



Whole-Genome Analysis of MR219 and Its Drought-Tolerant Mutant, NMR151 to Elucidate Drought Resistance Mechanisms

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Abstract: Background: Drought is among the most severe abiotic stresses limiting global rice (*Oryza sativa* L.) productivity and threatening food security, particularly in high-yielding but stress-sensitive cultivars such as MR219. Understanding the genomic basis of drought tolerance is essential to support the development of resilient rice varieties. This study aimed to identify genome-wide variants and drought-responsive genes associated with induced drought tolerance in the mutant line NMR151 derived from MR219. Methods: Whole-genome resequencing was performed on the drought-tolerant mutant NMR151 and its parental line MR219. High-quality paired-end reads totaling 92.1 million (MR219) and 78.5 million (NMR151) were mapped to the *O. sativa* cv. Nipponbare reference genome with an alignment efficiency of 91.4% and coverages of 35.4× and 29.6×, respectively. Variant calling, functional annotation using SnpEff, and visualization through the Integrative Genomics Viewer (IGV) were employed to detect and characterize single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Results: A total of 4.27 million and 4.18 million polymorphisms were identified in MR219 and NMR151, dominated by SNPs and InDels with a transition-to-transversion ratio of 2.4. Comparative analysis revealed 3.01 million shared and 1.17 million unique SNPs in NMR151, indicating mutation-driven allelic diversification. Most variants occurred in intergenic (33.2 %), upstream (29.1 %), and downstream (24.6 %) regions, while six drought-responsive genes (*OsDREB2A*, *OsNAC6*, *OsLEA3-1*, *OsAPX2*, *OsCPK21*, and *OsP5CS1*) were uniquely mutated in NMR151. Conclusions: The drought resilience of NMR151 results primarily from regulatory fine-tuning rather than extensive coding alterations. These findings provide valuable molecular insights and genomic markers for breeding climate-resilient rice varieties through mutation and marker-assisted selection.

Keywords: Drought-responsive genes; MR219; NMR151; SNPs; whole-genome resequencing

1. Introduction

Rice (*Oryza sativa* L.) belongs to the kingdom Plantae, order Poales, family Poaceae, sub-family Oryzoideae, and genus *Oryza*. It is the staple food

for more than half of the world's population, particularly in Asia, making its reliable and sustainable production critical for global food security [1]. Globally, rice is cultivated on over 160 million hectares and produces more than 500 million metric tons annually, with Asia accounting for nearly 90 % of both production and consumption [2]. The relatively compact and well-annotated rice genome has established it as a model cereal species for genomics research, enabling unprecedented advances in functional genetics and molecular breeding [3]. Nevertheless, rice production is continually threatened by abiotic stresses such as drought, salinity, and cold, as well as by biotic pressures from pests, weeds, and pathogens. Among these, drought is one of the most severe abiotic constraints; recent reviews estimate yield losses of 20–30 % or more in rain-fed systems, and this threat is increasing under climate change scenarios [4, 5]. In Malaysia, drought stress is a recurrent challenge, particularly within granary regions such as Kedah, Perlis, and Kelantan, where seasonal water shortages have led to major reductions in grain yield and crop failure during critical reproductive stages [6]. The recurrent drought events associated with climate change pose a rising threat to Malaysia's rice self-sufficiency (currently around 70 %) [7].

To safeguard rice production, the development of drought-tolerant varieties has become a national priority. However, conventional breeding approaches face limitations when addressing drought tolerance, a complex, quantitatively inherited trait strongly influenced by genotype $\times$ environment interactions [8,9]. Although marker-assisted breeding has been deployed to pyramid quantitative trait loci (QTLs) associated with drought tolerance (e.g., qDTYs) into elite cultivars, the process is often slow, resource-intensive, and yields only partial gains under severe field conditions [10]. These challenges underscore the need for complementary approaches, such as mutation breeding, to generate novel allelic variation within well-adapted genetic backgrounds without disrupting their elite traits. In Malaysia, gamma irradiation was applied to the widely grown high-yielding cultivar MR219 to induce new genetic variation. This effort produced promising mutant lines, including NMR151 (MR219-4) and NMR152 (MR219-9), which demonstrated enhanced tolerance to drought stress in greenhouse and field evaluations [11]. Among these, NMR151 stands out for its superior agronomic performance, including enhanced tillering, more panicles per plant, and higher grain yield potential, with reported yields reaching $\approx 15 \text{ t ha}^{-1}$ under optimized conditions, compared with the national average of $\approx 4.2 \text{ t ha}^{-1}$ [12, 13]. NMR151 has been officially registered (Plant Breeder's Certificate PBR0159, 2020), highlighting its agronomic and economic importance.

Despite these advances, the molecular mechanisms conferring drought tolerance in NMR151 remain poorly understood. With the advent of next-generation sequencing (NGS), whole-genome resequencing (WGRS) has enabled the genome-wide identification of single-nucleotide polymorphisms (SNPs), insertions/deletions (InDels), and structural variants, which are crucial resources for dissecting complex traits such as drought tolerance [14, 15]. Recent bioinformatic studies of drought-tolerant versus susceptible rice genotypes have identified differential variant and regulatory profiles, particularly in promoter regions, transcription-factor binding sites, and cis-regulatory elements [16]. However, a comprehensive genome-wide characterization of drought-associated variants in the MR219/NMR151 mutant background is still lacking. Therefore, this study aims to perform a whole-genome comparative analysis of MR219 and its drought-tolerant mutant NMR151. Specifically, we seek to (i) identify high-confidence SNPs and InDels associated with drought tolerance, (ii) annotate functional variants linked to drought-response pathways, and (iii) provide molecular resources to enhance marker-assisted and genomic selection for drought-resilient rice improvement in Malaysia. Collectively, our findings will elucidate the genetic basis of induced drought tolerance in NMR151 and contribute to the sustainable development of climate-resilient rice cultivars.

2. Materials and Methods

2.1 Plant material and DNA extraction

Seeds of the parental cultivar MR219 (a widely grown high-yielding Malaysian indica rice) were obtained from the Rice Genebank of the Malaysian Agricultural Research and Development Institute (MARDI). The Malaysian Nuclear Agency supplied seeds of the gamma-irradiation-derived drought-tolerant mutant line NMR151. Both genotypes were germinated under controlled growth chamber conditions (28 °C/16 h light – 24 °C/8 h dark, 70% relative humidity). Genomic DNA was extracted from young leaf tissue harvested

from 14–21-day-old seedlings using the QIAGEN DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol. DNA quality and concentration were assessed with a NanoDrop spectrophotometer (Thermo Scientific, USA) and quantified using a Qubit fluorometer (Thermo Fisher, USA). DNA integrity was confirmed by electrophoresis on a 1% agarose gel.

2.2 Whole-genome sequencing

High-quality genomic DNA from each genotype (MR219 and NMR151) was submitted to Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) for paired-end sequencing. Libraries were prepared using the Illumina DNA Prep Kit (Illumina, USA) with an average insert size of ~350 bp. Sequencing was conducted on an Illumina NovaSeq 6000 platform to generate 150 bp paired-end reads, targeting ~30× depth per genotype. Raw reads were delivered in FASTQ format and are deposited in the NCBI Sequence Read Archive (SRA) under accession numbers SRR21043964 and SRR20995190 [17].

2.3 Read preprocessing and mapping

Adaptor trimming and quality filtering were performed using Trimmomatic v0.39 (leading/trailing: 3; sliding-window: 4:30; minlen: 50) [18]. Reads with Phred quality score < 30 or containing > 10% ambiguous bases (N) were removed. Cleaned reads were aligned to the reference genome of *Oryza sativa* ssp. japonica (IRGSP-1.0, Nipponbare; RefSeq: GCF_001433935.1) using BWA-MEM v0.7.17 [19]. SAM files were converted to sorted BAM and indexed using SAMtools v1.14 [20]. Duplicate reads were marked with Picard v2.27.1 (Broad Institute). Mapping statistics (total reads, mapped reads, properly paired reads, singleton reads, and coverage) were calculated using SAMtools flagstat and coverage metrics (see Section 3.1 for details).

2.4 Variant calling and filtering

Variant detection (SNPs and small InDels) was performed using the Genome Analysis Toolkit (GATK v4.3.0; HaplotypeCaller) and BCFtools v1.14 (mpileup + call) to provide cross-validation of calls [21,22]. Raw variant calls from both pipelines were filtered using the following thresholds: minimum read depth (DP) ≥ 10, mapping quality (MQ) ≥ 30, variant quality (QUAL) ≥ 50, genotype quality (GQ) ≥ 20, and allelic support (alternate-allele ratio) ≥ 0.9. Variants concordant between both pipelines were retained for downstream analysis. Variant positions were output in VCF format and converted to tabular form using custom Python scripts. The number of SNPs and InDels, along with size distributions and substitution types, were summarized (see Sections 3.2 and 3.3).

2.5 Functional annotation of variants

Variants passing quality filters were annotated using ANNOVAR [23] or SnpEff v5.0 [24] against rice gene models from RAP-DB [3] and MSU Rice Genome Annotation Project v7.0. Variants were classified by genomic context (exonic, intronic, UTR, upstream, downstream, intergenic) and by effect (synonymous, missense, nonsense, frameshift, splice region). Identified variants were also cross-referenced with known drought-tolerance quantitative trait loci (qDTY) and candidate gene lists derived from recent resequencing studies of drought- and salt-tolerant rice [25, 26]. Summary statistics and high-impact variant counts are reported in Section 3.5.

2.6 Candidate drought-response gene annotation and visualization

To identify potentially functional polymorphisms within drought-responsive genes, annotated VCFs and the corresponding BAM alignments were visualized in the Integrative Genomics Viewer (IGV v2.12.3) [27]. Gene loci of interest (e.g., DREB, NAC, bZIP transcription factor families; osmoprotectant biosynthesis enzymes; ABA signaling components) were queried within the RAP-DB and Gramene databases for variant presence or absence between genotypes. Unique SNPs and InDels identified in NMR151 (absent in MR219) were flagged as candidate functional polymorphisms and further categorized (Section 3.6).

3. Results and Discussion

3.1 Whole-Genome Read Mapping and Variant Detection

After quality filtering, a total of 92.1 million reads for MR219 and 78.5 million reads for NMR151 were obtained and successfully aligned to the *Oryza sativa* cv. Nipponbare reference genome, achieving mapping

efficiencies of 91.9% and 91.5%, with mean coverages of 35.4× and 29.6×, respectively. These high mapping rates indicate robust sequencing quality and reliable genome representation in both genotypes (Table 1). Such depth and accuracy are comparable to those reported in high-coverage rice resequencing projects [14,4], ensuring sufficient sensitivity for variant discovery.

Table 1. Mapping statistics of short reads from MR219 and NMR151 mutant rice varieties aligned to the *Oryza sativa* Nipponbare reference genome.

Reference genome	Mapping statistics	MR219	NMR151
<i>Oryza sativa</i> Nipponbare	Total reads	92,097,778	78,541,072
	Mapped reads	90,190,469	76,295,108
	Properly paired	84,659,706	71,826,370
	Singleton	5,404,008	3,947,014
	Sequencing coverage	35.4	29.6

Variant calling revealed 4.27 million and 4.18 million polymorphisms in MR219 and NMR151, respectively, dominated by single-nucleotide polymorphisms (SNPs) and short insertions/deletions (InDels) (Table 2). The slightly lower variant density in NMR151 suggests that gamma irradiation induced localized point mutations without large-scale genomic rearrangements. The observed transition-to-transversion ratio ($Ts/Tv \approx 2.4$) for both genomes further confirms high variant call quality, consistent with typical values for well-assembled *Oryza sativa* datasets [14].

Table 2. Number of SNPs and INDELs identified in MR219 and NMR151 mutant rice varieties.

Reference genome	Variant	MR219	NMR151
<i>Oryza sativa</i> Nipponbare	SNPs	3,864,470	3,785,451
	Insertions	195,700	190,000
	Deletions	211,500	207,700
	Total	4,271,679	4,183,151

3.2 Variant Distribution and Functional Implications

Both genotypes exhibited comparable SNP and InDel distributions when aligned to the reference genome (Figures 1 and 2). Transitions ($A \leftrightarrow G$ and $C \leftrightarrow T$) were more frequent than transversions ($A \leftrightarrow C$, $A \leftrightarrow T$, $C \leftrightarrow G$, $G \leftrightarrow T$), exceeding 2.7 million substitutions per genome. The InDel length spectrum ranged from -12 bp to +12 bp, with 1 bp InDels most abundant—a typical pattern previously reported in rice resequencing studies [14]. NMR151 showed a slightly higher proportion of short deletions (-1 to -3 bp), which may influence gene-regulatory regions or post-transcriptional processing.

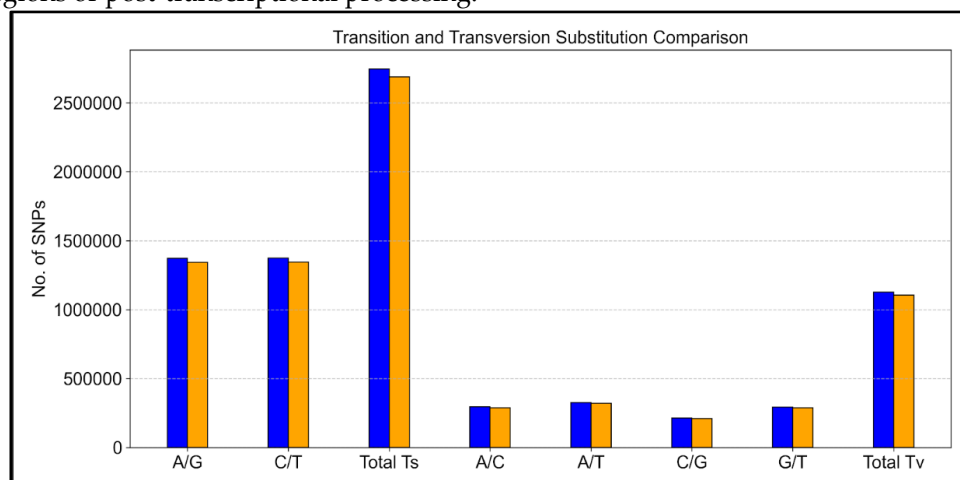


Figure 1. Distribution of nucleotide substitution types in MR219 (blue) and NMR151 (orange) aligned to the *Oryza sativa* Nipponbare reference genome. Transition substitutions ($A \leftrightarrow G$, $C \leftrightarrow T$) predominated over transversions, resulting in a Ts/Tv ratio of approximately 2.4 in both genotypes.

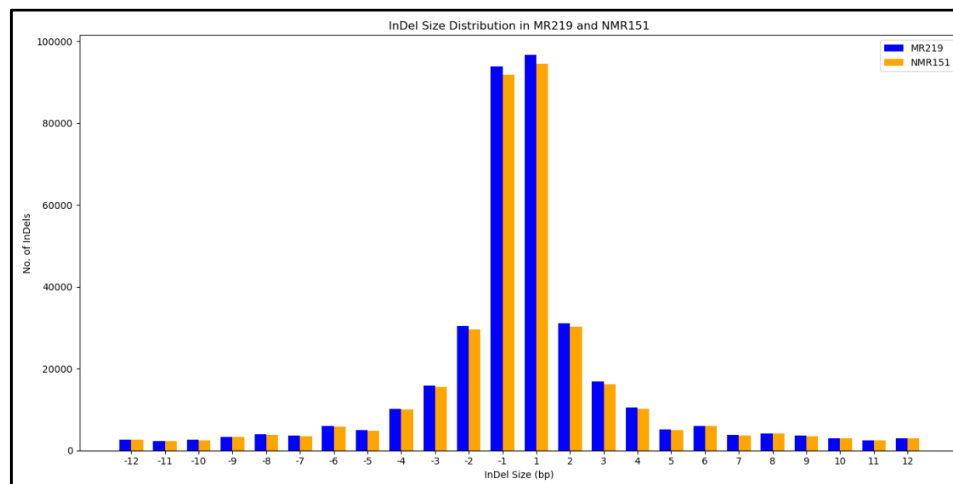


Figure 2. The graph shows the number of InDels ranging from -12 bp to +12 bp in the MR219 and NMR151 Mutant varieties using the *Oryza sativa* Nipponbare reference genome.

Comparative analysis revealed 3.01 million SNPs shared by both genomes, 1.26 million unique to MR219, and 1.17 million unique to NMR151, while 279,524 InDels were common, with 127,685 and 118,176 unique to MR219 and NMR151, respectively (Figure 3). The high proportion of shared variants confirms the genetic closeness of the mutant to its parental line, whereas the presence of private variants in NMR151 indicates irradiation-driven allelic diversification rather than hybridization-derived divergence [11].

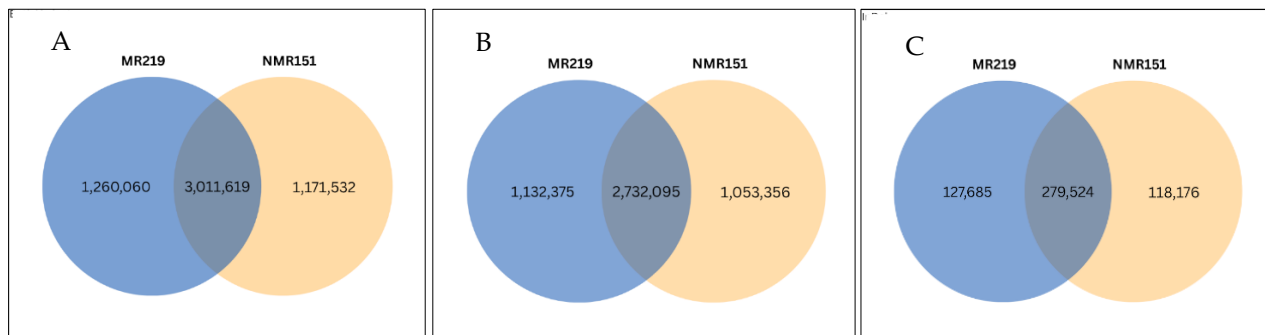


Figure 3. Number of shared variants (A), unique SNPs (B), and unique InDels (C) between MR219 and NMR151 aligned to the *Oryza sativa* Nipponbare reference genome

Functional annotation using SnpEff detected 78,341 high-confidence variants. The majority occurred in intergenic (33.2 %), upstream (29.1 %), and downstream (24.6 %) regions, with only 2.7 % in exons. Among exonic variants, missense (0.86 %), synonymous (0.76 %), and splice-region (0.11 %) substitutions were most common. This regulatory-region predominance supports the view that drought adaptation in rice is largely governed by cis-regulatory modulation rather than pervasive coding-sequence changes [15,4]. Previous greenhouse and field evaluations demonstrated that NMR151 maintained higher grain yield, tiller number, and survival rate under reproductive-stage drought compared to MR219 [11]. Physiological assessments showed improved relative water content and osmotic adjustment capacity, supporting its enhanced drought resilience.

3.3 Identification of Drought-Responsive Genes

Integrative Genomics Viewer (IGV) analysis identified six drought-responsive genes harboring unique polymorphisms in NMR151: *OsDREB2A*, *OsNAC6/SNAC2*, *OsLEA3-1*, *OsAPX2*, *OsCPK21*, and *OsP5CS1* (Table 3). These genes represent complementary roles in transcriptional regulation, antioxidative defense, signal transduction, and osmoprotectant biosynthesis.

Table 3. Functional effects of selected high- and moderate-impact variants predicted by SnpEff in MR219 and NMR151.

Gene ID	Variant type	Chromosome position	Predicted effect	Functional class	Remarks
<i>Os01g0718300</i>	SNP	Chr 1: 28,450,376 (A→G)	Missense	Moderate impact	Alters AP2/ERF DNA-binding domain in <i>OsDREB2A</i>
<i>Os03g0327800</i>	SNP	Chr 3: 9,612,844 (C→T)	Missense	Moderate impact	Substitution in the NAC-domain region of <i>OsNAC6</i>
<i>Os02g0782500</i>	SNP	Chr 2: 31,040,782 (G→A)	Synonymous	Low impact	Silent mutation in the <i>OsLEA3-1</i> coding sequence
<i>Os05g0477700</i>	InDel	Chr 5: 23,511,904 (+T)	Frameshift	High impact	Causes reading-frame change in <i>OsCPK21</i>
<i>Os09g0527200</i>	SNP	Chr 9: 17,788,605 (C→A)	Missense	Moderate impact	Substitution in the catalytic domain of <i>OsP5CS1</i>
<i>Os12g0630200</i>	SNP	Chr 12: 19,501,342 (G→T)	Stop-gained	High impact	A premature stop codon was detected in <i>OsAPX2</i> . While such mutations are typically associated with loss-of-function, compensatory upregulation of paralogous APX genes or altered regulatory feedback may contribute to stress adaptation. Functional validation is required to clarify its biological consequences.

OsDREB2A and *OsNAC6* act as key transcriptional hubs controlling ABA-independent and ABA-dependent drought responses, respectively [28,4]. *OsLEA3-1* and *OsAPX2* contribute to stress protection through desiccation tolerance and reactive oxygen species (ROS) detoxification [29,30]. *OsCPK21* functions in Ca²⁺-mediated ABA signaling, whereas *OsP5CS1* catalyzes proline biosynthesis for osmotic adjustment. The presence of NMR151-specific mutations in these loci indicates potential functional modulation; however, further transcriptomic, physiological, and functional validation studies are required to confirm their direct contribution to drought tolerance.

3.4 Integrative Interpretation and Breeding Implications

The combined variant analyses and functional annotation reveal that NMR151's enhanced drought tolerance is mainly derived from transcriptional and regulatory fine-tuning rather than large-scale coding disruptions. The prevalence of non-coding variants supports adaptive modulation of gene expression, while the identified genes form a coherent stress-response cascade encompassing perception (*OsCPK21*), regulation (*OsDREB2A*, *OsNAC6*), protection (*OsLEA3-1*, *OsAPX2*), and osmotic balance (*OsP5CS1*). These results complement previous genomic and QTL-based studies, which show that stable yield under drought is achieved through the integration of multiple regulatory networks [10,26]. The precise identification of candidate polymorphisms in an elite Malaysian background extends current drought-genomics knowledge and provides immediate targets for marker-assisted and haplotype-informed breeding. Future research incorporating transcriptomic and methylomic analyses, along with CRISPR or near-isogenic line validation, will further elucidate the causal role of these allelic variants in enhancing drought resilience. Gamma irradiation typically induces point mutations and small insertions/deletions rather than large chromosomal rearrangements. Some mutations may be neutral or even mildly deleterious; therefore, the drought-tolerant phenotype of NMR151 likely results from a combination of beneficial regulatory modifications and background genomic buffering. The proposed interactions among the key drought-responsive genes identified in this study are summarized in Figure 4.

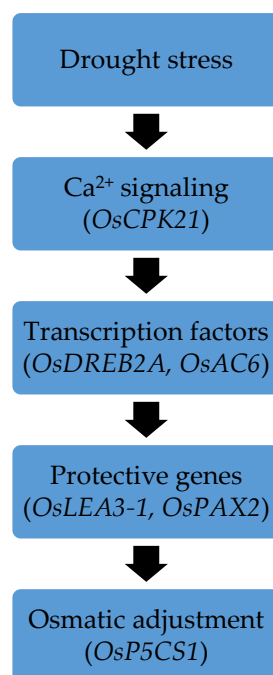


Figure 4. Proposed regulatory framework of drought tolerance in NMR151. Drought stress triggers Ca²⁺-mediated signaling (OsCPK21), which activates key transcription factors (OsDREB2A, OsNAC6), leading to induction of protective genes (OsLEA3-1, OsAPX2) and osmotic adjustment via proline biosynthesis (OsP5CS1). The model represents a conceptual interpretation based on genomic variant analysis.

4. Conclusions

Whole-genome resequencing of the drought-tolerant mutant NMR151 and its parental cultivar MR219 identified over 4.2 million high-quality variants, revealing irradiation-induced allelic diversification associated with drought resilience. Six drought-responsive genes (OsDREB2A, OsNAC6, OsLEA3-1, OsAPX2, OsCPK21, OsP5CS1) carried unique mutations in NMR151, highlighting key roles in transcriptional regulation, signaling, and oxidative protection. The data suggest that drought tolerance in NMR151 may be associated with regulatory fine-tuning of stress-responsive pathways, although functional validation is necessary to confirm causative relationships.

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