuosimiao), Gongliangyou Jingguizhan (Gong887S/Jingguizhan), Gong8Liangyou892 (Gong887S/R592), and Fengliangyou4 (serving as the control) were systematically evaluated for yield performance, grain quality, and heat tolerance. In this study, the photoperiod- and thermo-sensitive genic male sterile (PTGMS) lines, Gong 887S and 1892S, alongside restorer lines Jingguizhan, R592, and Yuenuosimiao—utilized in two-line or three-line hybrid rice breeding—were subjected to comprehensive whole-genome sequencing (WGS). Additionally, publicly accessible WGS data for extensively utilized male sterile lines such as AnnongS, Peiai64S, Zhu1S, C815S, Longke638S, and Y58S, together with the restorer line R9311, were integrated into our analysis to enhance genetic insights. Notably, the materials including Gong887S, Jingguizhan, R592, and their hybrid combinations (Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, and Gong8Liangyou892) were independently developed by Beijing Golden Nonghua Seed Technology Co., Ltd., demonstrating their proprietary advances in rice breeding.

2.2 Methods

2.2.1 Yield and grain quality traits

Data for the yield and grain quality traits of hybrid rice combinations Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, and Gong8Liangyou 892 were extracted from mean values obtained from multi-location regional trials conducted over two years. Gongliangyou Yuenuosimiao (National Certification No. Guoshendao20216105) was evaluated in regional trials for the late-maturing indica rice group across the middle and lower reaches of the Yangtze River during 2019–2020. Gongliangyou Jingguizhan (Jiangxi Provincial Certification No. Ganshendao20210072) participated in medium-season hybrid rice trials within Jiangxi Province for the same period. Gong8Liangyou892 (National Certification No. Guoshendao20253004) was assessed in consortium regional trials for the late-maturing indica rice group along the middle and lower Yangtze River stretches from 2022 to 2023.

2.2.2 Heat tolerance traits at the heading and flowering stages

The heat tolerance of Gongliangyou Yuenuosimiao, Gong Liangyou892, and control variety Fengliangyou4 during the heading and flowering stages was evaluated using both greenhouse and field-based staggered sowing methods.

Greenhouse Experiment. Seeds were sown on April 30 within a greenhouse setting. Seedlings at the five-leaf stage were transplanted into plastic pots (upper diameter: 26 cm, bottom diameter: 20 cm, height: 20 cm), each containing 7 kg of air-dried paddy soil augmented with 6 g of compound fertilizer (N-P₂O₅-K₂O, 12:18:15). Six pots were prepared per variety, with five hills (seedlings) per pot. At the heading stage, panicles were tagged, and three pots per variety were relocated to an artificial climate chamber for a 5-day extreme heat stress treatment, followed by transfer to a control chamber with optimal temperature settings until maturity. The remaining three pots per variety were kept in the control chamber continuously as controls. Upon reaching maturity, tagged panicles were harvested for seed setting rate analysis. The high-temperature treatment parameters included a daily maximum temperature of 40°C, a minimum of 30°C (average daily temperature: 33.17°C), relative humidity at 65%, a 12-hour photoperiod (8:00–20:00), and light intensity of 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1A).

Field Experiment. This trial was conducted under natural high-temperature conditions prevalent in Hunan Province from mid-July to late August using a staggered sowing method. Test varieties Gongliangyou Yuenuosimiao, Gong8Liangyou892, and Fengliangyou4 were sown on three separate dates: April 21, May 1, and May 11. Seedlings at the five-leaf stage were transplanted at a spacing of 16.7 cm × 26.7 cm (approximately 5 inches × 8 inches). Each variety was planted in two rows with ten plants, one seedling per hill. Standard field management protocols—including fertilization, irrigation, and pest and disease control—were applied throughout the trial. Field temperature and humidity were recorded automatically every 5 minutes using a DSR-TH digital temperature and humidity recorder (ZOGLAB Microsystem Co. Ltd.). Plants that headed between July 31 and August 6 faced extreme natural heat stress, characterized by daily maximum temperatures of 38.7°C to 40.3°C, daily minimum temperatures of 27.0°C to 28.5°C, an average daily temperature range of 32.0–32.4°C, and relative air humidity of 86.7% (Fig. 1B). Tillers entering the initial heading phase on July 31 were tagged for monitoring. At maturity, the seed setting rate of tagged panicles was determined for each test material.

2.2.3 Whole-Genome sequencing, sssembly, and snnotation

Sequencing data methodology

For the genomic analysis of Gong887S and Yuenuosimiao, a comprehensive multi-platform sequencing approach was employed, incorporating PacBio HiFi sequencing, second-generation sequencing (NGS), RNA sequencing (RNA-seq), and Hi-C sequencing methodologies. In contrast, for the rice varieties 1892S, Jingguizhan, R592, and Yuenuosimiao, solely NGS was utilized. Genomic DNA and total RNA were meticulously extracted from fresh leaf samples of the plant materials. High-quality DNA samples were fragmented using a Covaris ultrasonicator, followed by library preparation, which involved sequential steps of end-repair, A-tailing, adapter ligation, purification, and PCR amplification. The constructed libraries were subjected to paired-end (PE) sequencing on the Illumina Novaseq platform. Raw sequencing reads underwent stringent quality control using fastp, an open-source software tool, which filtered out: (1) reads containing adapter sequences; (2) reads with more than 10% ambiguous bases ('N'); and (3) paired reads where one read exhibited a base quality score below 5 over more than 20% of its length. Comprehensive post-quality control statistics, including read count, data yield, duplication rate, sequencing error rate, Q20/Q30 content, and GC content, were computed to ensure high data quality (iMeta 2023).

PacBio HiFi sequencing data were generated utilizing Single-Molecule Real-Time (SMRT) sequencing technology with Zero-Mode Waveguide (ZMW) cells (Jadhav et al. 2019; Schendel et al. 2020). This technology involves a laser illuminating the DNA polymerase anchored at the nanowells' base, generating fluorescent signals for nucleotide identification. Sequencing operations were performed on the PacBio Sequel II/Ile platform, ensuring high fidelity and accuracy in nucleotide sequence determination.

For RNA-seq, qualified RNA samples were processed to enrich eukaryotic mRNA using Oligo(dT) beads. Fragmented mRNA served as a template for first-strand cDNA synthesis with random hexamer primers. Subsequently, second-strand cDNA was synthesized in a reaction mixture consisting of buffer, dNTPs, DNA polymerase I, and RNase H. The resulting double-stranded cDNA was purified using AMPure XP beads, followed by end-repair, A-tailing, and adapter ligation. Final library preparation included fragment size selection and PCR amplification, culminating in highly purified sequencing libraries.

In the Hi-C sequencing workflow, qualified DNA samples were randomly fragmented using a Covaris ultrasonicator, adhering to a standard library preparation protocol encompassing end-repair, A-tailing, adapter ligation, purification, and PCR amplification. Sequencing was conducted on the Illumina Novaseq platform using PE mode. Raw data underwent image recognition, decontamination, adapter trimming, and duplicate removal processes. Summary statistical measures, including read count, data yield, duplication rate, sequencing error rate, and Q20/Q30 and GC content, were meticulously compiled to validate the integrity and reliability of the sequencing data.

Genome assembly and quality assessment

Library preparation for short-read sequencing was executed using the Illumina Novaseq platform, whereas long-read sequencing employed the PacBio Sequel II platform to ensure comprehensive data acquisition. De novo genome assembly was carried out utilizing Hifiasm v0.19.5-r590(Cheng et al. 2021), with subsequent error correction performed via NextPolish(Hu et al. 2020) to enhance the accuracy of the assembled genome. Hi-C sequencing data were carefully aligned and filtered through Chromap(Zhang et al. 2021). The assembly process included clustering, interaction matrix construction, and scaffolding using Yaho(Zhou et al. 2023), followed by meticulous manual review and correction facilitated by JuiceBox (<https://github.com/aidenlab/Juicebox>), allowing visualization-assisted error rectification.

To assess genome completeness, BUSCO was employed against the *embryophyta_odb10* database, providing a benchmark for evaluating the presence of conserved orthologous genes(Manni et al. 2021). The fidelity of repetitive sequence assembly was scrutinized using the Long-terminal Repeat Assembly Index (LAI) (Ou et al. 2018), offering insights into the assembly quality of repetitive elements. Furthermore, to evaluate assembly integrity, next-generation sequencing (NGS) reads were aligned to the assembled genome utilizing the BWA-MEM algorithm(Li and Durbin 2009), ensuring comprehensive coverage and alignment accuracy. Additionally, synteny analysis between rice genomes was performed employing Minimap2, facilitating comparative genomic assessments and elucidating evolutionary relationships(Li 2018). This multifaceted approach ensures the robust evaluation and refinement of genome assembly, underscoring its reliability and completeness.

The assembled genomes underwent thorough annotation encompassing repetitive elements, protein-coding genes, non-coding RNAs, and functional assignments. Repetitive Sequence Annotation: The identification of repetitive sequences, primarily transposable elements (TEs), was conducted using the Extensive de-novo TE Annotator (EDTA) (Ou et al. 2019). This tool integrates predictions from multiple sources to detect LTR retrotransposons, TIR transposons, MITEs, and Helitrons, facilitating the construction of a high-confidence, non-redundant TE library. Protein-Coding Gene Prediction and Integration: A tripartite approach was employed for gene prediction, integrating ab initio, homology-based, and transcriptome-based methods. Ab initio prediction was executed using Braker3(L et al. 2021). Homology-based predictions relied on GeMoMa(Keilwagen et al. 2016), which utilized data from closely related species. For transcriptome-based predictions, RNA-seq reads were aligned to the genome via Hisat2(Kim et al. 2015), and transcripts were assembled with Stringtie(Pertea et al. 2015). These assembled transcripts informed subsequent gene prediction using PASA(Haas et al. 2003). The gene models produced by these three

methodologies were synthesized into a consensus gene set through EvidenceModeler (EVM) (Haas et al. 2008), and this set underwent further refinement with PASA.

Non-Coding RNA Annotation: Non-coding RNAs, including tRNAs, rRNAs, snRNAs, and miRNAs, were identified based on their structural characteristics. tRNAs were detected using tRNAscan-SE (Lowe and Eddy 1997). The prediction of rRNAs, snRNAs, and snoRNAs involved searches against the Rfam database employing Infernal (Griffiths-Jones et al. 2005; Nawrocki and Eddy 2013).

Functional Annotation: Functional annotation of predicted protein sequences was performed using BLASTP searches across multiple public databases, such as NCBI Non-Redundant (NR), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Swiss-Prot. An E-value threshold of $< 10^{-5}$ was employed to ensure significant homology, providing insights into the functional roles of the annotated gene products.

A circular genome map was generated using Circos (Krzywinski et al. 2009) to visualize the distribution of gene density, repetitive sequence density, GC content, and synteny. To identify structural variations, whole-genome synteny and structural variation analysis between Gong 887S and Yuenuosimiao were performed using Syri (Goel et al. 2019), which detects inversions, translocations, and duplications.

Identification of QTNs in sequenced rice materials

In-depth analysis of re-sequencing data from six rice genotypes has led to the identification of Quantitative Trait Nucleotides (QTNs), encompassing single nucleotide polymorphisms (SNPs), insertions and deletions (Indels), and structural variants (SVs). These QTNs are associated with nine distinct trait categories: yield (64 QTNs), heading date (46 QTNs), secondary metabolism (17 QTNs), abiotic stress tolerance (38 QTNs), quality attributes (16 QTNs), biotic stress resistance (52 QTNs), seed morphology (23 QTNs), plant architecture (29 QTNs), and miscellaneous traits (17 QTNs) as detailed in Table S1. The identified QTNs were systematically classified according to three key criteria: (1) Genotype: categorized as wild-type (identical to the reference genome, Nipponbare), mutant (functional variant), or heterozygous; (2) Regulatory Direction: characterized by positive regulation (enhancing phenotypic values) or negative regulation (reducing phenotypic values); and (3) Allelic Effect: assessed as favorable (advantageous for yield, quality, or resistance enhancement), unfavorable (counterproductive to breeding objectives), or context-dependent (specific to particular breeding goals, such as optimizing heading date, fertility, plant height, or metabolic pathways).

Population-level comparison of QTNs

A comprehensive population-level comparison of Quantitative Trait Nucleotides (QTNs) was performed across all studied rice materials, with particular emphasis on genotype variations, regulatory direction, and allelic effects. Additionally, QTNs were identified within the extensive dataset from the 3,000 Rice Genomes Project (3K-RG). By integrating this data with our specific target materials, we were able to pinpoint key complementary loci that are crucial for advancing breeding efforts and enhancing desired phenotypic traits.

Indica-Japonica admixture analysis

The 3K-RG panel comprises two subspecies (indica and japonica) and several subpopulations, with the four most prominent being indica, tropical japonica, temperate japonica, and aus. The admixture analysis was performed as follows: (1) Haplotypes were constructed for the 3K-RG panel; (2) Normalized Allele Frequency (NAF) scores were calculated for the 3K-RG; (3) Haplotypes were constructed for the test materials; (4) The haplotypes of the test

materials were matched to the 3K-RG panel and assigned NAF scores; (5) The genome was divided into 100 kb windows (bins) for subpopulation classification; (6) An Indica-Japonica introgression genetic map (BinMap) was generated. Based on the BinMap derived from 3,734 bins, the subpopulation composition and genome-wide Indica-Japonica introgression proportions for each tested material were calculated. In this study, materials were classified into seven subpopulations: sp_ind (indica), sp_tej (temperate japonica), sp_trj (tropical japonica), sp_aus (aus), ss_INDx (indica-aus admixed), ss_JAPx (temperate-tropical japonica admixed), and admixed (indica-japonica admixed). These were further grouped into three major categories: Indica-type (XI), Japonica-type (GJ), and Admixed-type (Admix).

3. Results and analysis

3.1 Phenotypic characterization and genetic component analysis of Gong 887S

3.1.1 Phenotypic characterization of Gong 887S

Gong 887S is an indica-type, photo-thermo-sensitive genic male sterile (PTGMS) line that was developed through ten generations of systematic selection from a cross between 1892S (female parent) and 8-8S/93 – 11 (male parent), with 8-8S originating from Xiangling628S/Guangzhan63-4S (Fig. 2A). The germplasm resources, namely Xiangling628S, Guangzhan 63-4S, 1892S, and 93 – 11, were instrumental in providing the genetic foundation for the advancement of this novel germplasm (Li et al. 2015; Li et al. 2023; Zhang et al. 2025). Gong 887S is distinguished by its superior plant and leaf morphological traits, including a moderately compact plant type, long and erect flag leaves, dark green foliage and sheaths, and robust tillering capacity. In Changsha, Hunan Province, when sowing occurs in mid-to-late May, the line reaches heading in approximately 75 days. It attains an average height of 75 cm, characterized by sturdy culms and short uppermost three internodes, enhancing its resistance to lodging. Typically, Gong 887S yields an average of 17.0 productive panicles per plant, with a mean panicle length of 23.3 cm, 172 spikelets per panicle, a 1000-grain weight of 27.0 g, and a grain length-to-width ratio of 2.9 (Fig. 2B). Relative to its parental line 1892S, Gong 887S exhibits a markedly reduced growth duration, yet it also shows significant increases in plant height, number of productive panicles per plant, panicle length, spikelets per panicle, and 1000-grain weight (Figs. 2B, 2C).

Furthermore, Gong 887S demonstrates an exceptional stigma exertion rate, with a bilateral exertion rate of 35.2%, a unilateral exertion rate of 43.1%, and a cumulative exertion rate reaching 78.3%. These traits, combined with its favorable flowering habits, contribute to an outcrossing seed set rate exceeding 55% during hybrid seed production phases. In terms of grain quality, Gong 887S conforms to the Grade 1 standard outlined in the agricultural industry standard "Quality of Edible Rice Varieties" (NY/T 593–2013). Its specific quality metrics are as follows: a brown rice rate of 81%, milled rice rate of 72%, and head milled rice rate of 68.7%. The grain exhibits a length of 6.2 mm with a length-to-width ratio of 2.6. It has a chalky grain rate of 5% and a chalkiness degree of 1.0%. Additionally, its amylose content is 13.8%, with a gel consistency of 73 mm, an alkali spreading value of Grade 6, and transparency rated at Grade 1. These parameters underscore Gong 887S's superior grain quality, making it highly suitable for commercial rice production and consumption.

Leveraging Gong 887S as the female parent, over ten hybrid rice varieties have been successfully developed, including notable cultivars such as Gongliangyou Yuenuosimiao, Gongliangyou Jingguizhan, Gong8Liangyou 892, Gongliangyou Hesimiao, Gongliangyou17, and Gong8Liangyou 382. These varieties have achieved certification at

either the national or provincial level, underscoring their agronomic value and adaptability. Among these hybrids, Gongliangyou Yuenuosimiao (Gong887S/Yuenuosimiao) stands out as a premium cultivar for single-season rice cultivation in the middle and lower reaches of the Yangtze River. It has gained significant attention due to its remarkable traits, including high yield, superior grain quality, and resilience to high temperatures. By 2024, the cumulative cultivation area dedicated to this variety, as single-season rice, has exceeded 2 million mu (approximately 133,333 hectares), highlighting its widespread adoption and success in enhancing rice production in the region.

Moreover, Gongliangyou Yuenuosimiao has exhibited outstanding yield potential within the ratooning rice cropping system. In a 2022 field trial conducted in Zhangshu City, Jiangxi Province, this variety achieved a main crop yield of 13,545.00 kg·hm⁻², complemented by a ratoon crop yield of 8,610.00 kg·hm⁻². This culminated in an impressive total annual yield of 22,155.00 kg·hm⁻², underscoring its substantial high-yield advantage and its capacity to enhance overall productivity in diverse cropping systems.

3.1.2 High-quality genome assembly and gene annotation of Gong 887S and Yuenuosimiao

To explore the genetic foundation behind the exceptional traits of Gongliangyou Yuenuosimiao, such as high yield, superior grain quality, heat tolerance, and robust ratooning ability, comprehensive genome sequencing of its parental lines, Gong 887S and Yuenuosimiao, was conducted using a synergistic approach incorporating PacBio HiFi, next-generation sequencing (NGS), RNA-seq, and Hi-C technologies. The initial genome assembly produced 309 contigs for Gong 887S and 472 contigs for Yuenuosimiao, each exceeding 1 kb in length (Table 1). These initial assemblies were further refined: using Hi-C contact maps, the contigs for each genome were clustered, ordered, and oriented into pseudo chromosomes. For Gong 887S, the initial set of 309 contigs was successfully assembled into 12 pseudo chromosomes, comprising 20 contigs with an aggregate length of 392.79 Mb, which constituted 97.46% of the initial assembly. Likewise, Yuenuosimiao's 472 contigs were organized into 12 pseudo chromosomes, comprised of 16 contigs totaling 394.45 Mb, accounting for 96.13% of the initial assembly (Table S2).

Table 1

provides the statistical data for the genome assembly results of Gong 887S and Yue Nong Simiao. Table 2 presents the statistical results of gene function annotation for Gong 887S and Yue Nong Simiao. Table 1

Sample	CtgNum	CtgLen/bp	CtgN50/bp	CtgN90/bp	CtgMax/bp	GC/%
Gong887S	309	403,038,538	30,525,258	12,518,228	44,786,647	43.74
Yuenongsimiao	472	410,332,863	31,774,405	20,868,140	44,839,715	43.81
The metrics include tgNum, which represents the number of contigs greater than 1 Kb; CtgLen (bp), denoting the total length of contigs over 1 Kb; CtgN50 (bp), indicating the N50 length of the contigs; CtgN90 (bp), the N90 length of the contigs; CtgMax (bp), the length of the longest contig; and GC (%), the percentage of GC content.						

The quality of the genome assemblies was rigorously evaluated to ensure their reliability and accuracy. The Benchmarking Universal Single-Copy Orthologs (BUSCO) assessment indicated a completeness of over 98% for both Gong887S and Yuenuosimiao genomes, reflecting their high integrity. Additionally, the mapping rate of next-generation sequencing (NGS) reads back to their respective assembled genomes surpassed 97%, evidencing the precision of these assemblies (Table S3). Furthermore, the Quality Value (QV) for each chromosome exceeded 53.7, affirming the high base-level accuracy. The assemblies also demonstrated significant continuity, with a Longest Alignment Index (LAI) of 26.52 for Gong 887S and 27.12 for Yuenuosimiao. Both genomes exhibited similar profiles regarding gene density, repetitive sequence density, and GC content (Fig. 3 and Table S4). Following assembly, the

genomes of Gong 887S and Yuenuosimiao underwent comprehensive annotation, which included identifying repetitive sequences, protein-coding genes, non-coding RNAs, and conducting functional annotation. Functional analysis involved aligning predicted genes against public databases such as NR, GO, KEGG, and Swiss-Prot. In the Gong 887S genome, repetitive sequences constituted approximately 211 Mb, making up 55.65% of the total genome size, with Long Terminal Repeat (LTR) retrotransposons being the predominant elements at 40.14%. Similarly, in the Yuenuosimiao genome, repetitive sequences covered about 213 Mb, accounting for 54.05%, with LTR elements comprising 40.16% (Table S5). Protein-coding genes were predicted through an integrated approach combining homology-based, de novo, and transcriptome-based methods. Gene models were functionally annotated using BLAST searches against the NR, GO, KEGG, and SWISS-PROT databases. Gong887S's genome revealed 40,564 protein-coding genes, with 92.64% successfully annotated. In the Yuenuosimiao genome, 40,836 protein-coding genes were identified, with 94.74% receiving functional annotations. A BUSCO assessment of the annotated protein sets showed a completeness of over 95% for both genomes, underscoring the high quality and comprehensiveness of the gene annotations (Table 2).

Table 2

Annotation_Database	Gong887S		Yuenongsimiao	
	Annotated_Number	Annotated_Ratio	Annotated_Number	Annotated_Ratio
KEGG_Annotation	10928	24.91%	10,824	25.11%
Swissprot_Annotation	25809	58.83%	25,555	59.29%
nr_Annotation	40562	92.46%	40,068	92.96%
All_Annotated	40564	92.46%	40,836	94.74%

The columns include "Annotation Database," referring to the functional annotation databases used; "Annotated Number," which indicates the number of genes successfully annotated to each respective database; and "Annotated Ratio," representing the percentage of genes annotated to the database relative to the total number of genes.

3.1.3 Genetic component analysis of Gong 887S

A comparative analysis was conducted to examine the genetic components of Gong 887S by evaluating sequencing data from related lines: 1892S, GongxiangS, R9311, and Gong887S. This analysis involved counting the number of single nucleotide polymorphism (SNP) sites matching those in Gong 887S. Results showed genetic similarity percentages of 61.9% with 1892S, 82.3% with Gongxiang S, and 84.7% with R9311 (Table S5). To delve deeper into the subspecies composition, an indica-japonica component analysis was performed using data integrated with the 3K Rice Genomes Project dataset. Compared to Gongxiang S, Gong 887S possessed a higher proportion of tropical japonica (sp.trj), temperate japonica (sp.tej), and japonica-biased (JAPx) components. In contrast, 1892S displayed even greater levels of these japonica components than Gong 887S (Fig. 4A). A thorough examination of introgressed segments revealed that japonica-derived alleles in Gong 887S were primarily located on Chrs 1, 3, 7, 10, and 11, with large introgressed segments notably present on Chrs 1 and 3. Conversely, in 1892S, japonica introgressions were mainly found on Chrs 1, 4, 6, 8, 9, and 10, prominently featuring a large segment on Chr 4 followed by significant segments on Chrs 6, 9, and 10. This pattern explains its higher overall japonica component. For Gongxiang S, japonica introgressions were concentrated on Chrs 7 and 10, whereas R9311 exhibited introgressions primarily on Chr 7.

Tracing the origin of japonica segments in Gong 887S revealed that these alleles on Chrs 1, 2, 3, 5, 6, 8, and 10 were predominantly inherited from its parent Gongxiang S. The japonica components on Chr 7 and 9 mainly originated from the other parent, R9311. Additionally, genetic contributions from the female parent, 1892S, were detected to varying degrees. Notably, the sources of the large japonica segments on Chrs 1 and 3 likely trace back to ancestral parents such as Xiangling 628S and Guangzhan 63-4S (Fig. 4B). The breeding process for Gong 887S effectively integrated a significant portion of the japonica genetic background from 1892S and R9311, along with specific japonica traits from Xiangling 628S and Guangzhan 63-4S.

To further explore genetic differences between Gong 887S and other widely adopted hybrid rice male sterile lines, the study examined favorable Quantitative Trait Nucleotides (QTNs) or genes associated with yield, grain quality, and resistance to biotic and abiotic stresses across Gong 887S and eight other sterile lines: C815S, Longke 638S, AnnongS, Guangzhan 63-4S, Peiai 64S, Xiangling 628S, Y58S, and Zhu 1S. Findings indicated that the proportion of mutated QTNs shared between Gong 887S and the eight sterile lines ranged from 31.3% to 39.8%, with Xiangling 628S and Zhu 1S showing the highest proximity, nearly 40%. The proportion of heterozygous mutated QTNs spanned from 0.3% to 5.3%, with C815S exhibiting the highest at 5.3%. Meanwhile, wild-type QTNs ranged from 59.4% to 67.6%, with Peiai 64S having the highest percentage (Fig. 5A). Further classification of the mutated QTNs into favorable, unfavorable, and unknown categories revealed that across the eight sterile lines, favorable mutated QTNs constituted between 24.73% and 28.04%, while unfavorable mutated sites ranged from 38.8% to 42.5% (Fig. 5B).

A comprehensive comparative analysis of favorable alleles identified a total of 26 genes/loci consistently present across all nine male sterile lines. This shared genetic framework includes two yield-related genes, *LAX1* and *GS3*; six genes/loci associated with abiotic stress tolerance, such as *NRAT1* (contributing to aluminum tolerance), *qLTG3-1* (enhancing low-temperature germinability), and *OsHKT1*; five genes/loci conferring blast disease resistance, including *PiPR1*, *Pi25*, and *Pid2*; as well as six genes associated with plant architecture, such as *sd1*, *SCM2*, and *TIG1* (Fig. 5C, Table S6).

Moreover, a subset comparison between Gong 887S and the group comprising AnnongS, C815S, Guangzhan63-4S, and Longke638S revealed 32 favorable genes/loci shared among these lines. This subset includes three yield-enhancing genes: *LAX1* (linked to increased grain number), *OsCKX2* (also related to increased grain number), and *GS3* (associated with increased grain length); five genes pertinent to abiotic stress tolerance, including *NRAT1*, *qLTG3-1*, *OsHKT2;1* (which improves potassium utilization efficiency), *BET1* (conferring boron tolerance), and *OsHMA3* (involved in cadmium accumulation); five genes associated with blast resistance, such as *PiPR1*, *Pid2*, *Pi25*, and *LHCB5*; and seven genes influencing plant architecture, including *OsBRI1*, *sd1*, *TIG1*, *OsbHLH174*, and *DRO1* (Fig. 5D, Table S6).

Similarly, Gong 887S shares 33 favorable genes/loci with Peiai64S, Xiangling628S, Y58S, and Zhu1S. This collection encompasses four yield-related genes, including *LAX1*, *GS3*, *GNP1*, and *GW8*; seven genes/loci related to abiotic stress, such as *NRAT1*, *qLTG3-1*, and *OsHKT1*; seven genes/loci for blast resistance, including *PiPR1*, *Pi25*, and *Pid2*; and seven genes associated with plant architecture, such as *sd1*, *SCM2*, and *TIG1* (Fig. 3E, Table S6).

Overall, the analysis of all lines collectively delineated a core set of 26 favorable genes/loci ubiquitously present across all samples. This core set consists of two yield-related genes (*LAX1*, *GS3*); six genes/loci related to abiotic stress (including *NRAT1*, *qLTG3-1*, *OsHKT1*, among others); five genes/loci conferring blast resistance (such as *PiPR1*, *Pi25*, *Pid2*, etc.); and six genes influencing plant architecture (*sd1*, *SCM2*, *TIG1*, etc.) (Fig. 5F, Table S6).

3.2 Parental genetic composition analysis of Gong 887S-derived hybrid rice combinations

3.2.1 Genetic analysis of Gong 887S and its hybrid rice parents

A comprehensive analysis based on single nucleotide polymorphism (SNP) sites was performed using whole-genome sequencing data from six rice varieties: 1892S, GongxiangS, Gong 887S, Jingguizhan, R592, and Yuenongsimiao. The data revealed a total of 6,767,069 SNPs distributed across Chrs 1–12 among these six varieties (Fig. 6A). Genetic distance analysis indicated that Gong 887S demonstrated significant genetic divergence from 1892S, Jingguizhan, R592, and Yuenongsimiao. Notably, the greatest genetic distance was observed between Gong 887S and 1892S (0.96), followed by distances of 0.74 with R592 and 0.76 with Jingguizhan. In contrast, Gong 887S showed closer genetic proximity to its parental line, GongxiangS, with a distance of 0.21 (Fig. 6B). Further examination of the indica-japonica composition utilizing a panel of 4,584 genes revealed that Gong 887S, R592, Jingguizhan, and Yuenongsimiao were predominantly indica or indica-like, with these components constituting over 71% of their genomes. The proportion of japonica and japonica-like components ranged from 3.0% to 6.4%. Within this group, Gong 887S displayed the highest level of japonica and japonica-like introgression at 6.38%, followed by R592 at 4.26%, characterized by large introgressed segments on Chrs 2, 5, 7, and 8. Yuenongsimiao exhibited 3.05%, with substantial segments on Chrs 1, 2, 6, and 8, while Jingguizhan had 3.00%, with notable segments on Chr2, 7, and 8 (Figs. 6C, 6D; Table S7). The substantial japonica segments introgressed into Gong 887S, specifically on Chrs 1, 3, 10, and 11, played a crucial role in complementing the japonica components within the hybrid combinations Gongliangyou 592, Gongliangyou Jingguizhan, and Gongliangyou Yuenongsimiao. Additionally, japonica segments from R592 located on Chrs 5 and 11 further augmented the japonica composition of Gongliangyou 592. Similarly, japonica segments from Jingguizhan present on Chrs 2, 6, and 8, as well as those from Yuenongsimiao on Chrs 2, 6, 8, and 11, significantly enhanced the japonica composition within Gongliangyou Jingguizhan and Gongliangyou Yuenongsimiao, respectively. The complementary introgression of japonica and japonica-like components derived from the female parent Gong 887S and the male parents (Jingguizhan, R592, and Yuenongsimiao) across various chromosomes contributed to the formation of hybrid combinations Gongliangyou 592, Gongliangyou Jingguizhan, and Gongliangyou Yuenongsimiao. Noteworthy is the fact that Gongliangyou 592 exhibits the highest proportion of japonica and japonica-like components among these hybrids, which is hypothesized to enhance the high-yielding characteristics of these three hybrid combinations.

3.2.2 Genetic basis for superior agronomic traits in Gong 887S and its hybrid parents

An alignment analysis was conducted between favorable alleles from four parental rice lines (Gong 887S, Yuenongsimiao, Jingguizhan, and R592) and the 3K Rice Genomes Database. The hybrid variety Gongliangyou Yuenongsimiao was identified to possess a total of 68 advantageous alleles, including 55 alleles common to both parents, 3 unique to Gong 887S, and 10 exclusive to Yuenongsimiao. This composite allele structure is instrumental in endowing the hybrid with high yield, outstanding quality, and enhanced stress tolerance (Figs. 7A, 7D). The genetic configuration of Gongliangyou Yuenongsimiao comprises a broad spectrum of beneficial genes: 13 genes associated with yield (such as *LAX1*, *OsLG3*, *Gn1a*, *GS3*, *GNP1*, *GS5*, *GW5*, *GE*, *SGDP7*, *GW8*, *NOG1*, among others), 16 genes conferring resistance to abiotic stress (including *SKC1*, *LTG1*, *qLTG3-1*, *BET1*, *OsHKT2;1*, *OsHMA3*, *OsTPP7*, *qUVR-10*, *HAN1*, etc.), 14 genes for biotic stress resistance (such as *Bph3*, *BPH29*, *Pi21*, *PiPR1*, *Pid2*, *Pid3*, *Pia*, *OsCERK1*, *Xa1*, *Xa26*, *LHCB5*, *STV11*, and others), 10 genes related to seed morphology (e.g., *OsDfr*, *An-1*, *OsLOGL6*,

OsC1, *RAE2*, *GLA*, among others), 9 genes concerning plant architecture (including *OsBRI1*, *sd1*, *TAC3*, *SCM2*, *AP01*, *TIG1*, *OsHHLH174*, *DRO1*, etc.), and 3 genes affecting grain quality (such as *OsAAP6*, *Chalk5*, *OsGluA2*) (Fig. 7B, Table S6).

A total of fifty-two advantageous genes/loci are shared between Gong 887S and Jingguizhan. The hybrid Gongliangyou Jingguizhan further integrates six unique beneficial alleles from the female parent (Gong 887S) and fifteen from the male parent (Jingguizhan), resulting in a genetic combination that contributes to its superior agronomic performance (Figs. 7A and 7D, Table S6). These integrated loci are categorized functionally as follows: Thirteen loci are associated with yield, corresponding to twelve genes: *LAX1*, *OsLG3*, *Gn1a*, *GS3*, *GL3.2*, *GNP1*, *PTB1*, *GS5*, *GW5*, *GE*, *SGDP7*, and *GW8*, among others. Sixteen loci are linked to abiotic stress resistance, involving seven genes: *hbd2*, *qLTG3-1*, *BET1*, *OsHKT2;1*, *OsHMA3*, *OsTPP7*, and *qUVR-10*. Eighteen loci are associated with biotic stress resistance, derived from fourteen genes: *GH3-2*, *Bph3*, *BPH29*, *Pi21*, *PIPR1*, *Pi25*, *Pi-d2*, *OsCERK1*, *Pi56*, *Pia*, *Pi-ta*, *LHCB5*, *STV11*, and *Xa26*, among others. Ten loci regulate seed morphology, mapped to six genes: *Rd*, *An-1*, *An-2*, *OsC1*, *RAE2*, and *GLA*. Ten loci influence plant architecture, corresponding to ten genes: *D61*, *sd1*, *ILI3*, *SCM3*, *TAC3*, *SCM2*, *AP01*, *TIG1*, *OsHHLH174*, and *DRO1*. Lastly, three loci are associated with grain quality, each corresponding to a distinct gene: *OsAAP6*, *Chalk5*, and *OsGluA2* (Fig. 7B, Table S6).

A comparative analysis between Gong 887S and R592 identified fifty-four shared advantageous genes/loci. Additionally, the hybrid rice variety Gongliangyou 892 successfully incorporated four unique favorable alleles from Gong 887S and ten from R592. This combination of genes collectively contributes to the hybrid's outstanding properties (Figs. 7A, D, Table S6). The integrated loci are categorized as follows: Thirteen loci associated with yield, derived from ten genes including *LAX1*, *OsLG3*, *Gn1a*, *GS3*, *GNP1*, *GS5*, *OsEBS*, *SGDP7*, *GW8*, and *NOG1*; fifteen loci linked to abiotic stress tolerance, derived from nine genes such as *SKC1*, *HIS1*, *hbd2*, *qLTG3-1*, *BET1*, *OsHKT2;1*, *OsHMA3*, *OsTPP7*, and *qUVR-10*; fifteen loci conferring biotic stress resistance from ten genes like *Bph3*, *PIPR1*, *BPH29*, *Pi25*, *Pi-d2*, *Pi56*, *Pia*, *LHCB5*, *STV11*, and *Xa26*; ten loci regulating seed morphology from six genes including *Rd*, *An-1*, *An-2*, *OsC1*, *RAE2*, and *GLA*; eight loci related to plant architecture derived from six genes such as *D61*, *sd1*, *ILI3*, *SCM2*, *OsHHLH174*, and *DRO1*; and three loci pertaining to grain quality corresponding to the genes *OsAAP6*, *Chalk5*, and *OsGluA2* (Fig. 7B, Table S6).

In summary, the strategic integration of favorable genes from parental lines is an effective strategy for developing novel hybrid rice varieties with enhanced agronomic performance, encompassing high yield, superior grain quality, and increased resilience to both biotic and abiotic stresses.

3.3 Phenotypic characterization of the Gongliangyou series of hybrid rice varieties

The parental lines, Gong 887S, R592, Jingguizhan, and Yuenongsimiao, possess favorable alleles for several key yield-related genes, including *LAX1*, *LRK1*, *OsLG3*, *GS5*, *Gn1a/OsCKX2*, *GS3*, *GNP1*, *SGDP7/FZP*, and *GW8/OsSPL16*. As a result, the hybrid combinations derived from Gong 887S—specifically, Gongliangyou Yuenongsimiao, Gongliangyou Jingguizhan, and Gongliangyou 892—demonstrated high-yielding traits. In particular, during the regional trials conducted between 2019 and 2020 for the late-maturing indica rice group in the middle and lower reaches of the Yangtze River, Gongliangyou Yuenongsimiao achieved an average yield of 9736.35 kg·hm⁻² across 21 locations, marking a 2.6% increase over the control variety, Fengliangyou 4. Concurrently, Gongliangyou Jingguizhan was evaluated in the Jiangxi Xinggan Enterprise Rice Consortium regional trials, where it recorded an average yield of 9841.50 kg·hm⁻² across five locations, yielding an 8.1% increase over the control, Shenliangyou 5814, a difference that was statistically highly significant. Furthermore, in the 2022–2023 regional trials for the late-maturing indica

rice group in the middle and lower reaches of the Yangtze River, Gongliangyou 892 produced an average yield of 9714.00 kg·hm⁻² across 21 locations, surpassing the control, Fengliangyou 4, by 5.0% (Fig. 8A).

The parental lines, Gong 887S, R592, and Yuenongsimiao, possess favorable alleles for essential thermotolerance genes, including *TT2*, *TT3.1*, *TT3.2*, *TTL1*, *SLG1*, *SUS3*, and *HTH5*. To validate this genetic advantage, a controlled greenhouse experiment was conducted wherein plants were subjected to a 5-day heat stress treatment, characterized by daily maximum, minimum, and average temperatures of 40°C, 30°C, and 33.17°C, respectively. Under these conditions, Gongliangyou Yuenongsimiao demonstrated a mean seed-setting rate of 66.01%, significantly higher than that of the control variety, Fengliangyou 4 ($p < 0.001$) (Figs. 8B, D). Similarly, Gongliangyou 892 exhibited a mean seed-setting rate of 56.80%, also surpassing the control significantly ($p < 0.05$) (Figs. 8B, E). This enhanced thermotolerance was further confirmed through a multi-sowing field trial designed to expose plants to natural heat stress during the heading and flowering stages. During this period, ambient temperatures ranged from a maximum of 38.7–40.3°C to a minimum of 27.0–28.5°C, with a daily average of 32.0–32.4°C. Under these field conditions, both Gongliangyou Yuenongsimiao and Gongliangyou 892 maintained high mean seed-setting rates of 87.50% and 91.70%, respectively, both of which were significantly higher than those of the control (Fig. 8C). These results clearly demonstrate that Gongliangyou Yuenongsimiao and Gongliangyou 892 exhibit exceptional seed-setting stability under heat stress, indicating robust high-temperature tolerance.

Additionally, the parental lines—Gong 887S, R592, Jingguizhan, and Yuenongsimiao—carry favorable alleles for key grain quality genes, such as *OsAAP6*, *Chalk5*, *OsGluA2*, *Wxb*, and *ALKb*. Consequently, the hybrid combinations derived from these parents exhibit superior grain quality. Regional trial results indicate that Gongliangyou Yuenongsimiao and Gongliangyou Jingguizhan meet the Grade 2 rice quality standard as set by the Ministry of Agriculture, while Gongliangyou 892 achieves the Grade 3 standard, thereby highlighting the significant grain quality advantages of these three varieties. Specifically, Gongliangyou Yuenongsimiao demonstrated a head rice rate exceeding 70%, while the other two varieties, Gongliangyou Jingguizhan and Gongliangyou 892, both achieved head rice rates above 56.0%. Gongliangyou 892 exhibited an exceptionally low chalkiness degree (*Chalk5*) of only 1.9%, reflecting excellent grain appearance quality. Furthermore, the amylose content (*Wxb*) for all three varieties was found within the optimal range of 15% to 17%, ensuring desirable textural properties. Notably, Gongliangyou Jingguizhan displayed a gel consistency (*ALKb*) of 79 mm and a grain length-to-width ratio of 3.9, categorizing it as a long-grain, premium-quality rice (Fig. 9).

3.4 Genomic structural variation analysis between Gong 887S and Yuenongsimiao

Using the genome of Gong 887S as the reference and that of Yuenongsimiao as the query, a comprehensive analysis of synteny and structural variations (SVs) was conducted employing SyRI software. The analysis revealed a high degree of genomic synteny, with 99.5% of the genomes being collinear. Despite this overall collinearity, significant structural variations were identified, including inversions on Chrs 7 and 11, translocations on Chrs 4, 9, and 11, and duplications on Chrs 2, 4, 7, 9, and 11 (Fig. 10). Further investigation uncovered a total of 456,886 single nucleotide polymorphisms (SNPs) and 89,839 insertions and deletions (INDELs) between the two genomes. The distribution of these variants across Chrs was as follows: 284 variant genes on Chr 1, 82 on Chr 2, 137 on Chr 3, 133 on Chr 4, 31 on Chr 5, 48 on Chr 6, 144 on Chr 7, 194 on Chr 8, 28 on Chr 9, 161 on Chr 10, 21 on Chr 11, and 24 on Chr 12 (Table S8). Among these variant genes, several are associated with key agronomic traits. These include genes involved in heat tolerance during the heading stage, such as *SLG1* (*LOC_Os12g39840*), *OsTT1* (*LOC_Os03g26970*), *TT3.1* (*LOC_Os03g49900*), *TT3.2* (*LOC_Os03g49940*), *OsHsfA2c* (*LOC_Os10g28340*), *OsHSP17.0*

(*LOC_Os01g04380*), *NAL11* (*LOC_Os07g09450*), and *OsIFL* (*LOC_Os01g18840*); yield-related genes, such as *OsAP2-39* (*LOC_Os04g52090*), *PSR1* (*LOC_Os01g25484*), *OsNRT1.1B* (*LOC_Os10g40600*), *OsLa1* (*LOC_Os04g42010*), *OsTCP14* (*LOC_Os07g05720*), *SNG1* (*LOC_Os01g71320*), *FLO12* (*LOC_Os10g25130*), *OsPQT3* (*LOC_Os10g29560*), *OsCesA7* (*LOC_Os10g32980*), and *FLO16* (*LOC_Os10g33800*); and rice quality genes, such as *SSG4* (*LOC_Os01g08420*), *qGW8* (*LOC_Os08g41940*), *SNG1* (*LOC_Os01g71320*), *OsAlaAT1* (*LOC_Os10g25130*), *OsBURP16* (*LOC_Os10g26940*), *OsMDH10.1* (*LOC_Os10g33800*), *Du1* (*LOC_Os10g35550*), *OsPK3* (*LOC_Os04g58110*), and *OsSSIIIa* (*LOC_Os08g09230*).

Therefore, the synergistic effects of these multiple genes collectively confer the hybrid variety Gongliangyou Yuenongsimiao with its superior characteristics, encompassing high yield, premium grain quality, enhanced tolerance to both high and low temperatures during the heading stage, and robust ratooning ability.

4 Discussion

4.1 The genetic basis and breeding advantages of Gong 887S

The development of Gong 887S serves as a paradigm of the effective integration of traditional and molecular breeding methodologies. Its genetic composition epitomizes the benefits of a multi-parental pyramiding strategy, wherein the selection of parental lines—1892S, Xiangling 628S, Guangzhan 63-4S, and 93 – 11—was based not only on superior phenotypic traits but, more critically, on genomic complementarity. Specifically, the paternal parent 93 – 11 endowed the progeny with high-yield genes such as *Gn1a* and *GS3*, while Xiangling 628S contributed thermotolerance-associated genes, including *TT3.1* and *TTL1*. This gene pyramiding strategy addresses the inherent limitations of enhancing single traits in isolation (Fig. 4, Fig. 5). Through meticulous breeding and selection processes involving maternal lines (1892S, Xiangling 628S, Guangzhan 63-4S) alongside the paternal line 93 – 11, Gong 887S was developed with a japonica genetic introgression of 6.38%, whereas its indica genetic makeup comprises approximately 71% (Fig. 2). The japonica ancestry proportion in Gong 887S (6.38%) significantly exceeds that of conventional indica varieties, which typically exhibit < 3% japonica introgression. This enhanced intersubspecific hybridization suggests promising potential for trait improvement in indica rice. However, further investigation is required to elucidate the trade-off between yield and grain quality under high-temperature stress, potentially through CRISPR/Cas9-mediated editing of candidate loci (e.g., *TT3.1*, *Chalk5*) in elite genetic backgrounds. Chromosome introgression analysis identified that pivotal chromosomes, such as Chrs1, 3, 7, and 10, harbor japonica segments. These genomic regions are strongly linked with yield enhancement, lodging resistance, and stress tolerance. For example, *OsLG3* located on Chr3, a positive modulator of grain length, augments rice yield without detriment to grain quality. Concurrently, it enhances drought tolerance by facilitating the scavenging of reactive oxygen species (ROS) (Xiong et al. 2018; Yu et al. 2017). Similarly, *Gn1a* on Chr7 significantly influences panicle development, dictating grain number per panicle through the maintenance of cytokinin homeostasis in young panicles. It also reinforces lodging resistance by augmenting stem thickness and promoting crown root development (Guo et al. 2020; Rong et al. 2022). Gong 887S encompasses between 56 and 83 favorable quantitative trait nucleotides (QTNs), incorporating 26 functional loci aligned with core genes (Fig. 5). Within these, mutations in *LAX1* and *GS3* genes notably increased tiller number and grain length, with reported enhancements of 18% in tiller number and a 12% extension in grain length (Liu et al. 2018; Oikawa and Kyozyuka 2009). These genetic features are likely pivotal factors underpinning the high-yield and superior grain quality attributes of hybrid rice combinations derived from Gong 887S.

The impact of heat stress on pollen abortion in rice is mediated through multiple pathways, including those involving heat shock proteins (HSPs), the reactive oxygen species (ROS) scavenging system, and hormonal signaling. Within the genome of Gong 887S, we identified key thermotolerance-associated genes such as *TT1*, *TT3.1*, and *HTG3*, whose encoded proteins play pivotal roles in this process (Fig. 7, Table S6). Specifically, *TT1* modulates the activity of the 26S proteasome, thereby ensuring stable pollen development under elevated temperatures (Li et al. 2015). The cytoplasmic membrane-localized E3 ubiquitin ligase protein, *TT3.1*, responds to heat stress by translocating from the plasma membrane to multivesicular bodies, mitigating chloroplast damage caused by the accumulation of *TT3.2* in chloroplasts during heat stress and consequently enhancing the plant's thermotolerance (Zhang et al. 2022). Additionally, a MITE (Miniature Inverted-repeat Transposable Element) insertion in the promoter region of *HTG3* amplifies its expression during the flowering stage, resulting in a 35% increase in superoxide dismutase (SOD) enzyme activity (Wu et al. 2022). Furthermore, enhanced H3K4me3 modification in the promoter region of *HSP101* recruits the transcription factor *OsHSFA2d*, establishing a heat stress memory effect (Lin et al. 2014; Liu et al. 2009). Collectively, these sophisticated molecular mechanisms facilitate the maintenance of a seed-setting rate exceeding 85.0% in hybrid rice combinations derived from Gong 887S, even when subjected to high temperatures of 35°C (Fig. 8).

4.2 Innovations in rice molecular design breeding driven by genomic technologies

Recent advancements in modern rice breeding, particularly the significant progress made in genomics, have paved new paths for molecular design breeding (Li et al. 2024; Li et al. 2023; Wang et al. 2021; Zhang et al. 2019). Utilizing whole-genome sequencing, long-read sequencing, and Hi-C chromatin conformation capture technologies, we successfully developed high-quality, gapless genome assemblies for Gong 887S and Yuenong Simiao (Fig. 3). These cutting-edge technologies have identified numerous key structural variants (SVs) and their associated functional genes, effectively ushering rice genomics into a "gapless era" (Li et al. 2024; Huang et al. 2024; Jiang et al. 2023; Li et al. 2023; Zhang et al. 2025). Through the implementation of Telomere-to-Telomere (T2T) assembly, the Gong 887S genome revealed 12 major SVs linked to crucial agronomic traits (Fig. 10). Notably, a 2.1 Mb inversion on Chr 5 resulted in the formation of a fusion gene between *OsAAP6* and an adjacent transposon, thereby enhancing the protein content of rice grains (Peng et al. 2014). Similarly, on Chr11, a 3.4 Mb deletion reduced the physical distance between the *Chalk5* gene and its repressor, *OsMADS7*, thereby decreasing chalkiness through chromatin looping (Li et al. 2014; Zhang et al. 2018). Additionally, comparative genomic analysis uncovered a tandem repeat (TR) sequence unique to Gong 887S located upstream of the *GW8* gene. This TR sequence enhances promoter activity via a G-quadruplex structure, leading to a 0.3 mm increase in grain width. The synergistic effects of these SVs, alongside SNPs and Indels, construct a complex regulatory network, offering precise targets for molecular design breeding.

To enhance genome continuity and completeness, the integration of long-read sequencing technologies (PacBio HiFi, ONT) with Hi-C chromatin conformation capture achieved a Contig N50 of 25.7 Mb and a BUSCO completeness score of 98.3% for the Gong 887S genome. This approach notably resolved the highly repetitive CentO satellite sequences within the centromeric regions, providing essential insights for research into chromosome segregation mechanisms (Table 1). Third-generation sequencing technologies have demonstrated significant advantages in detecting complex structural variations (SVs). For example, a 3.2 Mb tandem repeat on Chr 4 of Yuenong Simiao encompasses genes such as *OsHKT2;1*, associated with salt stress response (H et al. 2021), and *OsNR2*, linked to both salt tolerance and nitrogen use efficiency (Gao et al. 2019; Yi et al. 2022). Comparative analysis with parental genomes revealed that this repeat originated from an introgressed japonica

segment, with its copy number variation (CNV) contributing to enhanced nitrogen use efficiency (NUE) (Fig. 6). Additionally, a single nucleotide polymorphism (SNP) in the intron of the *GW8/OsSPL16* gene in Yuenong Simiao promotes increased grain width. The hybridization between Gong 887S and Yuenong Simiao resulted in a synergistic “multi-grain + large-grain” effect, leading to an impressive average yield of 9,736 kg/ha in regional trials. Regarding grain quality, a non-synonymous mutation in the coding region of the *Chalk5* gene in Gong 887S reduced chalkiness to 1.0%. Concurrently, a splice-site variant in the *Wxb* gene intron in Yuenong Simiao reduced amylose content to 15.8%. This allelic complementarity resulted in a high head rice recovery rate of 68.7% in the hybrid. In terms of heat tolerance, the synergistic action of the *TT3.1* and *HTG3* genes from both parents is significant, enabling the hybrid to maintain a seed-setting rate of 87.5% under heat stress conditions at 35°C.

4.3 Molecular dissection of high-yield, high-quality, and high-temperature tolerance traits in the Gongliangyou hybrid rice varieties

The Gongliangyou hybrid rice varieties, comprising Gongliangyou Yuenongsimiao, Gongliangyou Jingguizhan, and Gongliangyou 892, have achieved synergistic enhancements in high yield, superior quality, and high-temperature tolerance by pyramiding elite alleles from the female parent Gong 887S and their respective male parents. Comprehensive whole-genome sequencing and functional annotation identified that Gong 887S contributes favorable alleles for yield-related genes such as *LAX1*, *GS3*, and *GNP1* (Fig. 7, Supplementary Table 7). Additionally, the strategic introgression of japonica components, particularly temperate japonica segments on Chrs 1 and 3, significantly augmented panicle grain number and grain plumpness (Fig. 4). The male parents—Yuenongsimiao, Jingguizhan, and R592—introduced advantageous mutations in genes like *GW8*, *OsCKX2*, and *PTB1* (Fig. 7), further enhancing the yield potential of the hybrid combinations. Notably, the *PTB1* gene, specific to Jingguizhan, increases photosynthate transport capacity by improving phloem loading efficiency, complementing the grain size-regulating function of the *GE* gene (*CYP78A*) from Gong 887S (F et al. 2015; Li et al. 2013). Furthermore, Gongliangyou Yuenongsimiao demonstrated a yield increase ranging from 2.6% to 8.1% over the control in regional trials (Fig. 4A), a trait attributed to the synergistic action of *LAX1*-regulated spikelet differentiation efficiency and *OsCKX2*-mediated cytokinin metabolism.

The molecular foundation for high-temperature tolerance in the Gongliangyou series is underpinned by a polygenic interaction network. Both Gong 887S and the male parents possess superior haplotypes of thermotolerance genes, including *TT3.1*, *TT3.2*, and *HTG3* (Fig. 7 and Table S6). Introgressed japonica segments on Chrs 1 and 7 of Gong 887S house the *TT3.1* and *SRL10* genes, while the *HTG3* gene, exclusive to the male parent R592, facilitates normal pollen development in Gongliangyou 892 under heat stress conditions. In the hybrids, co-expression of *SUS3* and *HTH5* markedly increased the efficiency of endosperm starch synthesis, maintaining a seed-setting rate of 56.80% even under 40°C stress (Fig. 8). A dominant complementary effect of the *TT2* gene was observed between Gong 887S and Yuenongsimiao, accounting for the stable yield performance of Gongliangyou Yuenongsimiao across diverse ecological zones. The dual regulatory network involving *GW8/OsSPL16* and *GS5* significantly increased the 1000-grain weight by coordinating the regulation of grain width and length (Fig. 8). This multi-gene synergistic enhancement mechanism effectively overcomes the conventional “trade-off” effect among yield components, offering a paradigm for constructing high-yield gene modules. Greenhouse and field trials further demonstrated that Gongliangyou Yuenongsimiao exhibited a significantly higher seed-setting rate than the control under 40°C stress, showcasing exceptional adaptability to high temperatures during the heading stage (Fig. 8), closely linked to this polygenic stacking effect. Additionally, analysis of genomic structural variations revealed a promoter insertion

mutation at the *TT2* locus in Gong 887S (Fig. 10), which may enhance its transcriptional activity and thereby improve the thermotolerance of its hybrid combinations.

With respect to grain quality, the Gongliangyou series achieves synergistic optimization of eating and cooking quality (ECQ) and appearance through the precise pyramiding of multiple quality-related genes. Favorable alleles of genes such as *Chalk5*, *Wxb*, and *ALKb* significantly reduce chalkiness (e.g., to a mere 1.9% in Gongliangyou 892) and optimize amylose content (15%-17%) and gel consistency (Fig. 7, Table S6). An A/G mutation in the *Chalk5* within the Gong 887S genome results in a denser arrangement of endosperm cells, further reducing chalkiness to 1.0%. The specific *Wxb* allele from male parents like Jingguizhan stabilizes the amylose content within the optimal range of 15.2% to 16.8% by modulating the chain length distribution of amylopectin. A single nucleotide polymorphism in the *ALKb* gene (*SSIIa*) of Gongliangyou Yuenongsimiao reduces amylose content to 15%-17%, aligning with the starch properties of high-quality indica rice (Li et al. 2014). Variations in amino acid transporter genes, such as *OsAAP6*, also significantly enhance grain protein content (Peng et al. 2014). Moreover, the moderate introgression of japonica components (6.38% in Gong 887S) potentially enhances both appearance and eating quality by regulating the expression of starch synthase genes (Fig. 4). Genomic comparisons have identified a non-synonymous mutation in *OsAAP6* in Gong 887S, which significantly reduces glutelin accumulation, while upregulation of *OsGluA2* in Yuenongsimiao improves cooked rice extensibility. This quality gene combination model, characterized by "low chalkiness, moderate amylose, and high gel consistency," establishes a novel paradigm for the molecular design breeding of high-quality rice.

5. Summary

This study systematically dissects the molecular mechanisms underlying high yield, superior grain quality, and heat tolerance in the thermo-sensitive genic male sterile (TGMS) line Gong 887S and its hybrid rice combinations. Through whole-genome sequencing, functional gene analysis, and multi-location phenotyping, we reveal that Gong 887S exhibits a 6.38% japonica introgression, significantly higher than conventional indica varieties, with key introgressed regions on Chrs 1, 3, and 7 harboring critical genes such as *OsLG3*, *Gn1a*, and *TT3.1*, which regulate grain number, grain length, and thermotolerance. Hybrid combinations (e.g., Gongliangyou Yuenong Simiao, Gongliangyou Jingguizhan, and Gong 8 Liangyou 892) were engineered to pyramid 56–83 favorable loci, integrating genes like *LAX1*, *GS3*, *TT1*, and *Chalk5*, resulting in yield increases of 2.6%–8.1% over controls. Under 40°C heat stress, these hybrids maintained seed-setting rates of 56.80%–66.01% in greenhouse trials and > 85% in field conditions, surpassing controls. Grain quality metrics—head rice rate (68.7%–72.0%), chalkiness (1.0%–1.9%), and amylose content (15%–17%)—exceeded premium standards. Structural variations, including an inversion on Chr7 and a deletion on Chr11, modulated *OsAAP6* and *Chalk5* expression, enhancing protein content and reducing chalkiness.

Declarations

Declaration of competing interest

The author declares no competing interests.

Author Contribution

Jianghui Yu performed major experiments and write the manuscript, Qiuping Yan, Yanyan Deng, Xingqiang Sun, Qinghua Yang, Tianyi Liao, Fuli Kang, Yunpeng Peng, Guowen Zhang, Chuanqing Sun performed the research study. Yangjun Zhang and Jianghui Yu conceived and supervised the study, funding acquisition, and revised the manuscript. All authors approved the final version of the manuscript.

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Figures

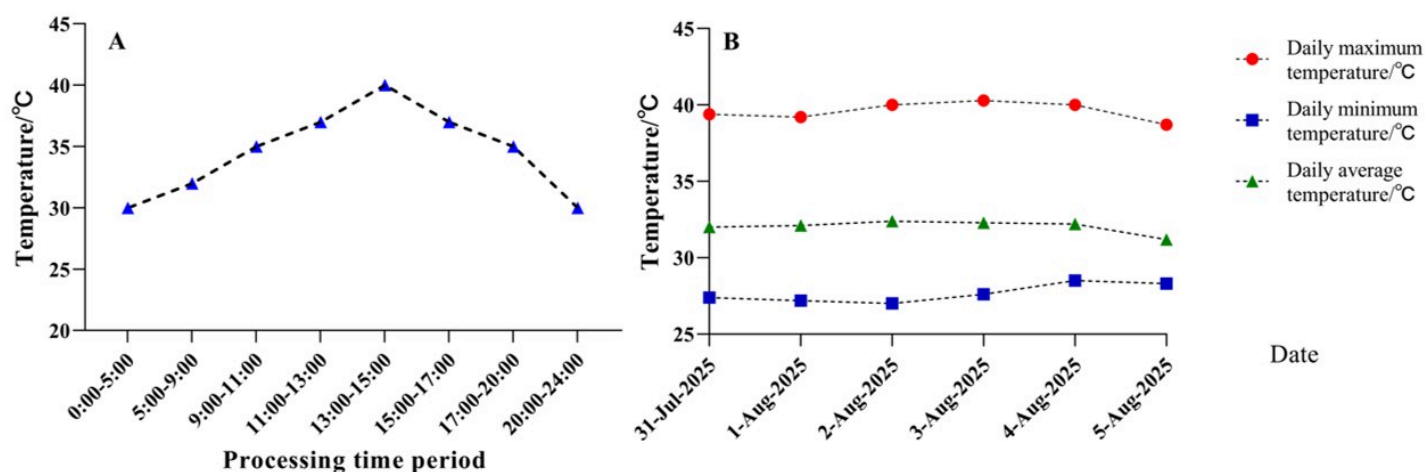


Figure 1

illustrates the temperature conditions during high-temperature stress periods in both greenhouse and field settings. (A) represents the temperature settings for the artificial climate chamber during the heading and flowering stages of the test materials, subjected to high temperature treatment for five consecutive days. The daily maximum temperature was set at 40°C, with a minimum of 30°C and an average of 33.17°C, while maintaining a relative humidity of 65%. The light cycle was from 8:00 AM to 8:00 PM, followed by darkness until 8:00 AM the next day, with a light intensity of 1200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$. (B) depicts the maximum, minimum, and average daily temperatures encountered during an extreme high-temperature stress event in the field from July 31 to August 6, coinciding with the heading and flowering stages of the test materials. Field temperature and humidity data were automatically recorded every five minutes using a ZOGLAB Microsystem Co. Ltd device. The field's daily maximum temperatures

ranged from 38.7°C to 40.3°C, minimum temperatures ranged from 27.0°C to 28.5°C, and average temperatures were between 32.0°C and 32.4°C, with a relative humidity of 86.7%.

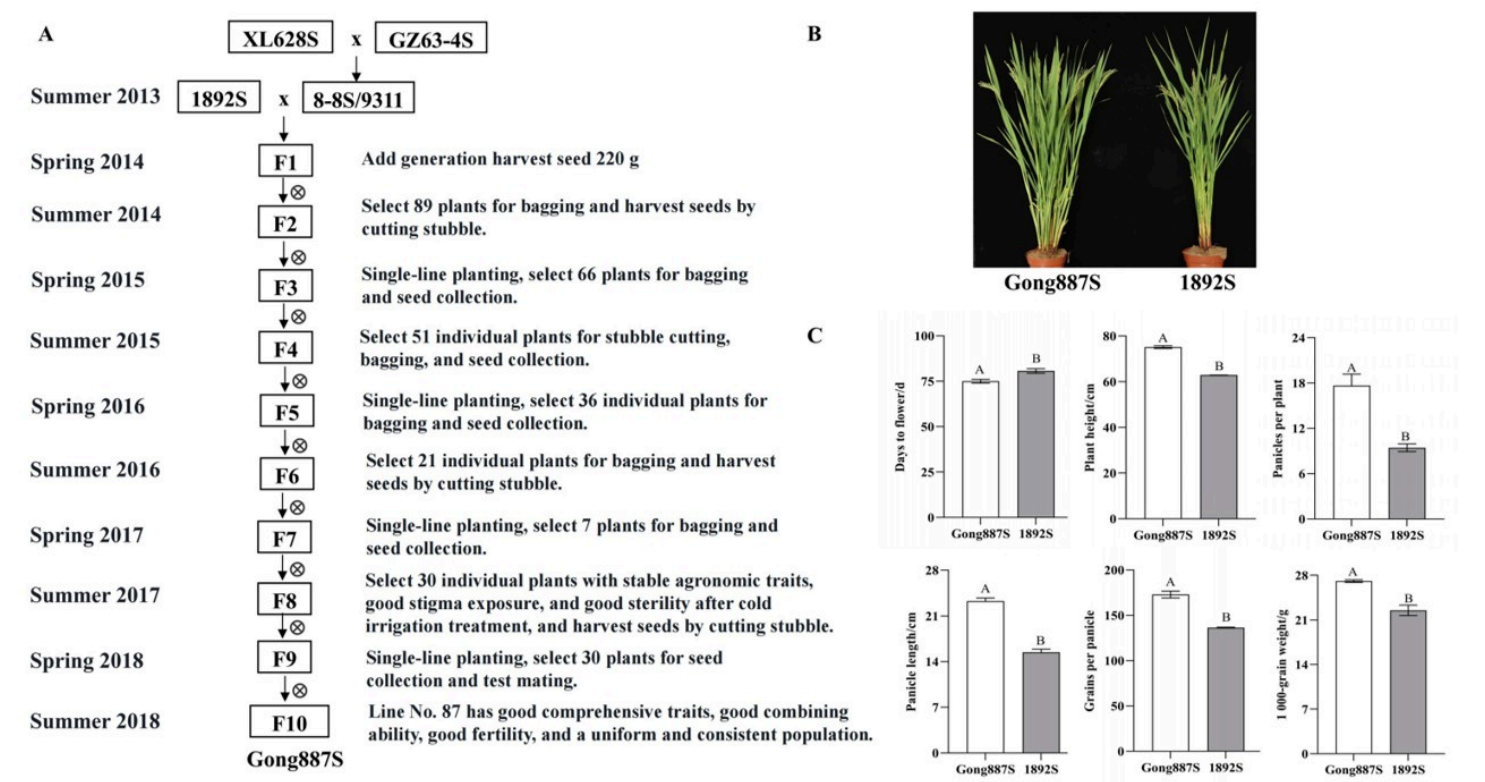


Figure 2

depicts the breeding process and agronomic traits of Gong 887S. (A) outlines the systematic breeding process of Gong 887S over ten generations. (B) provides a comparative analysis of the plant and leaf morphology between Gong 887S and its parental line, 1892S. (C) offers a comparative analysis of differences in various agronomic traits between Gong 887S and its parental line, 1892S, including growth duration, plant height, number of effective panicles per plant, panicle length, number of spikelets per panicle, and thousand-grain weight.

Gong887S

Yuenongsimiao

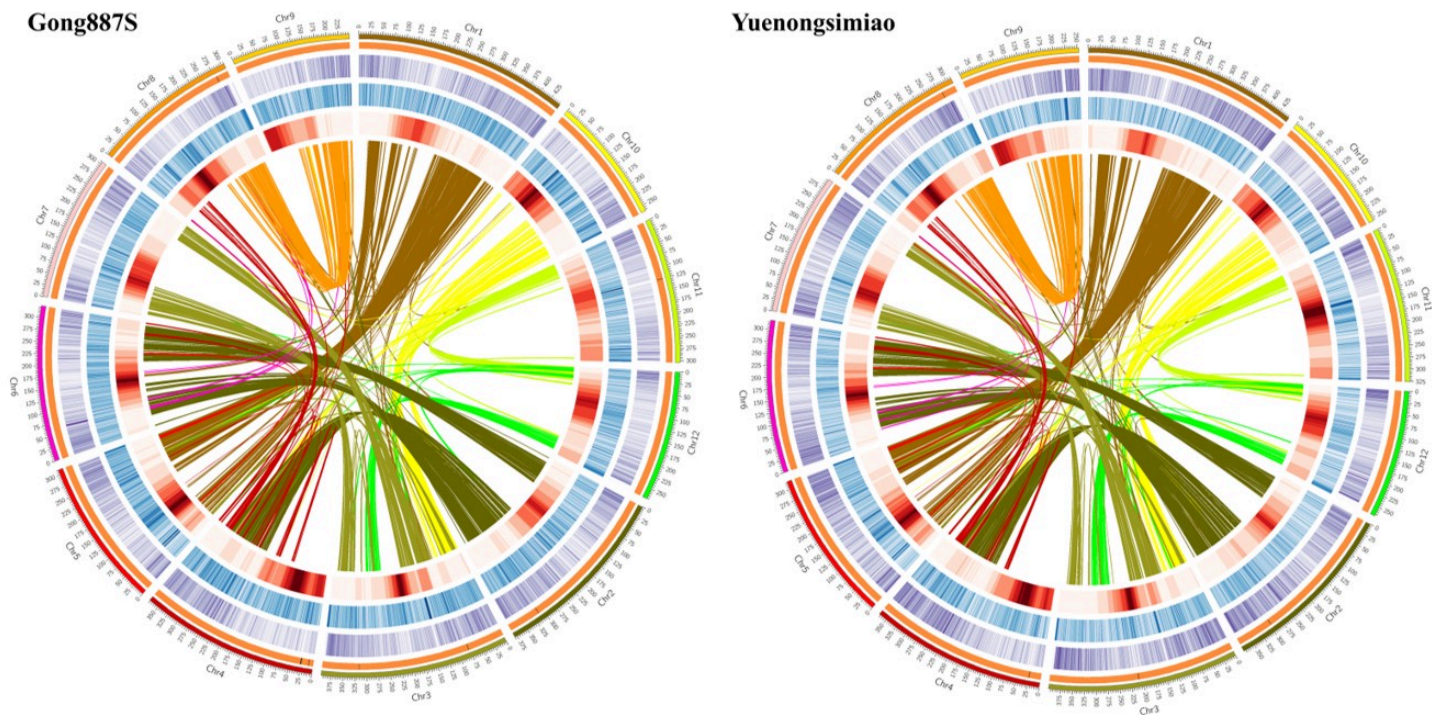


Figure 3

illustrates the genome assembly of Gong 887S and Yue nong simiao. In this circular plot, the golden-yellow color represents the karyotype of the chromosomes, while thin black lines denote gaps in the assembly. The purple heatmap indicates gene density, the blue heatmap reflects GC content, and the red heatmap shows the distribution of repetitive sequences. The innermost layer of the plot displays the syntenic relationships.

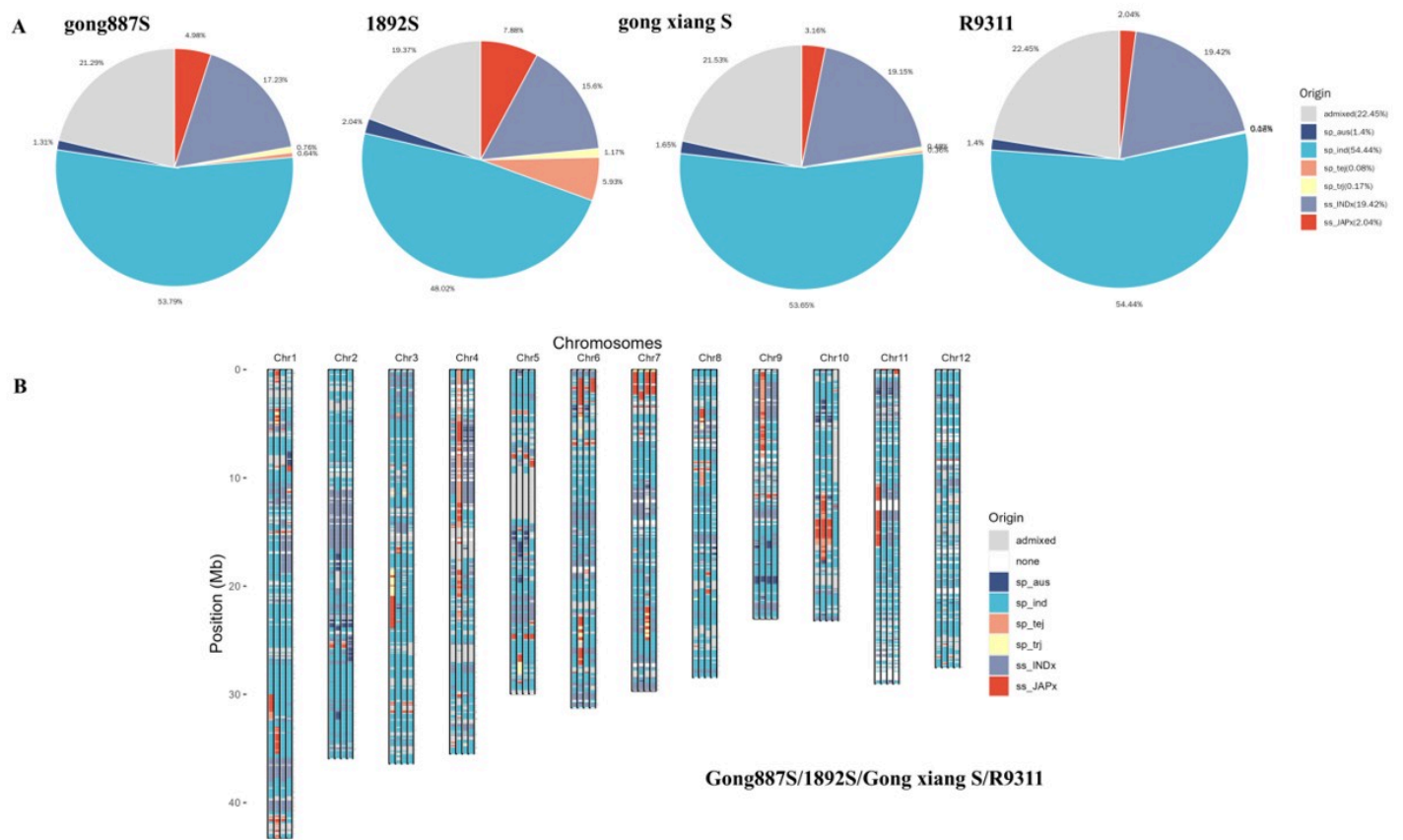


Figure 4

presents a comparative analysis of the genetic information between Gong 887S, 1892S, Gong Xiang S, and R9311.

(A) shows the proportion of components related to tropical japonica (sp_trj), temperate japonica (sp_trj), and intermediate japonica (JAPx) for Gong 887S, 1892S, Gong Xiang S, and R9311. (B) compares the introgression of indica and japonica components across different chromosomes among Gong 887S, 1892S, Gong Xiang S, and R9311.

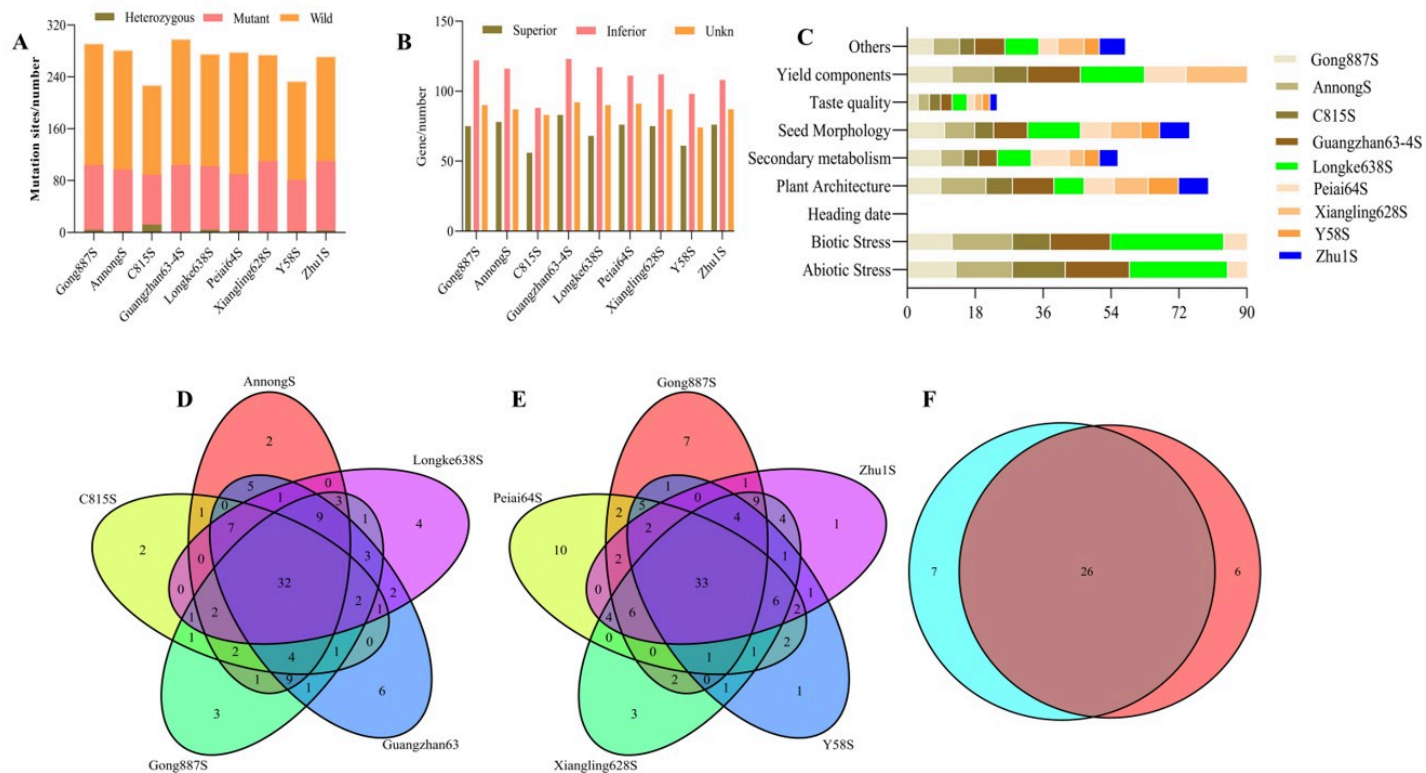


Figure 5

provides a comparative analysis of the gene profiles carried by nine male-sterile lines. (A) illustrates the number of mutated quantitative trait nucleotides (QTNs) in Gong 887S, C815S, Longke 638S, Annong S, Guangzhan 63-4S, Peiai 64S, Xiangling 628S, Y58S, and Zhu1S. (B) categorizes the QTNs in these nine sterile lines into favorable, unfavorable, and uncertain mutations. (C) examines the mutations related to genes affecting yield, abiotic stress tolerance, blast disease resistance, and plant architecture among the nine lines. (D) highlights the shared favorable gene mutations between Gong 887S and Annong S, C815S, Guangzhan 63-4S, and Longke 638S. (E) focuses on the common favorable gene mutations between Gong 887S and Peiai 64S, Xiangling 628S, Y58S, and Zhu1S. (F) presents the shared favorable gene mutations across all nine male-sterile lines.

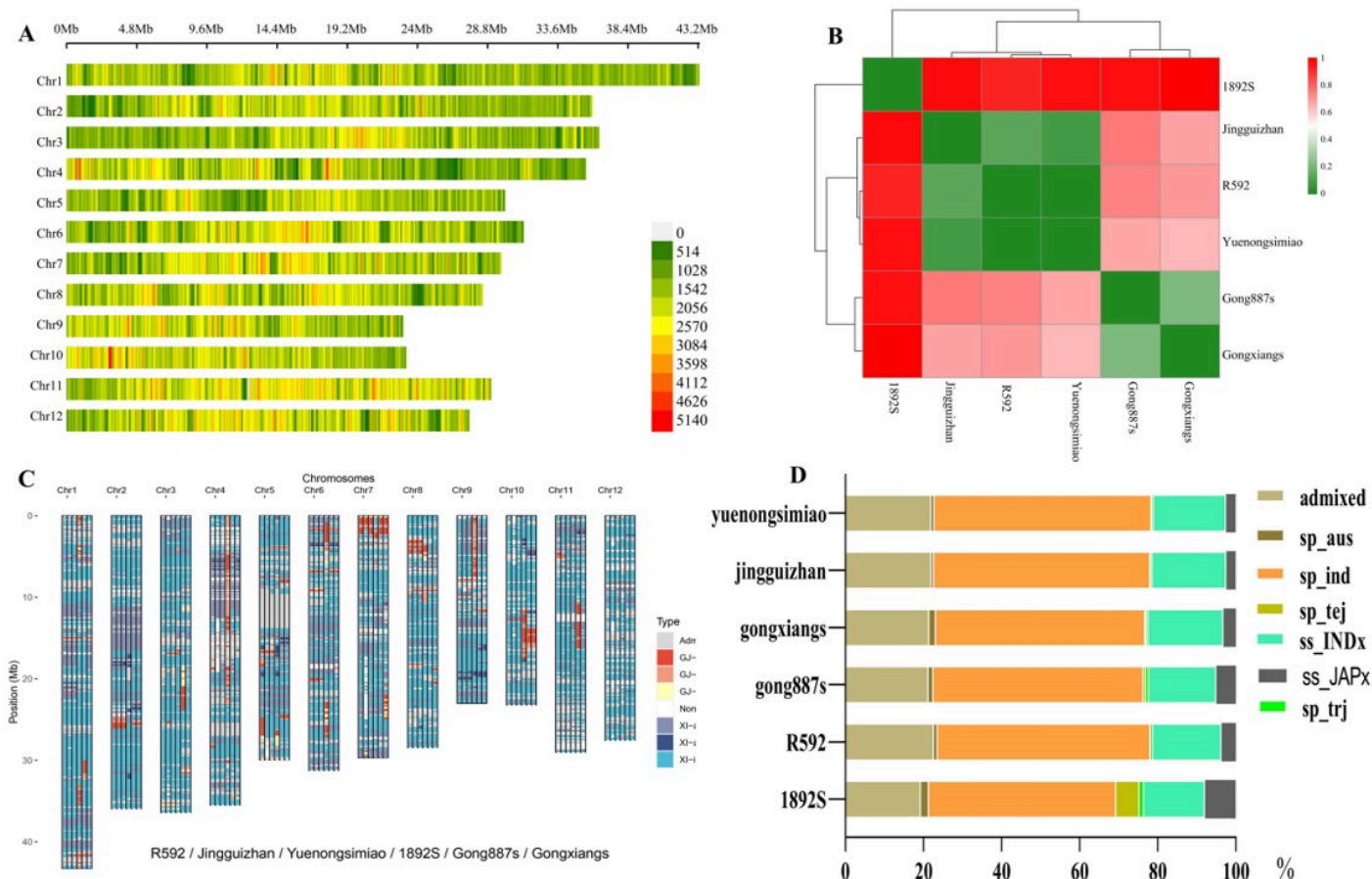


Figure 6

presents a comprehensive analysis of six rice varieties: 1892S, Gong Xiang S, Gong 887S, Jing Guizhan, R592, and Yue Nong Simiao. (A) depicts the marker density distribution of single nucleotide polymorphisms (SNPs) across different chromosomes for these six varieties, based on genome sequencing data. This distribution is shown using a sliding window approach with a size of 100 kb, where the number of variations per window is calculated and represented in the legend with varying colors indicating different quantities of variation. (B) provides a genetic distance analysis among the six sequenced rice varieties, presented as a heatmap of genetic distance clustering. The color intensity represents the magnitude of genetic distance—redder shades indicate greater distances, while greener shades depict smaller distances, clustering closely related materials together. (C) examines the introgression of *sp_tej*, *sp_trj*, and JAPx across different chromosomes for the six sequenced varieties. (D) showcases the distribution proportions of *sp_tej*, *sp_trj*, and JAPx among the six sequenced rice varieties.

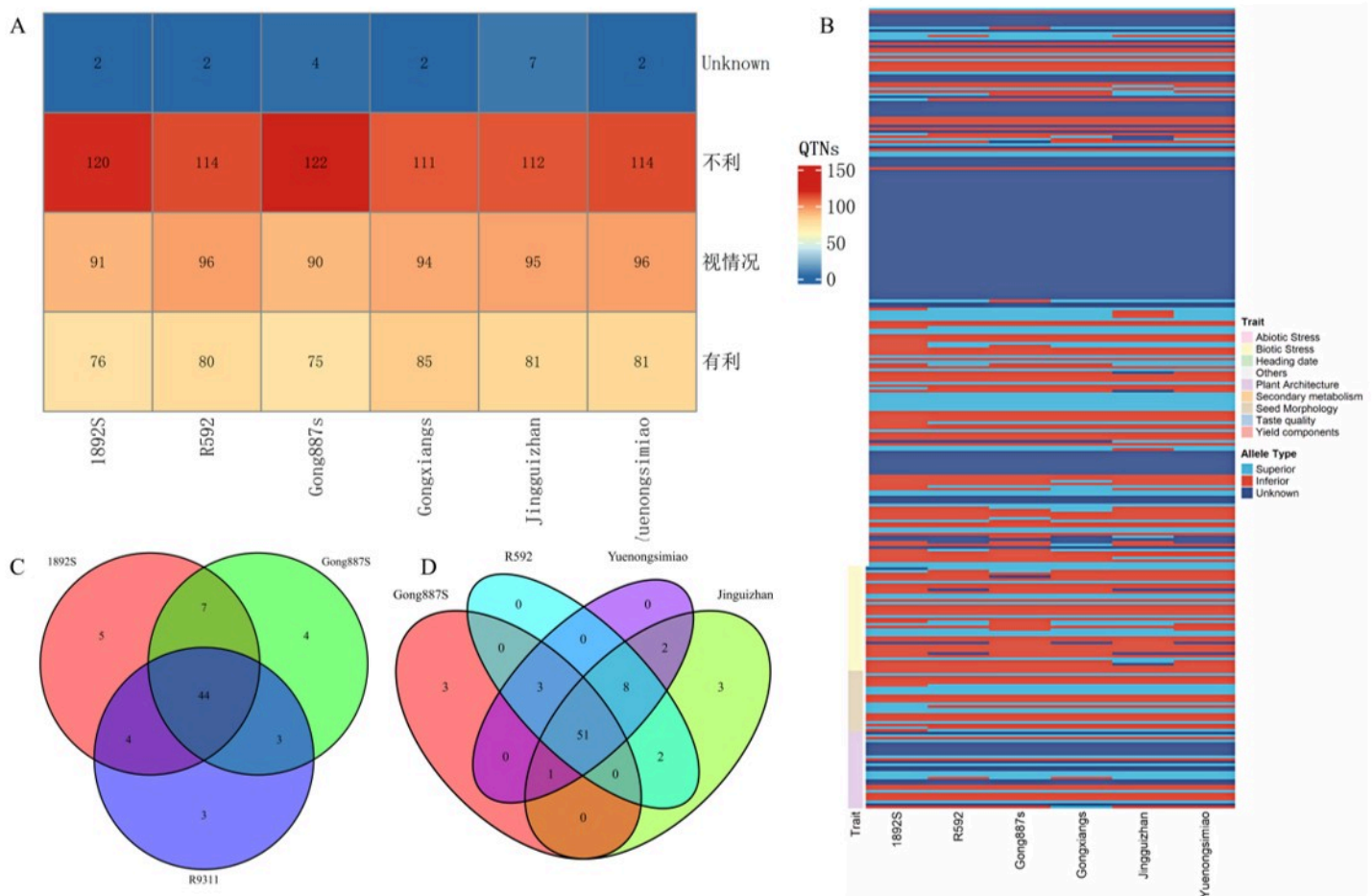


Figure 7

provides a statistical analysis of favorable genes in six sequenced parental rice lines. (A) presents an overview of favorable and unfavorable gene loci among these parental lines. (B) showcases a heatmap of the types of QTN alleles across the six sequenced parents, with different colors indicating various allele types. (C) illustrates the shared favorable gene presence between Gong 887S and both 1892S and 9311. (D) highlights the shared favorable gene presence between Gong 887S and R592, Jing Guizhan, and Yue Nong Simiao.

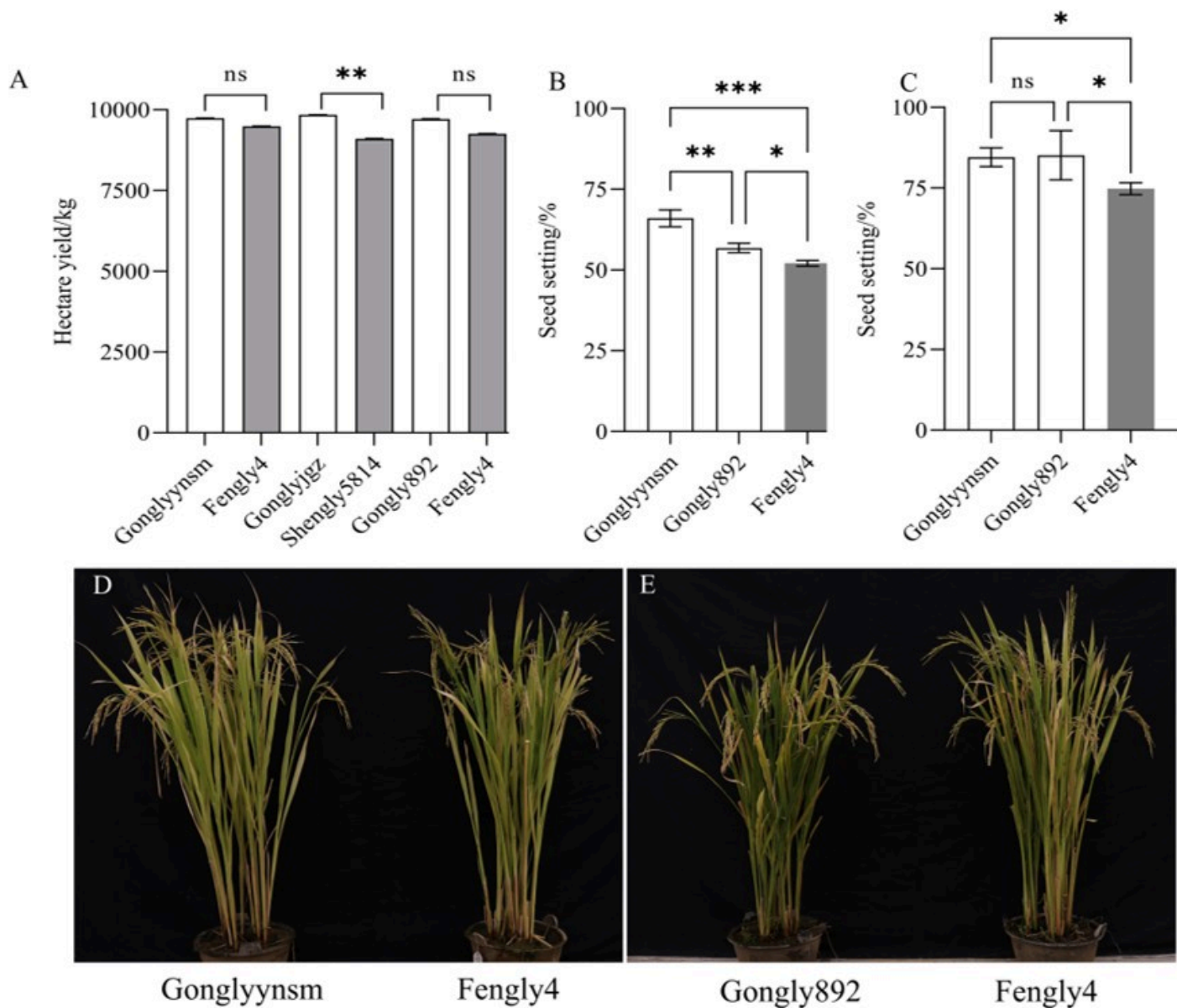


Figure 8

presents data on the yield and heat tolerance of three hybrid rice combinations. (A) displays the yield performance of Gong Liang You Yue Nong Simiao, Gong Liang You Jing Guizhan, and Gong Liang You 892 in regional trials. (B) analyzes the seed-setting rate of Gong Liang You Yue Nong Simiao and Gong Liang You 892 under greenhouse high-temperature stress for five consecutive days during the heading and flowering stages, with daily maximum temperatures at 40°C, minimum at 30°C, and an average of 33.17°C. Feng Liang You No. 4 is used as the heat-tolerant control variety. (C) provides a statistical analysis of the average seed-setting rates for Gong Liang You Yue Nong Simiao and Gong Liang You 892 under field conditions, where staged sowing trials subjected them to high-temperature stress during the heading and flowering stages, with peak temperatures ranging from 38.7-40.3°C, daily lows of 27.0-28.5°C, and average temperatures of 32.0-32.4°C. (D) illustrates the growth condition of Gong Liang You Yue Nong Simiao, compared to the control Feng Liang You No. 4, after exposure to high-temperature stress during the heading stage. Figure 8E shows the growth condition of Liang You 892 alongside the control Feng Liang You No. 4 following high-temperature stress during the heading stage.

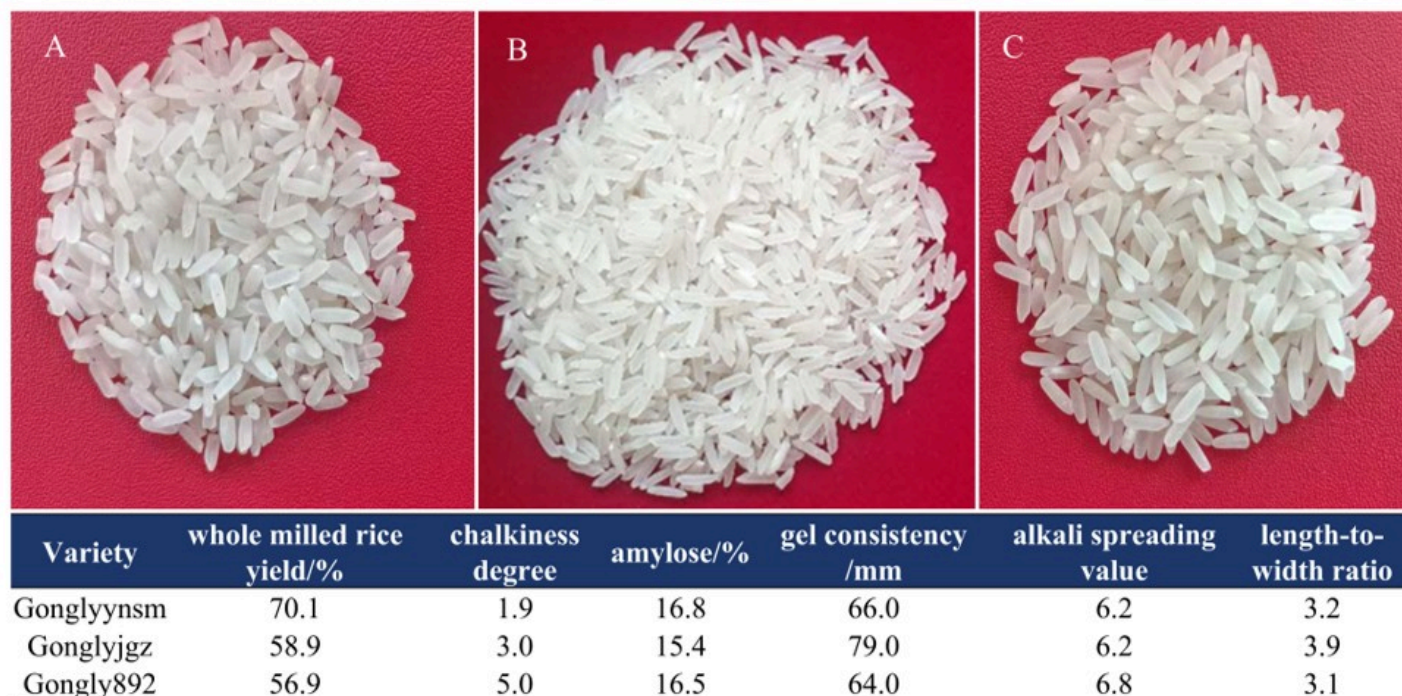


Figure 9

illustrates the rice quality of three hybrid rice combinations. The grain appearance of Gong Liang You Yue Nong Simiao (A), Gong Liang You Jing Guizhan (B), and Gong Liang You 892 (C) is presented. (D) details the rice quality parameters for these three hybrid rice varieties.

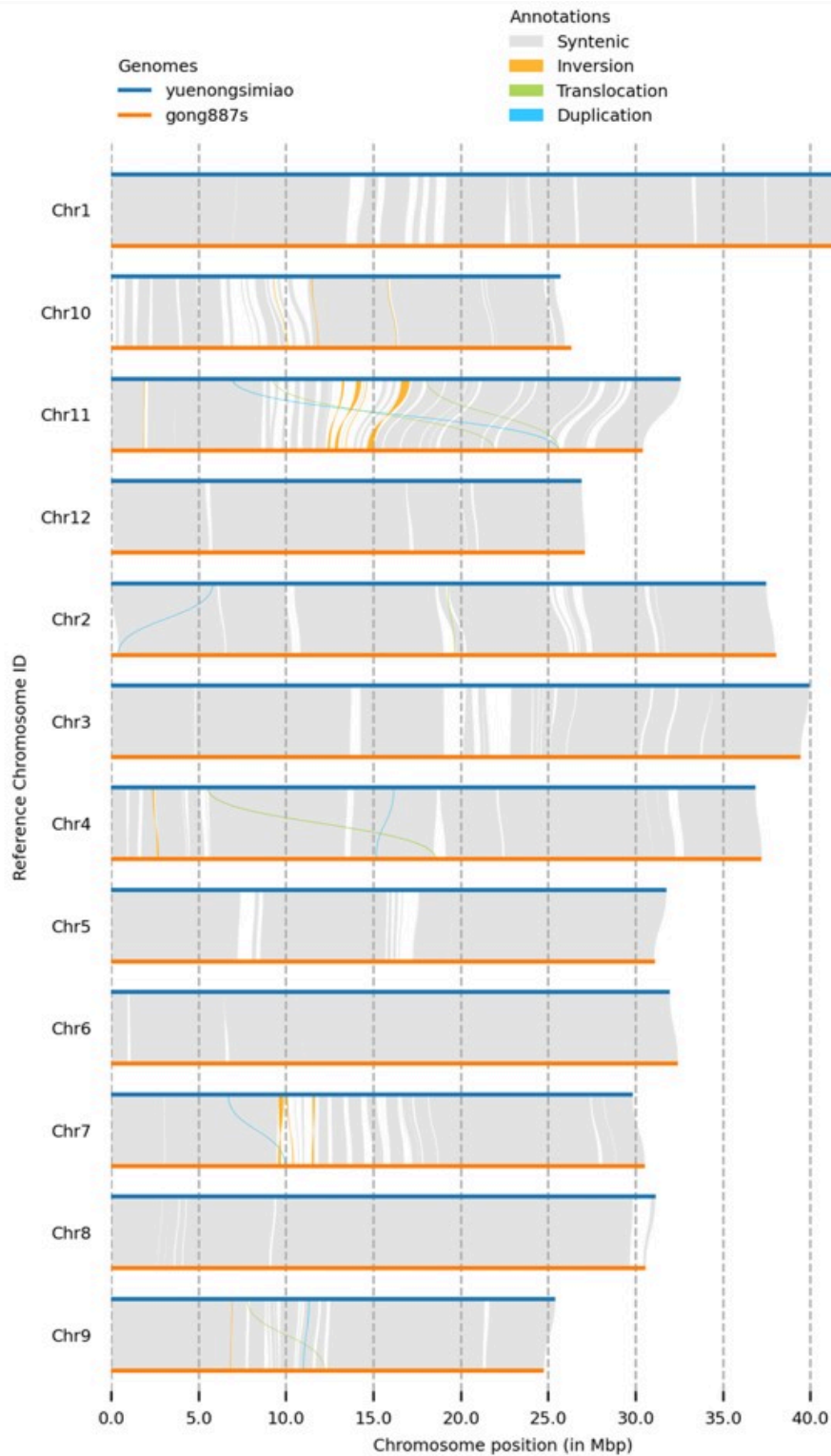


Figure 10

presents a comparative genomic analysis between Yue Nong Simiao and Gong 887S. Using Syri software, this figure highlights the synteny and structural variations between the genomes of Gong 887S and Yue Nong Simiao, illustrating instances of inversions, translocations, and duplications within the genome.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.zip](#)