

Article

Genome-Wide Identification and Abiotic Stress-Responsive Expression of the DMP Gene Family in Rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) production faces significant challenges from environmental stresses, necessitating the identification of functional genes to enhance crop resilience. The DUF679 membrane protein (DMP) family has been recently recognized for its diverse associations with plant development and stress adaptation, although much of the current research has focused on its involvement in doubled haploid (DH) technology in species such as maize and Arabidopsis. This study performed a comprehensive genome-wide analysis to characterize the 19-member OsDMP family in rice, integrating bioinformatics methods to analyze their physicochemical properties, phylogenetic relationships, and chromosomal distribution, complemented by qRT-PCR to validate expression patterns under abiotic stress and transient expression assays to verify subcellular localization of one representative member (*OsDMP16*). Our analysis classified *OsDMP* genes into four of five subfamilies, revealing significant functional diversification and closer evolutionary ties with other monocots. Furthermore, promoter analysis revealed abundant stress- and hormone-responsive cis-acting elements, correlating with diverse tissue-specific expression and significant responses to drought, salt, and hormonal stimuli. These findings provide a robust framework for understanding *OsDMP* evolution and expression regulation, revealing transcriptional responsiveness to abiotic stress and providing candidate genes for future functional validation and application in breeding programs.

Keywords: rice; DMP family; genome-wide analysis; evolution; abiotic stress

1. Introduction

Rice (*Oryza sativa* L.) is one of the most critical cereal crops globally, serving as the primary dietary staple for more than half of the world's population [1]. As the global population is projected to reach nearly 10 billion by 2050, the demand for rice production must increase by at least 70% to ensure global food security [2]. However, rice cultivation faces unprecedented challenges, including the shrinking of arable land, the depletion of water resources, and the increasing frequency of extreme weather events such as droughts, floods, and heatwaves driven by global climate change [3]. Furthermore, various biotic stresses, including blast, bacterial blight, and insect pests, continue to threaten stable rice yields [4]. Developing new rice varieties with higher yield potential, enhanced nutritional quality, and robust resilience to environmental stresses is imperative to address these multifaceted challenges [5]. Understanding the molecular mechanisms of stress-responsive gene families is crucial for developing such resilient varieties.



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The Domain of Unknown Function 679 (DUF679) membrane protein (DMP) family is a plant-specific group of genes encoding small integral membrane proteins characterized by a highly conserved DUF679 domain [6]. These proteins typically possess four transmembrane helices and are localized to various endomembrane systems, including the plasma membrane and the tonoplast [7]. While members of this family, such as *AtDMP8* and *AtDMP9* in Arabidopsis, have been identified as key players in gamete fusion and haploid induction [8], their broader biological functions, particularly in response to environmental stimuli, have gained increasing attention. Recent genome-wide studies across diverse plant taxa, including soybean (*Glycine max*), cotton (*Gossypium hirsutum*), pepper (*Capsicum annuum*), and sugar beet (*Beta vulgaris*), have reported transcriptional changes in *DMP* genes under abiotic stress conditions, raising the possibility of their involvement in stress adaptation [9–12]. For instance, in soybean, *GmDMP* genes were found to be significantly regulated by various abiotic stresses, with promoter analysis revealing an abundance of stress-responsive cis-acting elements [10]. Similarly, in cotton and pepper, the expression of several *DMP* members was markedly induced by drought, salt, and cold treatments, suggesting a potential association with plant responses to fluctuating environments, although direct functional evidence remains limited [9,13]. However, most studies to date have focused on their application in doubled haploid (DH) technology, particularly in maize where *ZmDMP* acts as an enhancer of haploid induction [14]. In rice, while some *OsDMP* homologs have been identified, research has primarily centered on their potential for haploid induction, often yielding inconsistent results [15]. Importantly, the potential involvement of the *OsDMP* family in rice abiotic stress responses—a critical factor for crop stability—has not been systematically investigated. In parallel to these emerging stress-related findings, the *DMP* family has attracted considerable attention for its role in haploid induction, another research direction with significant implications for rice breeding. Historically, DH lines were primarily generated through in vitro methods, such as anther or microspore culture [16]. While these techniques have been successfully applied in several crops, they are often limited by strong genotype dependency, low induction efficiency, and the high cost of specialized laboratory facilities [17]. Consequently, the development of in vivo haploid induction (HI) systems has become a major focus of research [18]. The most successful in vivo HI system was first established in maize (*Zea mays*), utilizing specific inducer lines such as Stock 6 [19,20]. A major breakthrough in understanding the molecular basis of this system was the identification of the *MATRILINEAL* (*MATL*), also known as *PHOSPHOLIPASE A1* (*ZmPLA1*) or *NOT LIKE DAD* (*NLD*), which encodes a pollen-specific phospholipase [21,22]. Mutations in *ZmMATL* trigger the formation of maternal haploids through sperm-mediated genome elimination [18]. The conservation of this mechanism allowed for the successful translation of HI technology to rice, where knockout of the orthologous *OsMATL* gene was shown to induce haploid seed formation, albeit at a relatively low rate of approximately 2–6% [23]. Recent research has revealed that the *DMP* family is one of the key players in vivo HI systems [8]. In the model plant Arabidopsis thaliana, the *DMP* family comprises 10 members (*AtDMP1–AtDMP10*). Functional studies have demonstrated that *AtDMP8* and *AtDMP9* are specifically expressed in sperm cells and play a redundant but essential role in gamete fusion during double fertilization. Simultaneous loss-of-function mutations in *AtDMP8* and *AtDMP9* lead to a significant reduction in fertilization success and trigger the development of maternal haploids, establishing the first *DMP*-based HI system in a dicotyledonous species [8].

The discovery of the *DMP* gene's role in haploid induction has sparked significant interest in its application in monocotyledonous crops. In maize, it was discovered that while the mutation of *ZmDMP* alone results in a very low haploid induction rate (HIR), its combination with the *ZmMATL* mutation significantly enhances the HIR, demonstrating a strong

synergistic effect [14]. This finding suggests that *DMP* genes may act as essential modifiers or enhancers of the HI process. Following these breakthroughs, *DMP* orthologs have been identified and characterized in several other important crops, including wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*), tobacco (*Nicotiana tabacum*), and soybean (*Glycine max*) [10,24,25]. For instance, in wheat, a genome-wide analysis identified 80 *TaDMP* genes, some of which were found to influence male sterility and potentially contribute to haploid induction [25]. In tomato, the knockout of *SIDMP* was shown to induce maternal haploids, further confirming the functional conservation of *DMP* genes across diverse plant taxa [24]. In rice, although several *OsDMP* homologs have been identified through comparative genomics, their comprehensive functional characterization remains incomplete. Preliminary studies on *OsDMP1*, *OsDMP2*, and *OsDMP3* have yielded mixed results regarding their independent ability to induce haploids [15]. However, these studies have often focused on a few specific members, and a systematic, genome-wide identification and evolutionary analysis of the entire *DMP* gene family in rice are still lacking.

In this study, we performed a systematic genome-wide analysis of the *DMP* gene family in rice. We identified all *OsDMP* members and characterized their physicochemical properties, gene structures, and conserved motifs. Furthermore, we conducted a detailed phylogenetic analysis to trace the evolutionary history of the *DMP* family across representative plant species and investigated the cis-acting elements in their promoter regions. Finally, we analyzed the expression profiles of *OsDMP* genes using public transcriptomic data and quantitative real-time PCR (qRT-PCR) under different abiotic stresses. Our findings provide a comprehensive foundation for further functional characterization of *OsDMP* genes and offer potential targets for improving abiotic stress resilience in rice breeding programs.

2. Materials and Methods

2.1. Identification of *DMP* Family Members in Rice

The genome sequences and GFF annotation files of rice were retrieved from the Rice Genome Annotation Project (RGAP, <https://rice.uga.edu/>, accessed on 2 March 2026). To identify potential members of the *DMP* gene family in rice, the amino acid sequences of Arabidopsis thaliana *DMP* proteins were downloaded from the Arabidopsis Information Resource (TAIR, <https://www.arabidopsis.org/>, accessed on 2 March 2026) and used as queries for BLASTp (NCBI, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 2 March 2026) searches against the rice genome. Additionally, the Hidden Markov Model (HMM) profile for the DUF679 domain was obtained from the Pfam database (<http://pfam.xfam.org>, accessed on 3 March 2026). HMMER 3.0 (<http://hmmerr.org/>, accessed on 3 March 2026) was employed to search for proteins containing the DUF679 domain within the rice genome [26]. All candidate sequences were further validated for the presence of the conserved *DMP* domain using Pfam, SMART, and InterPro. For genes with multiple transcripts, the longest coding sequence (CDS) was selected as the representative sequence for subsequent analysis. To avoid confusion with previously reported *OsDMP* genes, we cross-referenced our nomenclature with published studies. A comparison of our *OsDMP1–OsDMP19* nomenclature with previously reported names is provided in Table S2.

2.2. Physicochemical Property Prediction

The physicochemical properties of the identified *OsDMP* proteins, including sequence length, theoretical isoelectric point (pI), molecular weight (Mw), instability index, aliphatic index, and grand average of hydropathicity (GRAVY), were predicted using the ProtParam tool on the ExPASy server (<https://web.expasy.org/protparam/>, accessed on 11 March 2026). Subcellular localization was predicted using the WoLF PSORT online tool

(<https://wolfsort.hgc.jp/>, accessed on 11 March 2026), with the highest-scoring location selected as the predicted result [27].

2.3. Chromosomal Localization of OsDMP Genes

The chromosomal distribution of *OsDMP* genes was visualized using the “Gene Location Visualize from GTF/GFF” module in TBtools v2.441 [28], based on the rice genome annotation information.

2.4. Phylogenetic, Gene Structure, and Conserved Motif Analysis

To investigate the evolutionary relationships of the DMP family, a phylogenetic tree was constructed using DMP protein sequences from rice, Arabidopsis, maize (*Zea mays*), foxtail millet (*Setaria italica*), and sorghum (*Sorghum bicolor*). Multiple sequence alignment was performed using ClustalW. Subsequently, a Maximum Likelihood (ML) tree was generated using MEGA12 (v12.1) software with 1000 bootstrap replicates [29]. The resulting tree was visualized and refined using the Interactive Tree Of Life (iTOL, <https://itol.embl.de/>, accessed on 11 March 2026) [30]. Conserved motifs within the *OsDMP* proteins were identified using the MEME online tool (<https://meme-suite.org/meme/>, accessed on 11 March 2026) [31]. The parameters were set as follows: maximum number of motifs = 10, minimum motif width = 6, and maximum motif width = 50. The exon-intron structures of *OsDMP* genes, including CDS and untranslated regions (UTRs), were extracted from the rice GFF annotation file. Both motif patterns and gene structures were visualized using TBtools v2.441.

2.5. Synteny Analysis and Gene Duplication

Homologous *OsDMP* genes within the rice genome were identified using BLASTp with an E-value threshold of $<1 \times 10^{-10}$. Intrasppecific synteny and gene duplication events were analyzed using MCScanX (TBtools v2.446) with default parameters. For interspecific synteny analysis, the genome sequences and annotation files of Arabidopsis (TAIR10.55) and sorghum (*Sorghum bicolor* v3.1.1) were downloaded from Phytozome v13 (<https://phytozome-next.jgi.doe.gov/>, accessed on 11 March 2026). Syntenic relationships between rice and these two species were determined using MCScanX and visualized using TBtools (v2.446).

2.6. Analysis of Cis-Acting Elements in OsDMP Promoters

The 2000 bp sequence upstream of the start codon (ATG) for each *OsDMP* gene was defined as the promoter region and extracted using TBtools. These sequences were submitted to the PlantCARE database (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 11 March 2026) to predict cis-regulatory elements [32]. Elements related to stress responses, plant growth and development, phytohormone responsiveness, and light response were summarized and visualized using the “Basic Biosequence View” and “HeatMap” modules in TBtools.

2.7. Expression Profile Analysis of OsDMP Genes

Expression data for *OsDMP* genes across various tissues and under hormone treatments were retrieved from the Rice Expression Database (ROADv2, <https://roadv2.khu.ac.kr/>, accessed on 11 March 2026) [33]. Specifically, RNA-seq data from *japonica* rice cultivar Nipponbare were used. For tissue-specific expression analysis, DESeq2-normalized log₂ expression values were obtained. For hormone treatment analysis (ABA, ethylene, JA), log₂ fold change values relative to control conditions were used. All values represent averaged data downloaded directly from the database. The expression patterns were visualized as heatmaps using the “HeatMap” module in TBtools.

2.8. Plant Materials and Abiotic Stress Treatments

Rice (*Oryza sativa* L. *japonica* cv. Liaoxing 1, LX1) seedlings were cultivated hydroponically in Yoshida nutrient solution in an artificial climate chamber (QHX-1000L, Greenfafa, Wuhan, China) under controlled conditions (30 °C, 16 h light/8 h dark photoperiod) to investigate *OsDMP* transcriptional responses to environmental challenges. Two-week-old seedlings were subjected to salt stress and drought stress by transferring to solutions containing 300 mM NaCl and 20% polyethylene glycol (PEG 6000), respectively, while ddH₂O served as the control [34]. Leaf tissues from the second fully expanded leaf were collected at each time point. Each biological replicate consisted of pooled tissues from five individual seedlings, with three independent biological replicates per treatment. Seedling samples were harvested at 0, 3, 6, 12, and 24 h post-treatment, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent molecular analysis.

2.9. RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from the harvested samples using the Plant Total RNA Extraction Kit (TaKaRa, No. 9769, Kusatsu, Japan) and subsequently reverse-transcribed into cDNA using the PrimeScript RT Reagent Kit (TaKaRa, No. RR037Q) following the manufacturer's protocols. The resulting cDNA served as the template for qRT-PCR analysis, which was performed on an Applied Biosystems ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using the SYBR Premix Ex Taq II Kit (TaKaRa, No. RR820A). The reaction system and thermal cycling conditions were configured according to the manufacturer's instructions. Relative *OsDMP* gene expression was calculated using the 2- $\Delta\Delta C_t$ method [35], with the Actin gene serving as the internal reference for normalization. The stability of Actin expression under the tested stress conditions was validated by confirming that its C_t values remained constant (coefficient of variation < 5%) across all treatment time points and conditions. The experimental unit was individual seedlings. Three independent biological replicates ($n = 3$) were used, with each biological replicate consisting of pooled tissues from five seedlings. Technical replicates (three per biological sample) were averaged to yield a single expression value per biological replicate prior to statistical analysis. The heatmap displays the mean $\log_2$ fold change values averaged across biological replicates, with color intensity representing expression levels relative to the 0 h control. Eight representative *OsDMP* genes were selected for qRT-PCR validation based on their subfamily representation, detectable expression levels in public transcriptomic data, and abundance of stress-responsive cis-acting elements in their promoter regions. The specific primer sequences and their annealing temperatures (T_m) used for qRT-PCR are provided in Table S1.

2.10. Subcellular Localization Analysis

To experimentally verify the subcellular localization of *OsDMP* proteins, *OsDMP16* was selected as a representative member for transient expression analysis in rice protoplasts. The full-length coding sequence of *OsDMP16* (without the stop codon) was amplified by PCR and cloned into a GFP fusion expression vector to generate the *OsDMP16*-GFP construct. Rice protoplasts were isolated from 10-day-old etiolated seedlings of *Oryza sativa* L. *japonica* cv. LX1. The *OsDMP16*-GFP fusion construct was co-transformed with an endoplasmic reticulum (ER) marker (mCherry-HDEL) into rice protoplasts via PEG-mediated transformation. An empty GFP vector was co-transformed with the ER marker as a negative control. The protoplast transformation experiment was performed with three independent biological replicates, each yielding consistent localization patterns. After incubation at 28 °C in the dark for 12–16 h, GFP and ER marker fluorescence signals were observed using a confocal laser scanning microscope (Zeiss LSM 800, Carl Zeiss,

Oberkochen, Germany). The specific primer sequences used for subcellular localization analysis are provided in Table S1.

2.11. Statistical Analysis

For qRT-PCR expression analysis, differences between treatment and control groups at each time point were assessed using two-tailed Student's *t*-tests. A *p*-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics v26.0 (IBM, New York, NY, USA) or GraphPad Prism v9.0 (GraphPad Software, Boston, MA, USA). Data are presented as mean ± standard deviation (SD) from three independent biological replicates.

3. Results

3.1. Identification and Physicochemical Properties of the DMP Gene Family in Rice

A total of 19 *OsDMP* genes were identified in the rice genome and systematically named *OsDMP1* to *OsDMP19* (Table S2). The predicted protein lengths ranged from 147 (*OsDMP4*) to 565 amino acids (*OsDMP5*), with Mw spanning 15.65 to 62.04 kDa. The theoretical isoelectric points (pI) varied significantly from 4.83 (*OsDMP1*) to 9.10 (*OsDMP14*), indicating a broad range of charge properties among family members. Stability analysis revealed that 12 members (e.g., *OsDMP1*, 6, 18) are relatively stable (instability index < 40), while the remaining 7 are classified as unstable. Aliphatic index values (57.68 to 100.97) suggested varying thermal stabilities. Most *OsDMP* proteins (11 members) exhibited positive GRAVY values, reflecting their hydrophobic nature as membrane-associated proteins. Subcellular localization predictions indicated that the majority (10 members) are localized to the plasma membrane, while others are distributed in vacuoles, the endoplasmic reticulum, extracellular space, or cytoplasm, suggesting diverse functional roles across cellular compartments. To experimentally validate these predictions, we performed subcellular localization analysis of *OsDMP16* as a representative member (see below).

3.2. Phylogenetic Analysis of the DMP Gene Family

To investigate the evolutionary relationships and classification of the *DMP* gene family, a ML phylogenetic tree was constructed using protein sequences from five representative plant species: rice, *Arabidopsis thaliana*, maize (*Zea mays*), foxtail millet (*Setaria italica*), and sorghum (*Sorghum bicolor*). The phylogenetic analysis revealed that the *DMP* proteins from these species could be categorized into five distinct subfamilies, designated as Subfamily I to V, which are highlighted in different colors in the circular tree (Figure 1). The distribution of *OsDMP* proteins across these subfamilies was uneven, reflecting functional diversification during evolution. Subfamily V (green) was the largest group, containing a significant number of *OsDMP* members (e.g., *OsDMP1–8*, 14, 18, 19), which clustered closely with their orthologs from other monocot species like maize and sorghum. Subfamily III (blue) and Subfamily I (purple) also contained several rice members, such as *OsDMP10*, 12, 13, 17 and *OsDMP9*, 11, 15, respectively. In contrast, Subfamily II (red) contained fewer rice members, with *OsDMP16* being the representative rice protein among them. Notably, Subfamily IV (orange) did not contain any rice *DMP* proteins, suggesting a potential loss or divergence of these members in the rice lineage. The phylogenetic tree showed that *OsDMP* proteins generally clustered more closely with those from other monocotyledonous species than with the dicotyledonous model plant *Arabidopsis thaliana*. This observation suggests that the *DMP* gene family underwent significant expansion and differentiation after the divergence of monocots and dicots. The high bootstrap values across most branches further confirmed the reliability of the phylogenetic relationships established in this study. The presence of *OsDMP* members in four of the five subfamilies indicates that the rice *DMP*

family has maintained a high degree of structural and potentially functional diversity throughout its evolutionary history.

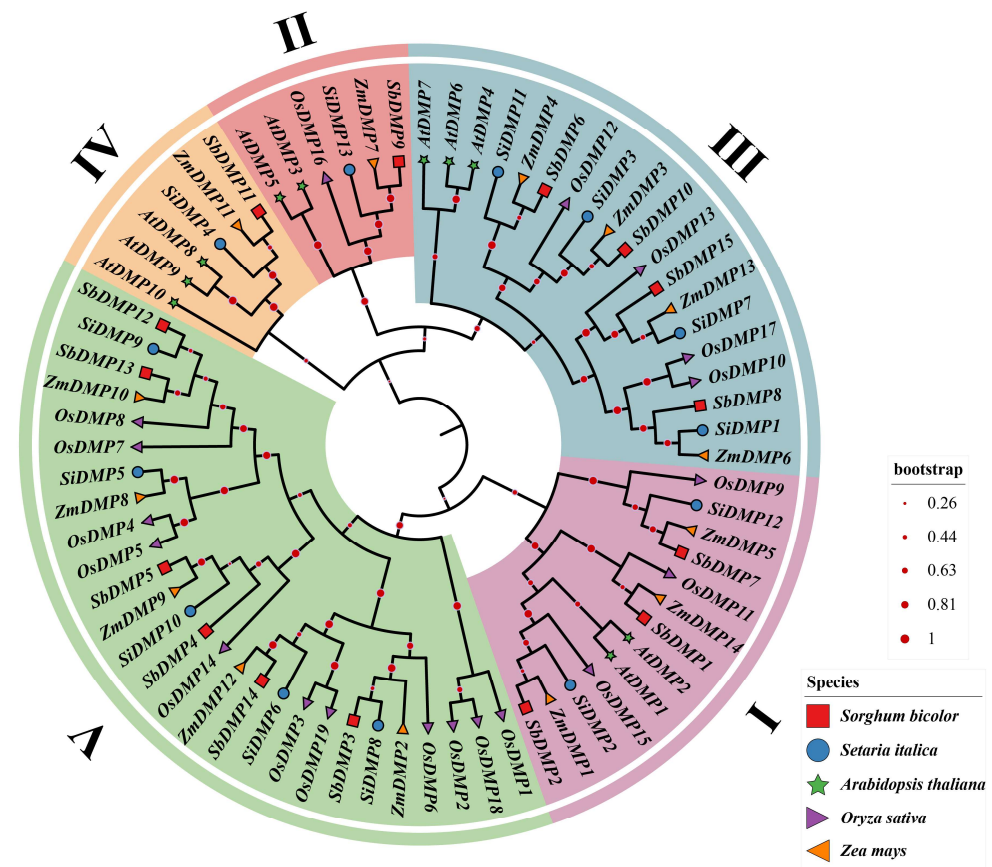


Figure 1. Phylogenetic analysis of DMP proteins from rice and four other plant species. The five subfamilies (I–V) are indicated by different background colors. Different symbols represent different species: purple triangles for rice, orange triangles for *Zea mays*, red squares for *Sorghum bicolor*, blue circles for *Setaria italica*, and green stars for *Arabidopsis thaliana*. The size of the red dots at the nodes represents the bootstrap support values.

3.3. Conserved Motif and Gene Structure Analysis of OsDMP Genes

To further understand the structural diversity and functional conservation of the *DMP* gene family in rice, we analyzed the conserved motifs, protein domains, and exon-intron structures of the 19 *OsDMP* members (Figure 2A–D). The results showed that the structural features of *OsDMP* genes were highly consistent with their phylogenetic classification. A total of 10 conserved motifs (designated as Motif 1 to 10) were identified using the MEME tool. Most *OsDMP* genes within the same subfamily exhibited similar motif patterns, suggesting functional conservation (Figure 2B). For instance, members of Subfamilies I, II, and III predominantly contained a core set of motifs, including Motifs 1, 2, 3, 4, 5, and 6, arranged in a conserved order. Subfamily V, the largest group, also showed a highly conserved motif composition, although some members like *OsDMP5* and *OsDMP18* possessed additional or duplicated motifs, potentially reflecting functional expansion. In contrast, *OsDMP4* and *OsDMP2* exhibited simpler motif patterns, lacking several core motifs found in other members.

All 19 *OsDMP* proteins were confirmed to contain the characteristic DUF679 domain, which is the defining feature of the *DMP* family (Figure 2C). The DUF679 domain was relatively conserved in length and position across most members, typically occupying the central region of the protein. Notably, based on the current genome annotation, *OsDMP5* stood out with a significantly longer protein sequence and was found to contain two

tandem DUF679 domains, a unique feature among the family members. However, it should be noted that this dual-domain architecture has not yet been experimentally verified (e.g., through RACE or full-length cDNA cloning), and thus the possibility that it represents a genome assembly or annotation artifact cannot be excluded at this stage. If confirmed, this structural duplication in *OsDMP5* may imply a specialized or more complex biological role compared to the single-domain members.

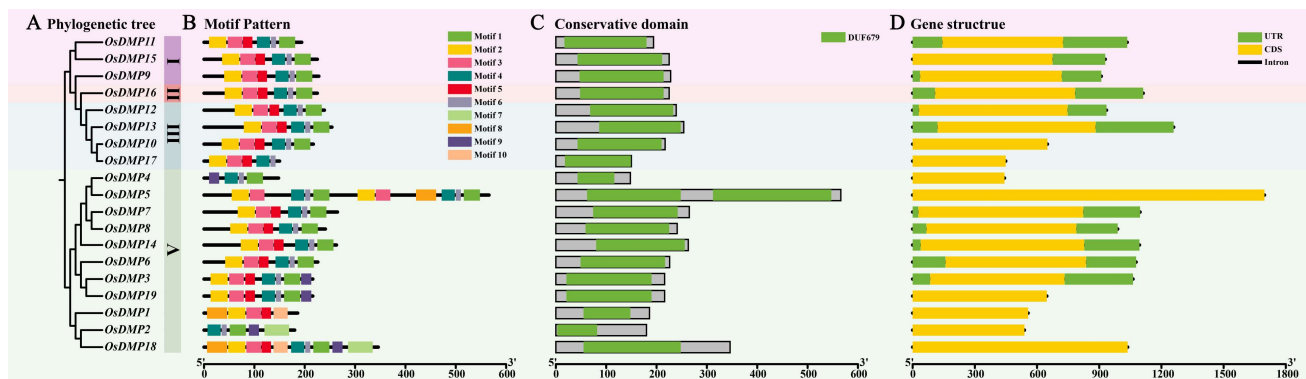


Figure 2. Phylogenetic relationships, conserved motifs, protein domains, and gene structures of *OsDMP* genes. (A) The ML phylogenetic tree of 19 *OsDMP* proteins. (B) Distribution of 10 conserved motifs identified by MEME. (C) Conserved DUF679 domains (green boxes) identified by Pfam. (D) Exon-intron structures of *OsDMP* genes. Yellow boxes represent CDS and green boxes represent UTRs. All *OsDMP* genes are intronless, each consisting of a single exon.

All 19 identified *OsDMP* genes were found to be intronless, each consisting of a single exon (Figure 2D). This simple gene structure is a common characteristic of certain membrane protein families and may facilitate rapid transcriptional responses. The distribution of UTRs varied among members, with some genes lacking identified 5' or 3' UTRs in the current annotation.

3.4. Chromosomal Distribution of *OsDMP* Genes

To understand the genomic distribution of the *DMP* gene family in rice, we mapped the 19 *OsDMP* genes onto the rice chromosomes (Figure S1). The *OsDMP* genes were unevenly distributed across 8 of the 12 rice chromosomes and one unanchored scaffold (ChrUn). Chromosome 1 (Chr1) exhibited the highest density of *OsDMP* genes, harboring 9 members (*OsDMP1* to *OsDMP9*). Notably, a prominent gene cluster consisting of *OsDMP1* through *OsDMP8* was identified on Chr1, which likely originated from tandem duplication events. Other chromosomes contained fewer members: Chromosome 7 (Chr7) had two genes (*OsDMP14* and *OsDMP15*), while Chromosomes 2, 3, 5, 6, 8, and 12 each contained a single *OsDMP* gene (*OsDMP10*, 11, 12, 13, 16, and 17, respectively). Additionally, two genes (*OsDMP18* and *OsDMP19*) were located on the unanchored scaffold ChrUn.

3.5. Synteny and Evolutionary Analysis of *OsDMP* Genes

Gene duplication analysis revealed that both tandem and segmental duplications contributed to the expansion of the *OsDMP* family. In addition to the tandem duplication cluster on Chr1, two segmental duplication pairs were identified: *OsDMP1*/*OsDMP18* and *OsDMP3*/*OsDMP19*, as indicated by the red lines in the Circos plot (Figure 3). To further understand the evolutionary constraints acting on the duplicated *OsDMP* gene pairs, the non-synonymous substitution rate (K_a), synonymous substitution rate (K_s), and the K_a/K_s ratio were calculated (Table S3). The K_a/K_s ratio for the *OsDMP1*/*OsDMP18* pair was 0.54, which is significantly less than 1, suggesting that this gene pair has undergone purifying selection to maintain its functional conservation. Interestingly, the *OsDMP3*/*OsDMP19*

pair exhibited a Ka/Ks ratio of 1.02, which is slightly greater than 1, indicating that this pair may have been subjected to positive selection or relaxed selective constraints, potentially leading to functional diversification. These results suggest that while tandem duplication on Chr1 was the primary driver for the expansion of the *OsDMP* gene family in rice, segmental duplication followed by different selective pressures also played a crucial role in the evolution and functional divergence of the family.

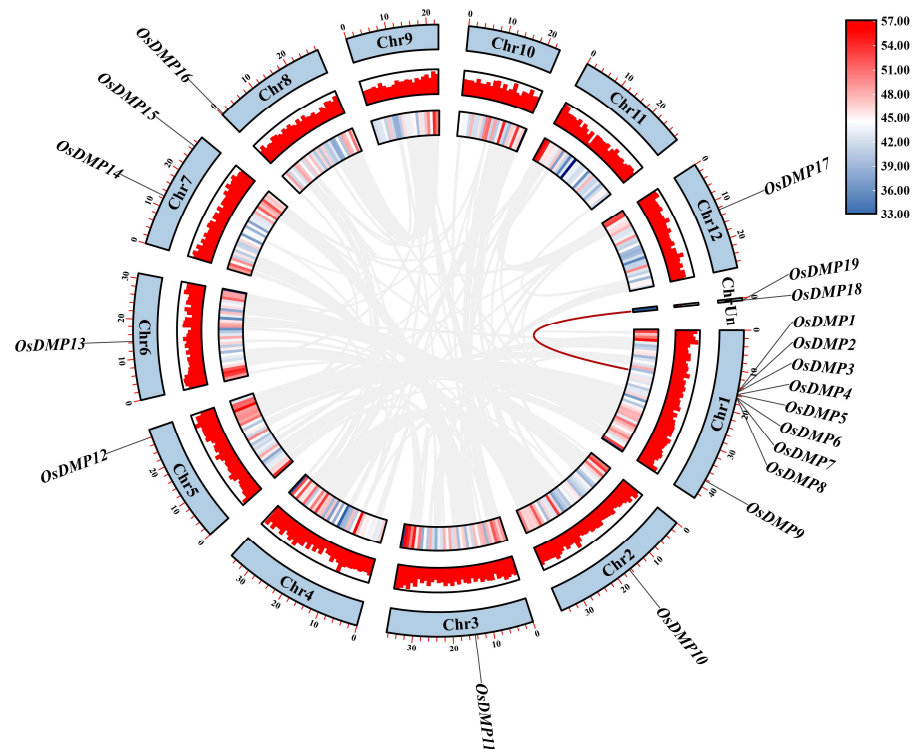


Figure 3. Chromosomal distribution and synteny analysis of *OsDMP* genes in rice. Grey lines in the center indicate whole-genome duplication or segmental duplication blocks in the rice genome, while the red lines highlight the specific segmental duplication pairs within the *OsDMP* family.

3.6. Interspecies Synteny Analysis of *OsDMP* Genes

To explore the evolutionary conservation of the *DMP* gene family, a comparative synteny analysis was performed between rice and two representative species: the dicot *Arabidopsis thaliana* and the monocot *Sorghum bicolor* (Figure 4). Multiple syntenic *DMP* gene pairs were identified between rice and sorghum, as indicated by the red lines connecting their chromosomes, which is consistent with their close phylogenetic relationship within the Poaceae family. In contrast, no syntenic *DMP* gene pairs were detected between rice and *Arabidopsis*, reflecting the substantial evolutionary divergence between monocots and dicots. These results suggest that the *OsDMP* genes share a more recent common ancestor with their sorghum orthologs and that the *DMP* family may have undergone distinct evolutionary trajectories following the monocot-dicot split.

3.7. Cis-Acting Element Analysis of *OsDMP* Gene Promoters

To investigate the potential regulatory mechanisms of *OsDMP* genes, the 2000 bp upstream promoter regions were analyzed using the PlantCARE database (Figure 5A–C). The stacked bar chart revealed that biological and abiotic stress elements were the most abundant category in nearly all *OsDMP* promoters, followed by hormone-responsive elements (Figure 5C). Notably, the total number of cis-acting elements varied considerably among family members. *OsDMP3*, *OsDMP10*, and *OsDMP17* contained the highest number of elements (exceeding 50), suggesting that these genes may be subject to more complex

transcriptional regulation. In contrast, *OsDMP11* harbored relatively fewer elements. The heatmap analysis further highlighted the differential distribution of specific elements across *OsDMP* genes (Figure 5B). *OsDMP17* contained a notably high number of STRE elements (16), which are general stress-responsive elements involved in multiple stress signaling pathways, while *OsDMP14* was enriched in ABRE elements (9), indicating a potential strong response to abscisic acid. In terms of hormone responsiveness, CGTCA-motif and TGACG-motif were widely distributed across most members, whereas ABRE elements showed a more uneven distribution, being particularly enriched in *OsDMP9*, *OsDMP14*, *OsDMP12*, and *OsDMP13*. Growth and development-related elements, including those involved in meristem expression (CAT-box) and seed-specific regulation, were relatively sparse and restricted to specific members.

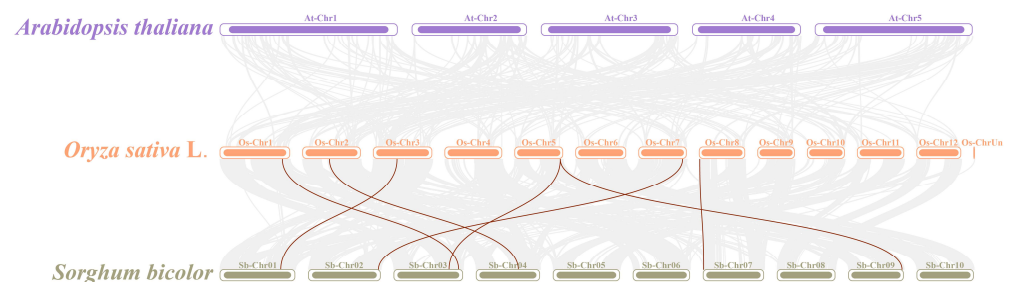


Figure 4. Interspecies synteny analysis of *DMP* genes among *Arabidopsis thaliana*, *Oryza sativa*, and *Sorghum bicolor*. The chromosomes of the three species are displayed as colored bars: purple for *A. thaliana*, orange for *O. sativa*, and olive for *S. bicolor*. Grey lines in the background indicate genome-wide collinear blocks, while red lines highlight the syntenic *DMP* gene pairs between rice and sorghum.

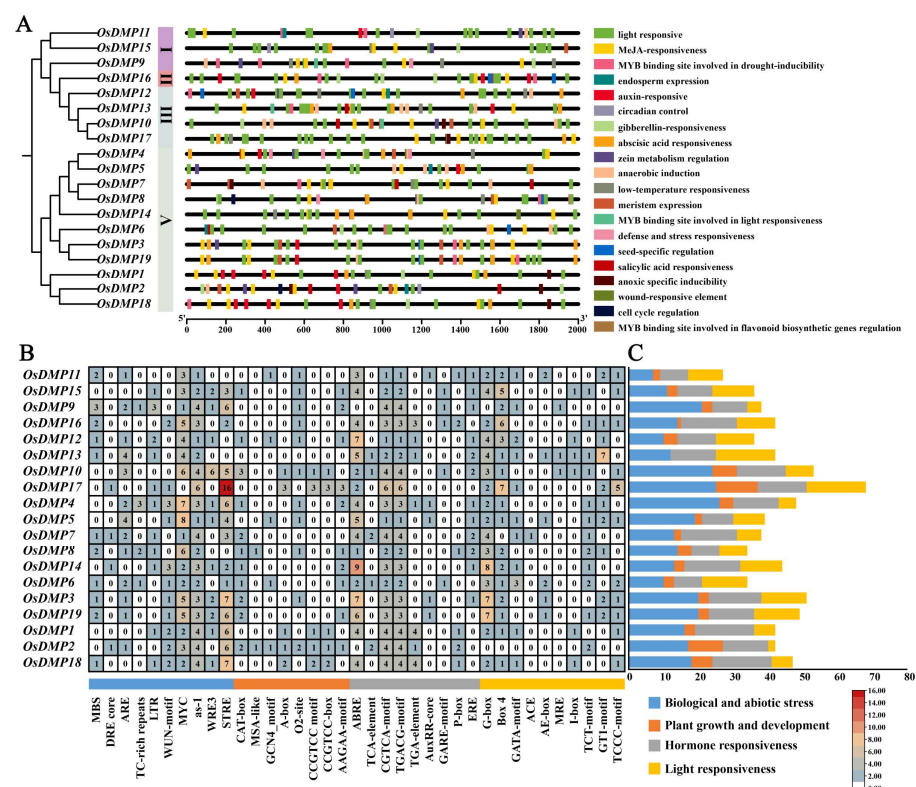


Figure 5. Cis-acting element analysis of *OsDMP* gene promoters. (A) Distribution of cis-acting elements in the 2000 bp upstream regions of *OsDMP* genes. (B) Heatmap showing the number of each element type per gene. (C) Stacked bar chart summarizing the total number of elements classified into four categories.

3.8. Tissue-Specific Expression Patterns of OsDMP Genes

To characterize the expression profiles of *OsDMP* genes, we analyzed their transcript abundance across 24 tissues and developmental stages using publicly available RNA-seq data (Figure 6). The expression patterns showed clear divergence among subfamilies and individual members. Subfamily I members (*OsDMP11* and *OsDMP15*) exhibited the highest expression levels among all *OsDMP* genes, particularly in vegetative tissues. *OsDMP11* and *OsDMP15* showed the strongest expression in root and roothair, *OsDMP11* also displayed moderate expression in shoot, leaf, ligule and sheath, suggesting their involvement in vegetative growth and development. In contrast, the majority of Subfamily V members (*OsDMP4*, 5, 7, 8, 14, 6, 3, 1, 2) showed consistently low expression across nearly all examined tissues, as indicated by the predominant dark blue coloring. This widespread transcriptional silence suggests that these genes may be expressed under specific conditions not represented in the dataset, or may function at very low transcript levels. Notably, *OsDMP6* showed a detectable expression signal specifically in pollen, implying a potential role in male gametophyte development. Subfamily II member *OsDMP16* displayed moderate expression in several tissues including root, roothair, anther, antherwall, seed, and endosperm. Subfamily III members (*OsDMP12*, 13, 10, 17) exhibited variable expression patterns: *OsDMP12* and *OsDMP13* showed relatively broad expression across vegetative tissues such as root, roothair, shoot, and leaf, while *OsDMP10* was preferentially expressed in pollen. Overall, the expression analysis revealed that *OsDMP* genes from different subfamilies exhibited distinct tissue-specific expression patterns. These divergent expression profiles are consistent with the phylogenetic classification and suggest functional differentiation among *OsDMP* subfamilies.

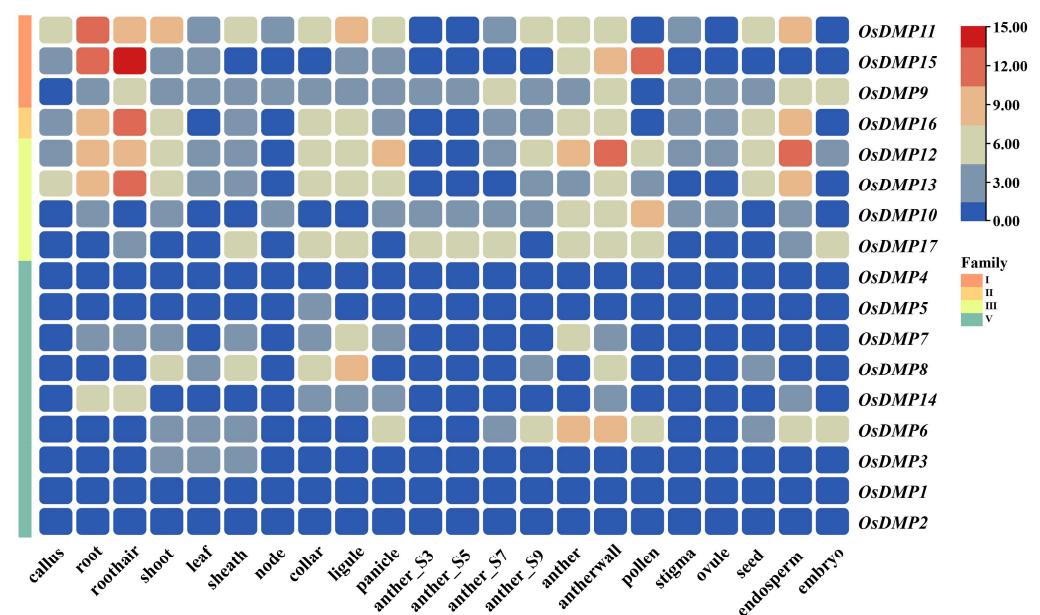


Figure 6. Expression profiles of *OsDMP* genes across different tissues and developmental stages in rice. The heatmap displays DESeq2-normalized $\log_2$ expression values. The color scale ranges from blue (low expression) to red (high expression). The left color bar indicates the subfamily classification (I, II, III, V).

3.9. Expression Profiles of *OsDMP* Genes in Response to Phytohormone Treatments

To investigate the potential roles of *OsDMP* genes in phytohormone signaling pathways, we analyzed their expression patterns under abscisic acid (ABA), ethylene, and methyl jasmonate (JA) treatments using RNA-seq data (Figure 7). The results revealed that several *OsDMP* genes exhibited distinct and time-dependent responses to these hormonal

stimuli. The most prominent response was observed under JA treatment, particularly for members of Subfamily V. *OsDMP8* showed a rapid and strong induction following JA application, with its expression level peaking at 3 h and 6 h and remaining highly elevated up to 1 day. *OsDMP7* also exhibited a significant, though more moderate, up-regulation in response to JA treatment from 1 h to 1 day. In contrast, Subfamily III members *OsDMP12* and *OsDMP11* (Subfamily I) showed a down-regulation trend under JA treatment, especially at the 3 h and 6 h time points. Under ABA treatment, *OsDMP14* (Subfamily V) displayed a gradual induction, with its highest expression level observed at 1 day post-treatment. *OsDMP13* (Subfamily III) showed a slight up-regulation at early time points (3 h to 12 h). The response to ethylene treatment was relatively less pronounced for *OsDMP* genes compared to JA and ABA. In summary, the differential expression of *OsDMP* genes under various phytohormone treatments, especially the strong induction of *OsDMP8* and *OsDMP7* by JA, suggests that these genes are transcriptionally integrated into hormone signaling networks, although their specific functional contributions remain to be experimentally determined. These findings are consistent with the presence of numerous hormone-responsive cis-acting elements identified in their promoter regions.

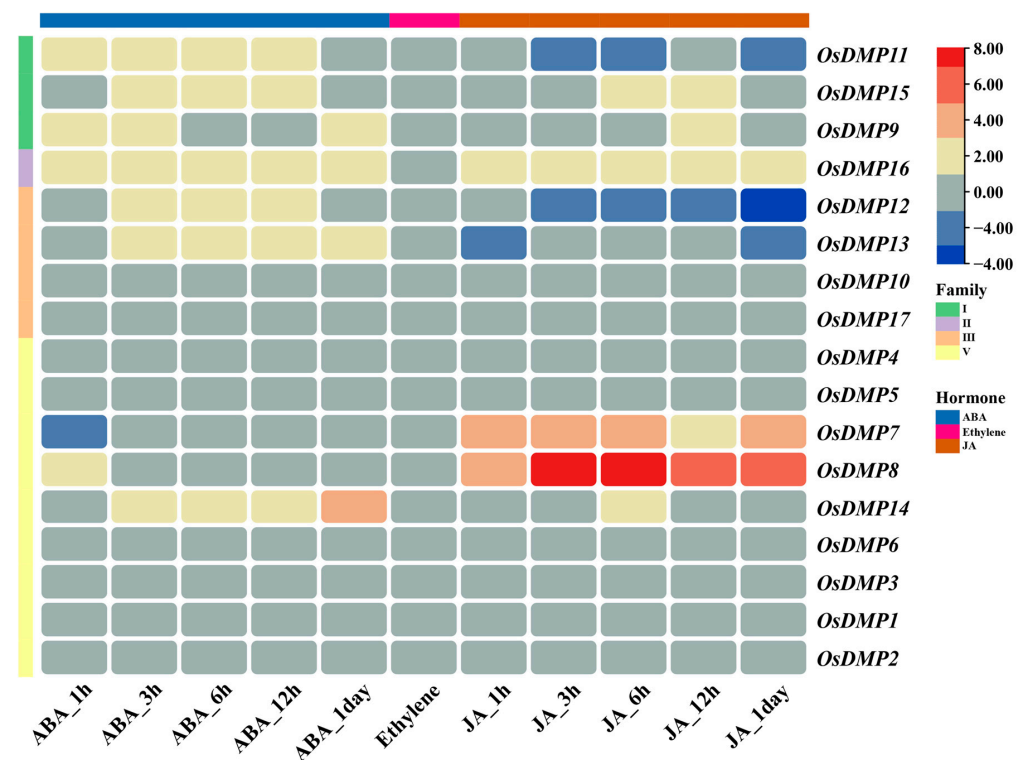


Figure 7. Expression profiles of *OsDMP* genes in response to different phytohormone treatments. The heatmap displays $\log_2$ fold change values relative to control conditions. The color scale ranges from blue (down-regulation) to red (up-regulation). The left color bar indicates the subfamily classification (I, II, III, V), and the top color bar indicates the hormone treatment.

3.10. Expression Analysis of *OsDMP* Genes Under Abiotic Stress via qRT-PCR

To validate the response of *OsDMP* genes to abiotic stresses, we performed qRT-PCR analysis on eight representative members from different subfamilies under PEG-induced drought stress and NaCl-induced salt stress (Figure 8A,B). Under PEG-induced drought stress, members of Subfamily II and Subfamily V showed the most significant up-regulation (Figure 8A). Subfamily II member *OsDMP16* exhibited a dramatic response, with its expression level increasing rapidly and peaking at 12 h. Within Subfamily V, *OsDMP5* displayed a strong and sustained up-regulation, reaching its maximum at 24 h, while *OsDMP3* showed a transient induction peaking at 3 h. *OsDMP4* remained relatively stable under

PEG treatment. In Subfamily III, *OsDMP10* showed a strong induction peaking at 6 h, whereas *OsDMP17* showed only minor fluctuations. In Subfamily I, *OsDMP9* showed a steady increase in transcript abundance from 6 h to 24 h, while *OsDMP15* remained stable. Under NaCl-induced salt stress, the expression patterns were markedly different and often subfamily-specific (Figure 8B). Subfamily V members showed highly divergent responses: *OsDMP3* and *OsDMP5* were significantly induced, both peaking at 6 h, whereas *OsDMP4* showed no significant change. In Subfamily III, *OsDMP10* and *OsDMP17* exhibited a rapid and severe decrease in expression, with *OsDMP10* transcript levels becoming almost undetectable by 6 h. In Subfamily I, *OsDMP15* showed a significant down-regulation, with its expression becoming nearly undetectable by 24 h, while *OsDMP9* remained relatively stable. Subfamily II member *OsDMP16* showed only minor fluctuations under salt stress. In summary, the qRT-PCR results confirm that *OsDMP* genes are highly responsive to abiotic stresses, with their expression patterns being closely linked to their phylogenetic classification. These findings provide a solid foundation for further functional characterization of the *OsDMP* family, and future studies employing genetic approaches will be necessary to determine whether these transcriptional responses contribute to enhanced stress tolerance.

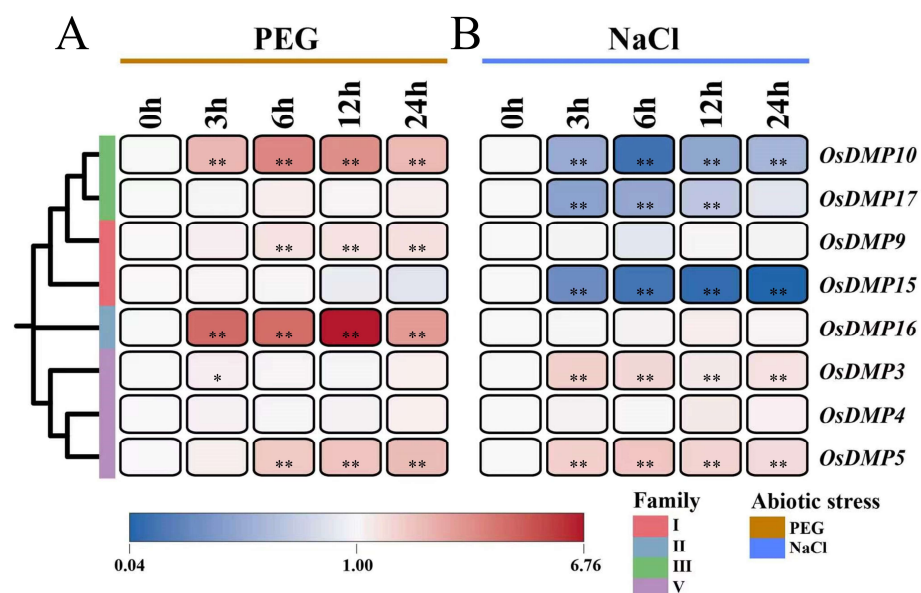


Figure 8. Expression analysis of eight *OsDMP* genes under PEG and NaCl treatments. (A) PEG-induced drought stress (yellow). (B) NaCl-induced salt stress (blue). Values calculated from three biological replicates each with three technical replicates. * Significance at $p < 0.05$, ** extremely significance at $p < 0.01$.

3.11. Subcellular Localization Verification of *OsDMP16*

To experimentally validate the computationally predicted subcellular localization of *OsDMP* proteins, we performed transient expression of *OsDMP16*-GFP in rice protoplasts. *OsDMP16* was selected as a representative member due to its significant transcriptional response to drought stress and its membership in Subfamily II. Confocal microscopy revealed that the *OsDMP16*-GFP fusion protein exhibited a perinuclear reticular fluorescence pattern that completely overlapped with the mCherry-HDEL ER marker signal, with no detectable signal in the nucleus (Figure 9A). In contrast, the empty vector control showed free GFP distributed in both the nucleus and partial overlap with the ER marker, while the ER marker itself showed the characteristic perinuclear continuous structure without nuclear signal (Figure 9B). The distinct patterns—exclusive ER localization for *OsDMP16*-GFP versus nucleocytoplasmic distribution with partial ER association for free GFP—confirm

the specific ER targeting of OsDMP16. This result experimentally confirms that OsDMP16 is an ER-resident membrane protein, which is consistent with the WoLF PSORT prediction for this member. The ER localization of OsDMP16 suggests that it may function in membrane-associated processes within the secretory pathway, potentially participating in protein folding, lipid metabolism, or stress-related signaling at the ER. Whether other OsDMP family members share similar ER localization or exhibit distinct subcellular distributions remains an open question that warrants further investigation.

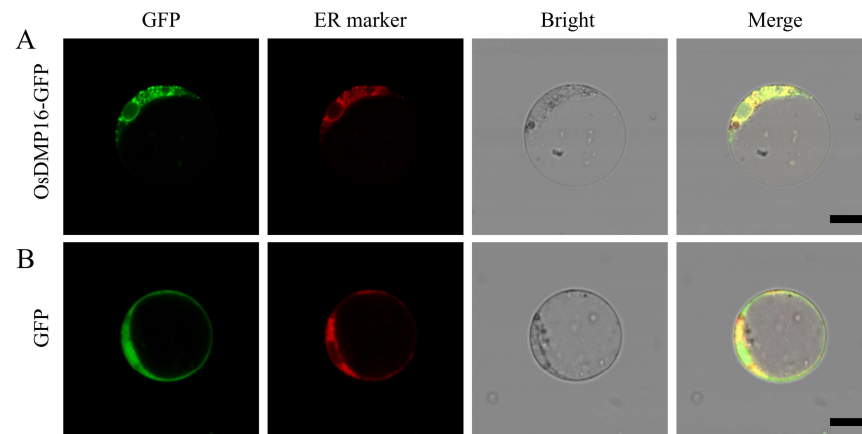


Figure 9. Subcellular localization of OsDMP16 in rice protoplasts. (A) OsDMP16-GFP fusion protein co-expressed with an ER marker in rice protoplasts. (B) Empty GFP vector control co-expressed with the ER marker. Scale bars = 10 μ m.

4. Discussion

The *DMP* gene family represents a unique class of plant-specific membrane proteins that play critical roles in reproductive development, particularly in vivo HI, as well as in various physiological and stress response processes [14,36,37]. Despite their significance, a comprehensive genome-wide characterization of the *DMP* family in rice has remained limited. In this study, we identified 19 *OsDMP* genes and performed a systematic analysis of their evolution, structure, and expression patterns. Our findings provide novel insights into the functional diversification and evolutionary trajectory of the *DMP* family in one of the world's most important cereal crops [38]. Notably, this study was designed as a genome-wide, family-level characterization to generate a prioritized candidate list for future functional work, rather than as a single-gene mechanistic investigation. We therefore focused experimental validation on drought and salt stress—the predominant constraints on rice production in northeastern China where the study germplasm originates—while acknowledging that cold-response testing, additional hormone validation, and genetic manipulation of individual candidates remain important directions for subsequent studies.

4.1. Evolution, Expansion, and Structural Characteristics of the *OsDMP* Family

The identification of 19 *OsDMP* genes in the rice genome reveals a family size comparable to other major crops, such as 21 in peanut (*Arachis hypogaea*) [39] and 33 in wheat (*Triticum aestivum*) [25], but significantly larger than the 10 members found in the model dicot *Arabidopsis thaliana* [8]. This expansion suggests that the *DMP* family has undergone extensive diversification in monocots, potentially driven by the complex genomic landscapes of these species [40]. Our results indicate that tandem duplication was a primary driver of this expansion, particularly on Chromosome 1, where a prominent cluster of eight genes (*OsDMP1* to *OsDMP8*) was identified. Such clusters are often associated with rapid adaptation to environmental changes or specialized developmental processes [6,41]. Additionally, segmental duplication events, such as the

OsDMP1/OsDMP18 and *OsDMP3/OsDMP19* pairs, further contributed to the family's growth. The Ka/Ks analysis revealed that while *OsDMP1/OsDMP18* underwent strong purifying selection (Ka/Ks = 0.54) to maintain functional conservation, the *OsDMP3/OsDMP19* pair (Ka/Ks = 1.02) showed signs of positive selection or relaxed constraints, potentially leading to functional neo-functionalization or sub-functionalization [42]. Despite this expansion, the OsDMP family maintains high structural conservation, with all 19 proteins harboring the characteristic DUF679 domain. A notable observation from the genome annotation was the predicted presence of two tandem DUF679 domains in *OsDMP5*. While most DMP proteins are relatively small and contain a single DUF679 domain, this putative dual-domain architecture of *OsDMP5* is unusual among characterized DMP family members. However, we acknowledge that this structural feature is derived solely from computational prediction based on the current rice genome assembly, and experimental verification through full-length cDNA cloning or RACE has not yet been performed. Therefore, the possibility that this represents a genome assembly or annotation artifact cannot be ruled out. If experimentally confirmed, this dual-domain structure may represent an evolutionary innovation in rice, possibly enhancing its interaction with other membrane-associated proteins [13,43]. Notably, all 19 *OsDMP* genes were found to be intronless, a hallmark characteristic of the DMP family that is thought to facilitate rapid transcriptional responses to environmental stimuli by bypassing the time-consuming splicing process [6,39]. Furthermore, our subcellular localization experiment using transient expression of *OsDMP16*-GFP in rice protoplasts confirmed that *OsDMP16* is localized to the endoplasmic reticulum. While this provides direct experimental evidence for one representative member, it should be noted that this result cannot be generalized to the entire *OsDMP* family, as individual members may exhibit distinct subcellular distributions depending on their sequence divergence and functional specialization. The ER localization of *OsDMP16* suggests that this protein may participate in ER-associated processes such as membrane lipid metabolism, protein quality control, or stress-related signaling within the secretory pathway, which could be relevant to its observed transcriptional responsiveness to drought stress. Phylogenetic analysis categorized the *OsDMP* genes into five distinct subfamilies (I–V), consistent with previous reports in other species [6,15,44]. The interspecies synteny analysis further highlighted the evolutionary divergence between monocots and dicots, underscoring the significant differentiation of the DMP family following the monocot-dicot split [45].

4.2. Regulatory Mechanisms and Response to Abiotic Stress

The promoter analysis revealed a high density of stress-responsive cis-acting elements, such as STRE, MYC, and ABRE, across the *OsDMP* family. This suggests that *OsDMP* genes may be transcriptionally responsive to environmental challenges, although it remains to be determined whether these expression changes reflect an active adaptive mechanism or a secondary consequence of stress-induced cellular perturbations. Our qRT-PCR results confirmed this, showing significant up-regulation of several members under PEG-induced drought and NaCl-induced salt stress. Notably, *OsDMP16* (Subfamily II) exhibited a dramatic 7-fold induction under drought stress, while *OsDMP5* (Subfamily V) showed sustained up-regulation under both drought and salt stress. These findings are consistent with recent reports in sugar beet and peanut, where specific DMP genes were found to be transcriptionally responsive to abiotic stress [12,39]. Furthermore, the strong induction of *OsDMP8* and *OsDMP7* by methyl jasmonate (JA) treatment, coupled with the abundance of JA-responsive elements (CGTCA-motif) in their promoters, points to a role in the jasmonate-mediated signaling pathway. JA is a key hormone involved in both defense against pathogens and response to abiotic stresses like wounding and cold [15,46]. The

differential response of *OsDMP* genes to various hormones and stresses highlights the functional specialization within the family, where different subfamilies or individual members have evolved to respond to specific environmental cues. The presence of these elements, combined with the observed expression patterns, suggests that the *OsDMP* family may play a broader role in plant resilience than previously recognized, extending beyond its established functions in reproductive development [47]. In this study, although our promoter analysis identified various cis-acting elements related to cold response and hormone signaling (such as ABA and JA), we specifically prioritized the experimental validation of drought and salt stress responses. This focus reflects the regional agronomic context of northeastern China, where drought and salinity represent the most prevalent environmental constraints on rice production, and where the study germplasm (cv. Liaoxing 1) is cultivated. Validation of cold-responsive and additional hormone-mediated pathways remains an important direction for future work. However, it is important to note that transcriptional responsiveness alone does not establish a causal role in stress tolerance; the observed expression changes could also represent downstream consequences of general stress signaling rather than active adaptive mechanisms. Future functional studies, such as overexpression or knockout analyses, will be essential to distinguish between these possibilities.

4.3. Potential Roles in Reproductive Development and Haploid Induction

One of the most exciting aspects of DMP research is its application in in vivo HI. In maize and Arabidopsis, mutations in *DMP* genes have been shown to trigger or significantly enhance HI rates [8,14]. Our tissue-specific expression analysis identified pollen-specific expression for *OsDMP6* and *OsDMP10*, suggesting their involvement in male gametophyte development, a key stage for HI. Recent studies in rice have demonstrated that while *OsMATL* is the primary gene for HI, specific *OsDMP* genes, such as *OsDMP16* (corresponding to previously reported *DMP1*) or *OsDMP6* (corresponding to previously reported *DMP3*), can exert a synergistic effect, significantly increasing the induction efficiency when mutated alongside *OsMATL* [15,48]. The development of efficient HI systems is a major goal in rice breeding to accelerate the production of DH lines [20]. *OsDMP16* (Subfamily II) and *OsDMP6* (Subfamily V) have been proposed as potential synergistic partners for *OsMATL*, suggesting possible functional specialization within specific subfamilies. However, it should be emphasized that this synergistic role is derived from published external data rather than from original experimental evidence in the present study [15]. The pollen-specific expression of *OsDMP6* and *OsDMP10* further supports their potential role in male gametophyte development and fertilization processes. Unlike the primary HI gene *OsMATL*, which is essential for triggering the initial induction, these *OsDMP* members have been hypothesized to function as modulators of fertilization efficiency or membrane stability during gamete fusion, based on their characteristic DUF679 transmembrane structure and published functional studies [8,14]. This hypothesis, however, awaits direct experimental validation in future work. Furthermore, the synergistic effect observed between *OsMATL* and specific *OsDMP* genes suggests a complex molecular network governing in vivo HI in rice. Future breeding strategies could leverage CRISPR/Cas9-mediated multiplex genome editing to simultaneously target *OsMATL* and its *OsDMP* partners, such as *OsDMP16* and *OsDMP6*, to achieve higher induction rates while minimizing negative impacts on seed set and plant vigor. Additionally, exploring the natural allelic variation in these *OsDMP* candidates across diverse rice germplasm may reveal superior alleles for developing more robust HI platforms. Beyond gene knockout, promoter engineering to fine-tune the expression levels of *OsDMP6* or *OsDMP10* in a tissue-specific manner could offer a more precise approach to balancing induction efficiency and reproductive

fitness. Future research focusing on the protein–protein interactions between OsMATL and OsDMP proteins, as well as their downstream signaling components, will be essential to fully decipher the molecular mechanism of HI and to harness the full potential of the DMP family for accelerating rice genetic improvement and global food security [49].

4.4. Limitations of the Study

While this study provides a comprehensive genome-wide characterization of the OsDMP family, several aspects remain to be addressed in future work. The atypical dual-domain architecture observed in OsDMP5 is currently inferred from genome annotation and domain prediction algorithms; full-length cDNA cloning or RACE experiments would help confirm whether this structure is genuinely encoded or reflects an annotation artifact, and we have flagged this as a priority for follow-up investigation. Regarding subcellular localization, our transient expression assay confirmed ER residency for OsDMP16 as a representative member. The partial overlap between free GFP and the ER marker in control experiments may reflect non-specific ER accumulation of highly expressed free GFP or spectral overlap between channels; however, the exclusive perinuclear ER localization of OsDMP16-GFP (without nuclear signal) clearly distinguishes it from the nucleocytoplasmic distribution of free GFP. Extending this validation to additional family members across different subfamilies would provide a more complete picture of the spatial distribution of OsDMP proteins within the cell. Similarly, the tissue-specific expression patterns discussed herein were retrieved from well-curated public transcriptomic databases, which offer broad coverage across developmental stages and tissues; nonetheless, complementary expression analyses using the authors' own experimental material under defined conditions would further strengthen these observations. Finally, while our qRT-PCR results clearly demonstrate transcriptional responsiveness of several *OsDMP* candidates to drought and salt stress, establishing whether these expression changes confer measurable stress tolerance will require future functional approaches, including overexpression, loss-of-function mutants, or CRISPR/Cas9-mediated gene editing, which are currently underway in our laboratory.

5. Conclusions

In summary, this study identified and characterized 19 *OsDMP* genes in rice, which elucidated their evolutionary relationships, structural features, and expression patterns under abiotic stress. A notable finding was that all 19 *OsDMP* members are intronless, a characteristic that likely contributes to their rapid transcriptional response capabilities. Phylogenetic analysis classified these genes into five subfamilies, highlighting significant functional diversification within the family. Tandem and segmental duplications were identified as major drivers of OsDMP family expansion, suggesting adaptive evolution in response to environmental pressures. Promoter analysis unveiled abundant stress- and hormone-responsive cis-acting elements, which correlated with diverse tissue-specific expression profiles and significant transcriptional responses to drought, salt, and hormonal stimuli. Additionally, our study identified promising candidates, particularly *OsDMP16* and *OsDMP6*, whose expression patterns and phylogenetic positions suggest their potential as candidate genes for future functional studies on stress adaptation and reproductive development. These genes represent candidate genes for future functional studies to validate their roles in stress tolerance and haploid induction.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16181844/s1>, Figure S1: Chromosomal distribution of *OsDMP* genes in rice; Table S1: List of primers used in this study; Table S2: Physicochemical properties and subcellular localization predictions of OsDMP proteins; Table S3: Ka/Ks analysis of duplicated OsDMP gene pairs.

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