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Genomic identification and expression profiling of *DMP* genes in oat (*Avena sativa*) elucidate their responsiveness to seed aging

Yuan Ma[1†], Huan Liu[1*†], Jinglong Wang[2], Guiqin Zhao[1], Kuiju Niu[1], Xiangrui Zhou[1], Ran Zhang[3] and Ruirui Yao[1]

Abstract

Background The Domain of unknown function 679 membrane protein (DMP) family, which is unique to plants, plays a crucial role in reproductive development, stress response and aging. A comprehensive study was conducted to identify the *DMP* gene members of oat (*Avena sativa*) and to investigate their structural features and tissue-specific expression profiles. Utilizing whole genome and transcriptome data, we analyzed the physicochemical properties, gene structure, cis-acting elements, phylogenetic relationships, conserved structural (CS) domains, CS motifs and expression patterns of the *AsDMP* family in *A. sativa*.

Results The *DMP* family genes of *A. sativa* were distributed across 17 chromosomal scaffolds, encompassing a total of 33 members. Based on phylogenetic relationships, the *AsDMP* genes were classified into five distinct subfamilies. The gene structure also suggests that *A. sativa* may have undergone an intron loss event during its evolution. Covariance analysis indicates that genome-wide duplication and segmental duplication may be the major contributor to the expansion of the *AsDMP* gene family. Ka/Ks selective pressure analysis of the *AsDMP* gene family suggests that *DMP* gene pairs are generally conserved over evolutionary time. The upstream promoters of these genes contain several cis-acting elements, suggesting a potential role in abiotic stress responses and hormone induction. Transcriptome data revealed that the expression patterns of the *DMP* genes are involved in tissue and organ development. In this study, the *AsDMP* genes (*AsDMP1*, *AsDMP19*, and *AsDMP22*) were identified as potential regulators of seed senescence in *A. sativa*. These genes could serve as candidates for breeding studies focused on seed longevity and anti-aging germplasm in *A. sativa*. The study provides valuable insights into the regulatory mechanisms of the *AsDMP* gene family in the aging process of *A. sativa* germplasm and offers theoretical support for further function investigation into the functions of *AsDMP* genes and the molecular mechanisms underlying seed anti-aging.

Conclusions This study identified the *AsDMP* genes as being involved in the aging process of *A. sativa* seeds, marking the first report on the potential role of *DMP* genes in seed aging for *A. sativa*.

Keywords *Avena sativa*, *DMP* gene family, Gene expression, Seed aging, Expression profiling

[†]Yuan Ma and Huan Liu contributed equally to this work and are considered co-first authors.

*Correspondence:
Huan Liu
liuhuan@gsau.edu.cn

[1]Key Laboratory of Grassland Ecosystems, College of Grassland Science, Gansu Agricultural University, Lanzhou 730070, China
[2]Tibet Grassland Science Research Institute, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850000, China
[3]Institute of Ecological Protection and Restoration, Chinese Academy of Forestry, Grassland Research Center, National Forestry and Grassland Administration, Beijing 100091, China

Introduction

Membrane proteins are essential for numerous biological processes, such as cell proliferation and differentiation, signal transduction and recognition, and material transport [1–4]. The DMP (Domain of unknown function 679 membrane proteins) family comprises plant-specific genes that encode membrane proteins [5]. The DMP family genes commonly contains four transmembrane domains with cytoplasmic amino and carboxyl termini. It is involved in various physiological processes, particularly in plant reproductive development and senescence [6, 7]. At the whole-genome level of *Arabidopsis thaliana*, a total of 10 members of *DMP* gene family have been identified, each exhibiting distinct expression patterns across various tissues and organs. Among these, *AtDMP1*, *AtDMP2*, *AtDMP3*, *AtDMP4*, and *AtDMP7* are involved in various types of programmed cell death, including organ senescence, silique dehiscence, and abscission of floral organs and siliques, while *AtDMP8* and *AtDMP9* promote gamete fusion during double fertilization [5, 8, 9]. The *AtDMP1* is reported to be up-regulated during both developmental senescence and darkness-induced senescence, with its expression increasing from the onset to late stages of senescence. This suggests that *AtDMP1* plays a consistent role in developmental senescence and induced senescence. In contrast, *AtDMP3* and *AtDMP4* are upregulated specifically during leaf senescence, indicating that they may have overlapping functions in this process [9, 10]. Studies have shown that both DMP1 T-DNA insertion mutants and DMP1 overexpressing plants age earlier than the wild type. Additionally, the aging-specific transcriptional activation of DMP1 is regulated by WRKY transcription factors [11]. Mutations in the two W-boxes (homologous binding sites for WRKY proteins) within the DMP1 promoter cause the loss of DMP1 expression during aging [11, 12]. In addition, DMP1 directly or indirectly participates in membrane division associated with endoplasmic reticulum decomposition in leaf aging, membrane fusion during root vacuole formation [7], and dual targeting of the vacuole membrane and plasma membrane [13]. The *DMP* gene has been extensively studied for haploid induction. Haploid induction systems have been established for polyploid mothers in *A. thaliana*, *Zea mays*, *Brassica napus*, and *Nicotiana tabacum* [14–16]. The ability of the *DMP* gene to induce haploids in polyploid monodicotyledonous crops was further confirmed. In *Gossypium hirsutum*, a systematic analysis identified 58 *DMP* genes. Analysis of their expression patterns revealed potential involvement in key biological processes, including plant aging, reproductive development, and stress response [17]. Developmentally programmed cell death (dPCD) genes that have been reported include *BIFUNCTIONAL NUCLEASE1*(*BFN1*), aspartic protease *PASPA3*, *RIBONUCLEASE3*(*RNS3*), *CYSTEIN ENDOPEPDITASE*(*CEP1*), *DOMAIN OF UNKNOWN FUNCTION679 MEMBRANE PROTEIN4*(*DMP4*), and *EXITUS1*(*EXI1*) [18]. During stigma senescence in *A. thaliana*, *AtDMP4*, along with BFN1, RNS3, EXI1, CEP1 and PASPA3, promotes both senescence and the dPCD process [19]. Importantly, DMP4 is the only protein among DMP proteins that interacts with DMP1 [11]. Studies have also identified genes associated with seed aging in several other crops. For example, *WRKY* genes in *Medicago sativa* have been shown to influence seed vigor [20]. In oat (*Avena sativa*), the *Glutathione Reductase*(*GR*) gene family is recognized for its role in seed aging [21]. Furthermore, the lipoxygenase family in *Cicer arietinum* is involved in regulating the artificial aging process [22].

Seed "aging", or "deterioration", is a common phenomenon during the storage of agricultural and pasture seeds. As storage time increases, it often results in irreversible declines in seed viability vigor, and germination ability [23, 24]. Seed aging can occur through natural or artificial accelerated processes. Natural aging involves the gradual loss of seed vigor after maturity under typical environmental conditions. In contrast, artificial accelerated aging accelerates the aging process through controlled conditions, leading to a rapid decline in seed vigor. This method is widely used to study seed deterioration and evaluate storage resistance [25, 26].

Avena sativa is an important grass crop known for its excellent nutritional quality and high economic value. It plays a significant role in livestock production [27]. Additionally, it helps reduce grazing pressure on grasslands, aids in the recovery of degraded grasslands, and contributes to ecological protection. In areas with fragile ecological conditions, it is regarded as an irreplaceable food and fodder crop in [28]. The aging of *A. sativa* seeds affects not only seed and seedling growth, yield and quality, but also the conservation, utilization and development of germplasm resources. Therefore, mitigating the economic losses caused by the decline in quality of *A. sativa* seeds due to aging and deterioration is of great significance in agricultural production. DMP proteins, which are membrane proteins found almost exclusively in green plants, have been systematically analyzed in *A. thaliana* [6], *G. hirsutum* [17], and *Glycine max* [9]. In addition, DMP proteins have been characterized in several species, including *Oryza sativa* (20), *Vitis vinifera* (7), *Z. mays* (15), *Sorghum bicolor* (15), and *Ananas comosus* (9). These studies have highlighted the involvement of DMPs in various biological processes, such as promoting gamete fusion during double fertilization, inducing maternal haploid production, and contributing to plant senescence, reproductive development, and stress response [17]. To date, most research on *DMP*

genes has focused on haploid breeding, with relatively few studies investigating their role in aging. The response of *DMP* gene family members in *A. sativa* to aging stress remains unknown, and there is a notable lack of studies on the expression and function of *DMP* genes in *A. sativa* seeds during aging. In this study, we identified 33 members of the *DMP* gene family from the *A. sativa* genome and analyzed their phylogenetic relationships. using bioinformatics tools, we conducted a comprehensive analysis of physicochemical properties, protein structures, subcellular localization, conserved motifs, and chromosomal location of the AsDMP family members. We also characterized their expression patterns, which will provide theoretical support for further investigation into the molecular mechanisms of the *AsDMP* genes and breeding research.

Results

Identification of the DMP proteins in oat

Thirty-three DMP sequences were identified and designated as *AsDMP1* through *AsDMP33* based on their chromosomal positions (Table 1). In addition, we determined the physicochemical properties of these *AsDMP* genes, including amino acid sequence length (aa), isoelectric point (pI), protein molecular weight (kDa), instability index, aliphatic index, and subcellular localization. All 33 *DMP* genes encode proteins with amino acid lengths ranging from 169 (*AsDMP6*) to 325 (*AsDMP24*) aa. Theoretical isoelectric points (pI) range from 5.26 (*AsDMP28*) to 9.04 (*AsDMP33*). Molecular weight range 17.88 (*AsDMP6*) to 35.50 (*AsDMP24*) kDa. The predicted aliphatic index range was 76.04 to 107.62. The aliphatic index reflects the thermal stability of proteins. Additionally, hydrophilicity and hydrophobicity of proteins is one of the most important factors affecting the structural stability of proteins. All AsDMP proteins were analyzed and found to be hydrophilic (Fig. 1), suggesting their transmembrane nature. In addition, all AsDMP proteins contained between 3 and 4 transmembrane structural domains and lacked signal peptides, suggesting that they are transmembrane, non-secretory proteins. Predictions of the subcellular localization of DMP proteins indicate that all proteins are localized to the plasma membrane, aligning with the known function of DMPs. In addition, AsDMP14, AsDMP19, AsDMP24, AsDMP25, AsDMP26 and AsDMP32 were localized extracellularly, while AsDMP15 and AsDMP21 were found in chloroplasts. These findings suggest that DMPs also regulate biological processes both inside and outside the plasma membrane.

Protein structure prediction of oat DMP family members

The main secondary structures of proteins inlcude alpha-helixces(α-helixes), beta-sheets(β-sheets), beta-turns(β-turns), random coils, and extended chains. The predicted secondary structure of AsDMP, based on its amino acid sequence, mainly consists of α-helices, β-turns, random coils and extended chains (Table 2). Furthermore, α-helixes and random coils account for a significant proportion of the secondary structure in all AsDMPs members, suggesting that the amino acid secondary structure of AsDMPs is primarily composed of α-helices and random coils. By predicting the tertiary structures of AsDMP family proteins, it was observed that AsDMP1, AsDMP2, AsDMP3, AsDMP4, AsDMP18, AsDMP20, AsDMP28, AsDMP29, AsDMP5, AsDMP7, AsDMP8, AsDMP13, AsDMP31, AsDMP10, AsDMP16, AsDMP22, AsDMP11, AsDMP12, AsDMP15, AsDMP21, AsDMP23, AsDMP24, AsDMP19, AsDMP25, AsDMP26, AsDMP27, and AsDMP30 proteins showed a high degree of similarity. This high degree of structural similarity suggests potential functional similarities. However, these groups displayed distinct morphologies from one another and differed from the tertiary structures of other family members, suggesting a diversity of tertiary structures within the AsDMP family (Fig. 2).

Phylogenetic analysis of *DMP* gene family in oat

To analyze the phylogenetic relationships among DMP family members from different species, we constructed phylogenetic trees for *A. sativa*, *A. thaliana*, *Z. mays*, *O. sativa*, and *S. bicolor* using the neighbor-joining (NJ) method with MEGA 11 software (version 11.0.10). The phylogenetic tree analysis revealed that the plant *DMP* gene family can be classified into five subfamilies: I, II, III, IV and V (Fig. 3). Subfamily V contains the largest number of DMPs, with a total of 40 genes, including 15 AsDMPs. This was followed by subfamilies III, I, IV, and II, with 23, 15, 10, and 5 genes, respectively, and 8, 7, 3, and 0 of these being AsDMPs. The *AsDMP* gene has experienced a significant expansion compared to the *DMP* genes in *A. thaliana*, *Z. mays*, *O. sativa*, and *S. bicolor*. Additionally, the branching clustering pattern of AsDMP proteins closely resembles that reported for *G. hirsutum* [17].

Conserved motifs of the oat *DMP* gene and analysis of gene structure

To further elucidate the diversity and conservation of the *AsDMP* gene family during evolution, we analyzed the conserved motifs, conserved domains and gene structures based on phylogeny (Fig. 4). A total of 10 motifs were predicted using the MEME website (http://meme-suite.org/tools/meme) (Figs. 4B and 5), with each DMP protein containing between 4 and 9 motifs. All DMP members shared Motif 3, 4, and 5, suggesting that these motifs are highly conserved among the 33 AsDMP members. The basal composition and distribution of DMP members within the same subfamily are largely

Table 1 Physico-chemical properties of DMP family in *A. sativa*: an in-depth overview of amino acid sequence length, isoelectric point, molecular weight, instability index, aliphatic index, transmembrane domains, and subcellular localization

Gene name	Gene ID	Protein length (aa)	Isoelectric point (PI)	Molecular weight (MW)/kDa	Instability index	Aliphatic index	Transmembrane domains	signal peptide	Predicted subcellular localization
AsDMP1	*AVESA.00010b.r2.1AG0036510.1*	239	6.50	25.35	50.20	96.78	4	NO	PlasmaMembrane
AsDMP2	*AVESA.00010b.r2.1AG0004840.1*	236	6.03	25.01	44.79	97.58	4	NO	PlasmaMembrane
AsDMP3	*AVESA.00010b.r2.1DG0155630.1*	235	6.28	24.95	47.51	99.23	4	NO	PlasmaMembrane
AsDMP4	*AVESA.00010b.r2.2CG0263910.1*	284	6.22	30.45	34.11	97.29	4	NO	PlasmaMembrane
AsDMP5	*AVESA.00010b.r2.2DG0399840.1*	215	8.40	22.92	37.84	90.37	4	NO	PlasmaMembrane
AsDMP6	*AVESA.00010b.r2.2DG0382070.1*	169	7.58	17.88	47.24	86.75	3	NO	PlasmaMembrane
AsDMP7	*AVESA.00010b.r2.3AG0415880.1*	216	6.03	22.95	31.51	90.37	4	NO	PlasmaMembrane
AsDMP8	*AVESA.00010b.r2.3CG0462960.1*	216	6.17	23.21	28.56	95.32	4	NO	PlasmaMembrane
AsDMP9	*AVESA.00010b.r2.3CG0495930.1*	173	7.73	18.60	25.81	103.12	4	NO	PlasmaMembrane
AsDMP10	*AVESA.00010b.r2.3CG0514780.1*	219	7.69	24.54	50.08	98.36	4	NO	PlasmaMembrane
AsDMP11	*AVESA.00010b.r2.3CG0515720.1*	231	6.82	24.79	43.59	83.25	4	NO	PlasmaMembrane
AsDMP12	*AVESA.00010b.r2.3CG0515740.1*	237	7.69	25.30	32.07	83.21	4	NO	PlasmaMembrane
AsDMP13	*AVESA.00010b.r2.3DG0517300.1*	216	6.03	22.93	30.89	92.18	4	NO	PlasmaMembrane
AsDMP14	*AVESA.00010b.r2.3DG0543000.1*	226	5.92	24.87	42.77	96.73	3	NO	PlasmaMembrane、Extracellular
AsDMP15	*AVESA.00010b.r2.4AG0601280.1*	230	9.01	24.65	42.54	76.04	4	NO	PlasmaMembrane、Chloroplast
AsDMP16	*AVESA.00010b.r2.4AG0640750.1*	218	8.30	24.35	44.69	99.72	4	NO	PlasmaMembrane
AsDMP17	*AVESA.00010b.r2.4AG0641780.1*	233	6.29	24.95	46.68	83.82	4	NO	PlasmaMembrane
AsDMP18	*AVESA.00010b.r2.4CG1263690.1*	185	6.70	19.46	22.93	107.62	4	NO	PlasmaMembrane
AsDMP19	*AVESA.00010b.r2.4CG1314570.1*	193	8.38	20.80	32.02	82.44	4	NO	PlasmaMembrane、Extracellular
AsDMP20	*AVESA.00010b.r2.4DG0745150.1*	181	8.46	20.13	39.60	84.09	4	NO	PlasmaMembrane
AsDMP21	*AVESA.00010b.r2.4DG0745170.1*	229	7.75	24.56	44.47	76.42	4	NO	PlasmaMembrane、Chloroplast
AsDMP22	*AVESA.00010b.r2.4DG0768120.1*	177	6.41	19.36	42.26	86.05	3	NO	PlasmaMembrane
AsDMP23	*AVESA.00010b.r2.4DG0769210.1*	233	6.07	24.97	46.92	81.33	4	NO	PlasmaMembrane
AsDMP24	*AVESA.00010b.r2.4DG0769220.1*	325	8.27	35.50	30.29	77.78	4	NO	PlasmaMembrane、Extracellular
AsDMP25	*AVESA.00010b.r2.5AG0809890.1*	193	8.39	20.78	34.74	84.46	4	NO	PlasmaMembrane、Extracellular
AsDMP26	*AVESA.00010b.r2.5DG0998080.1*	193	8.39	20.78	34.74	84.46	4	NO	PlasmaMembrane、Extracellular
AsDMP27	*AVESA.00010b.r2.6AG1010130.1*	210	7.60	21.65	39.73	82.90	4	NO	PlasmaMembrane
AsDMP28	*AVESA.00010b.r2.6AG1060270.1*	212	5.26	21.92	25.48	97.22	4	NO	PlasmaMembrane
AsDMP29	*AVESA.00010b.r2.6AG1060280.1*	185	8.38	19.38	23.78	106.05	4	NO	PlasmaMembrane
AsDMP30	*AVESA.00010b.r2.6CG1110230.1*	243	8.85	26.01	39.66	80.78	4	NO	PlasmaMembrane
AsDMP31	*AVESA.00010b.r2.7AG1213710.1*	214	8.78	22.84	36.62	92.62	4	NO	PlasmaMembrane
AsDMP32	*AVESA.00010b.r2.7CG0697010.1*	227	6.37	24.56	43.24	76.96	4	NO	PlasmaMembrane、Extracellular
AsDMP33	*AVESA.00010b.r2.7DG1394100.1*	213	9.04	22.80	40.14	90.75	4	NO	PlasmaMembrane

consistent, suggesting functional similarities among these members. It is noteworthy that there are variations in the motif composition among some proteins, both within the same subfamily and between different subfamilies. For instance, Motif 8 and Motif 10 are present only in certain proteins within subfamily V, highlighting their functional variability. In addition, some motifs are absent in certain subfamily members, while others are found exclusively in specific genes. For example, Motif 1 and Motif 7 are absent in all AsDMP members of subfamily III, while Motif 7 is also missing in subfamily IV. Motif 6 is present only in subfamily III, and Motif 8, 9, 10 are present only in some members of their respective subfamilies. Determining whether the presence or absence of these specific motifs confers unique functional roles to the *DMP* genes requires further study. The variation in motif composition among subfamilies may be attributed to their functional diversity. Gene structure analysis revealed that

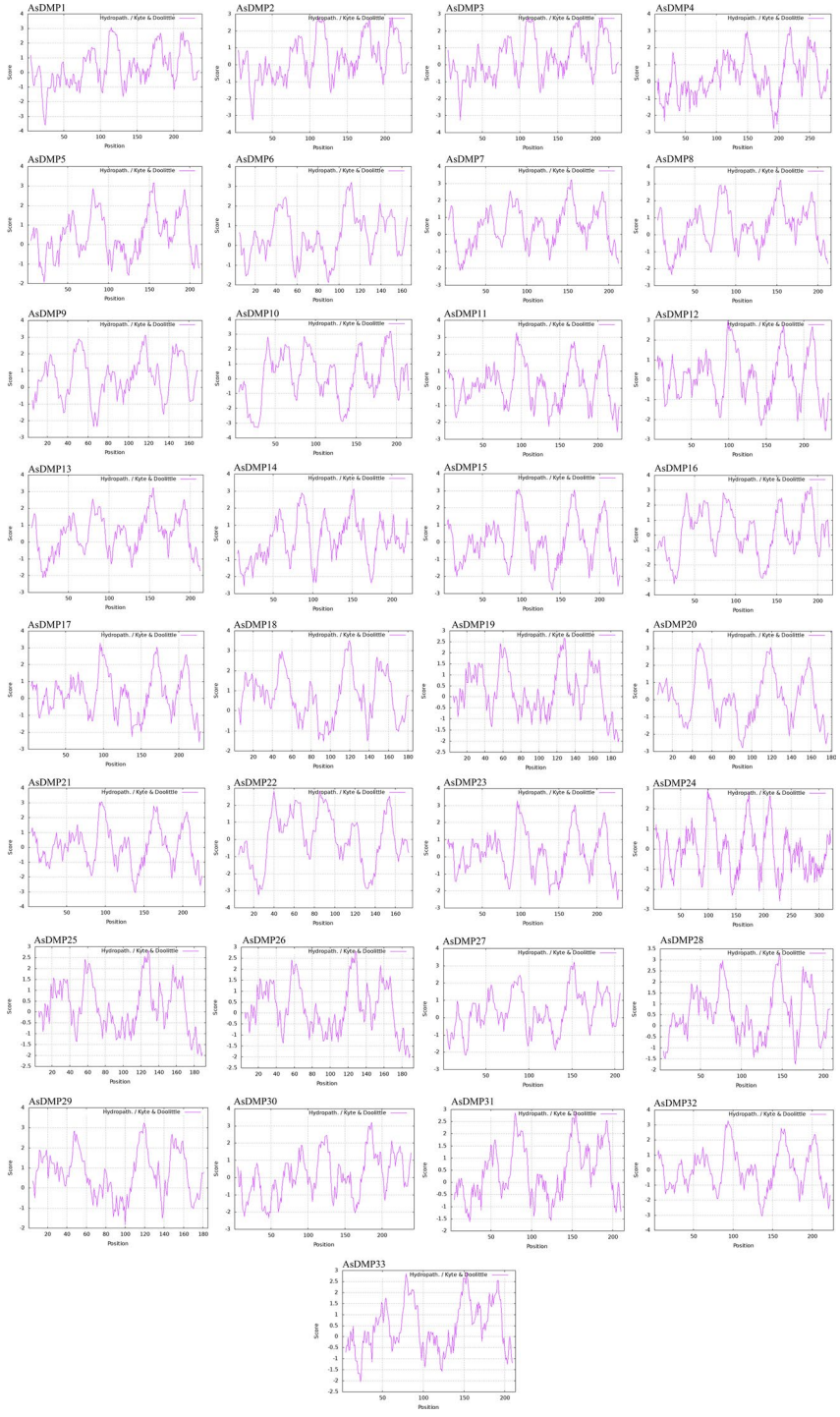

Fig. 1 The hydropathicity map of the AsDMP protein: analysis of amino acid residue hydrophobicity and hydrophilicity. The x-axis represents the position of amino acid residues, while they-axis indicates the hydrophobicity or hydrophilicity score. Peaks and troughs in the curve correspond to regions with high hydrophobicity or high hydrophilicity respectively

introns were present only in AsDMP4 from subfamily I and AsDMP24 from subfamily V (Fig. 4D). This indicates that, despite the functional similarities among DMP family members, there are notable differences among individual genes.

Chromosome localization, collinear analysis and Ka/Ks selection pressure analysis

To better understand the distribution of *AsDMPs* genes across chromosomes, a chromosome map of 33 *AsDMP* genes was constructed (Fig. 6). These genes were found

Table 2 AsDMP proteins secondary structure characterization (%): alpha-helix, extended strand, random coil and beta turn

name	Alpha-helix	Extended strand	Random coil	Beta turn
AsDMP1	41.84	11.72	40.17	6.28
AsDMP2	41.53	11.86	40.68	5.93
AsDMP3	40.43	11.91	41.70	5.96
AsDMP4	46.83	13.32	35.92	4.93
AsDMP5	39.53	15.35	38.60	6.51
AsDMP6	45.56	15.38	32.54	6.51
AsDMP7	40.28	13.43	41.67	4.63
AsDMP8	41.20	12.04	41.67	5.09
AsDMP9	44.51	16.18	32.37	6.94
AsDMP10	42.01	17.35	36.99	3.65
AsDMP11	35.93	16.88	42.42	4.76
AsDMP12	34.18	18.99	41.77	5.06
AsDMP13	36.57	16.20	43.06	4.17
AsDMP14	45.13	11.06	37.17	6.64
AsDMP15	33.91	15.22	45.22	5.65
AsDMP16	39.45	18.81	35.78	5.96
AsDMP17	39.48	15.02	40.34	5.15
AsDMP18	43.78	14.59	34.59	7.03
AsDMP19	37.82	16.06	41.45	4.66
AsDMP20	44.20	14.92	34.25	6.63
AsDMP21	37.12	14.85	41.05	6.99
AsDMP22	31.64	21.47	40.68	6.21
AsDMP23	40.77	16.31	37.34	5.58
AsDMP24	41.54	14.15	39.38	4.92
AsDMP25	39.90	13.99	38.86	7.25
AsDMP26	39.90	13.99	38.86	7.25
AsDMP27	35.71	14.76	44.29	5.24
AsDMP28	41.98	13.21	38.68	6.13
AsDMP29	47.03	12.97	34.05	5.95
AsDMP30	39.51	12.76	42.39	5.35
AsDMP31	36.45	18.22	39.72	5.61
AsDMP32	37.00	15.86	40.97	6.17
AsDMP33	38.50	16.43	41.31	3.76

to be distributed across 17 chromosomes. Five genes are distributed on chromosomes chr 3 C and chr 4D each, three genes on chr 4 A and chr 6 A each, two genes on chr 1 A, chr 2D, chr 3D, and chr 4 C each, and one gene is found on each of the remaining chromosomes. Tandem duplications were observed on chromosomes 3 C, 4D, and 6 A.

Gene family evolution primarily involves whole genome duplication(WGD), segmental duplication and tandem duplication [29]. Most plants have undergone ancient genome-wide replication events, also known as polyploidy, which led to the duplication of all genes across the genome [30]. This large-scale chromosomal doubling event resulted in the retention of numerous duplicated genomic fragments [31]. Tandem repeats occur on the same chromosome, adjacent to each other, and often have similar sequences and functional clusters [32]. Segmental duplications involve gene copies that are located distantly from each other or on different chromosomes. These duplications are a primary driver of gene family expansion and amplification [33, 34].

Through homology analysis of *AsDMP* genes, we visualized their relationships to elucidate the mechanisms underlying the expansion of the *AsDMP* gene family (Fig. 7). Among the 35 homologous gene pairs, four pairs were identified as resulting from tandem duplication events (*AsDMP11*/*AsDMP12*, *AsDMP20*/*AsDMP21*, *AsDMP23*/*AsDMP24*, *AsDMP28*/*AsDMP29*) (Fig. 6). Thirty-one gene pairs underwent WGD or segmental duplication. We therefore hypothesized that WGD or segmental duplication events are the major drivers of gene amplification in the evolution of the *DMP* gene family.

To further understand the evolutionary relationship of *AsDMP* genes, we constructed collinearity maps for the DMP families in *A. sativa*, *S. bicolor* and *Z. mays* (Fig. 8). Fourteen *AsDMP* genes were found to be collinear with at least two or more homologous genes. Specifically, there were 19 pairs of collinear relationships between 14 *AsDMPs* and five *SbDMPs*, and 14 pairs of collinear relationships between 14 *AsDMPs* and four *ZmDMPs*. Notably, the 14 *AsDMP* genes showing collinearity with both *S. bicolor* and *Z. mays* were identical, suggesting a higher degree of conservation among *A. sativa*, *S. bicolor* and *Z. mays* within the Poaceous plant. This suggests that these genes may have played an important role in evolution by participating more frequently in gene duplication events.

Selection pressure analysis was performed by evaluating the ratio of nonsynonymous substitutions per nonsynonymous site (Ka) to synonymous substitutions per synonymous site (Ks) (Ka/Ks) (Table 3). Not a Number (NaN) indicates that these gene pairs exhibit almost exclusively synonymous mutations at sites where such mutations are possible, indicating significant sequence divergence and a considerable evolutionary distance between them. We found that the Ks value for the gene pairs *AsDMP-1*/*AsDMP-9*, *AsDMP-1*/*AsDMP-14*, *AsDMP-2*/*AsDMP-9*, *AsDMP-2*/*AsDMP-14*, *AsDMP-3*/*AsDMP-9*, *AsDMP-3*/*AsDMP-14* are NaN, resulting in a NaN Ka/Ks ratio. Among the gene pairs with WGD or segmental duplication, 22 pairs have Ka/Ks ratios less than 0.5, while three pairs have Ka/Ks ratios between 0.5 and 1.0, with all Ka/Ks ratios being less than 1. Additionally, four tandemly duplicated genes also have Ka/Ks ratios below 1. These findings indicate that these *AsDMP* genes experienced purifying selection during evolution, with functional differentiation occurring following WGD or segmental duplication.

Cis-element analysis of *AsDMP* gene promotors

Gene expression was typically regulated by cis-elements in the upstream promoter sequence. Exploring the

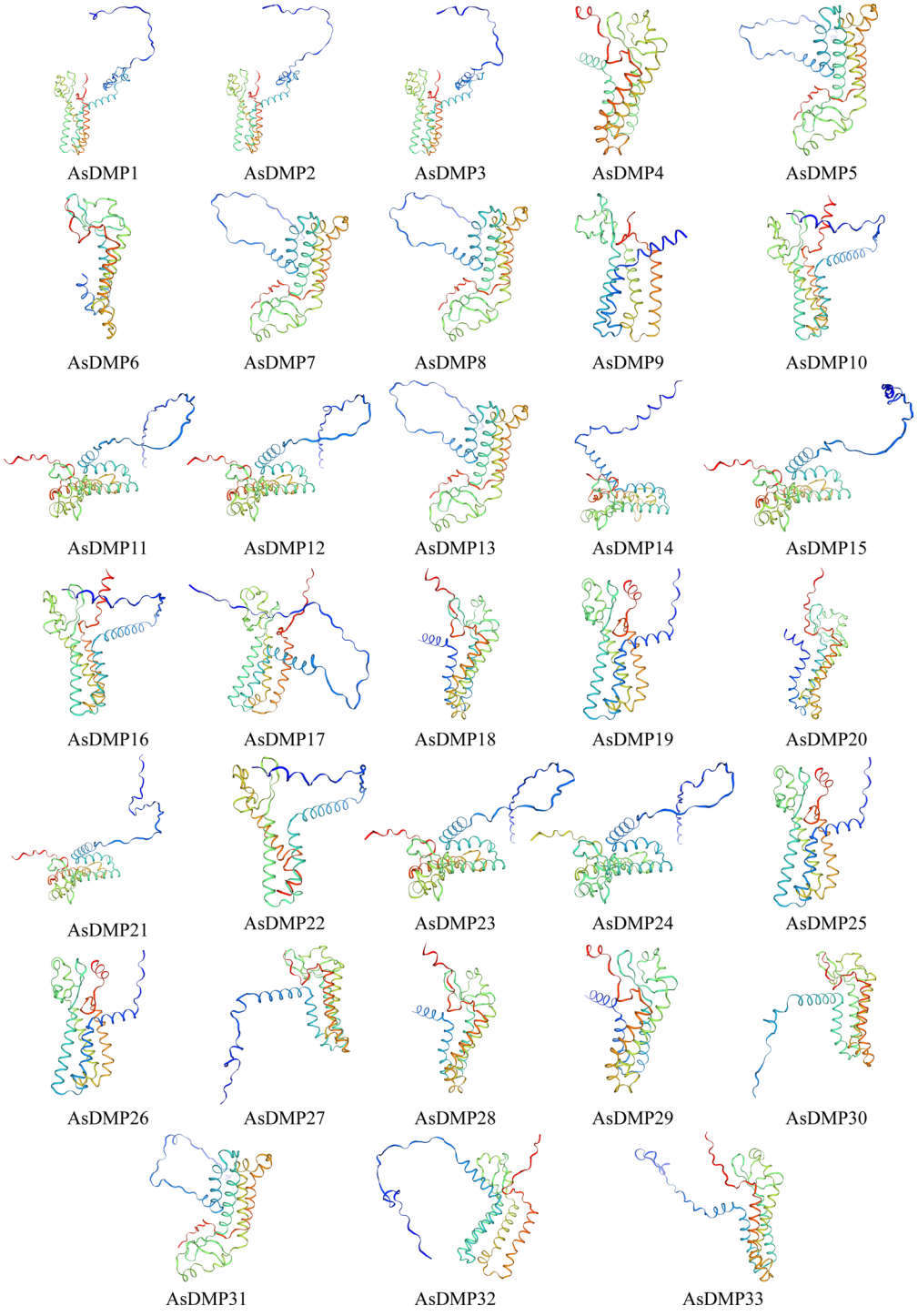

Fig. 2 Three-dimensional structure of putative DMP proteins from *A. sativa* generated through homology modelling. All AsDMP proteins were modeled using SWISS-MODEL (https://swissmodel.expasy.org)

cis-regulatory elements contained within the promoter region of the *AsDMP* gene will help to understand the regulatory mechanism of the *DMP* gene and speculate on its potential functions. We used the PlantCARE database to perform a predictive analysis of promoter cis-regulatory elements. Based on their roles and functions, cis-regulatory elements are categorized as follows: hormone-responsive elements, which include auxin responsive elements, gibberellin-responsive elements, abscisic acid (ABA)-responsive elements, salicylic acid-responsive elements, and MeJA-responsive elements. Abiotic stress response elements include those responsive to low

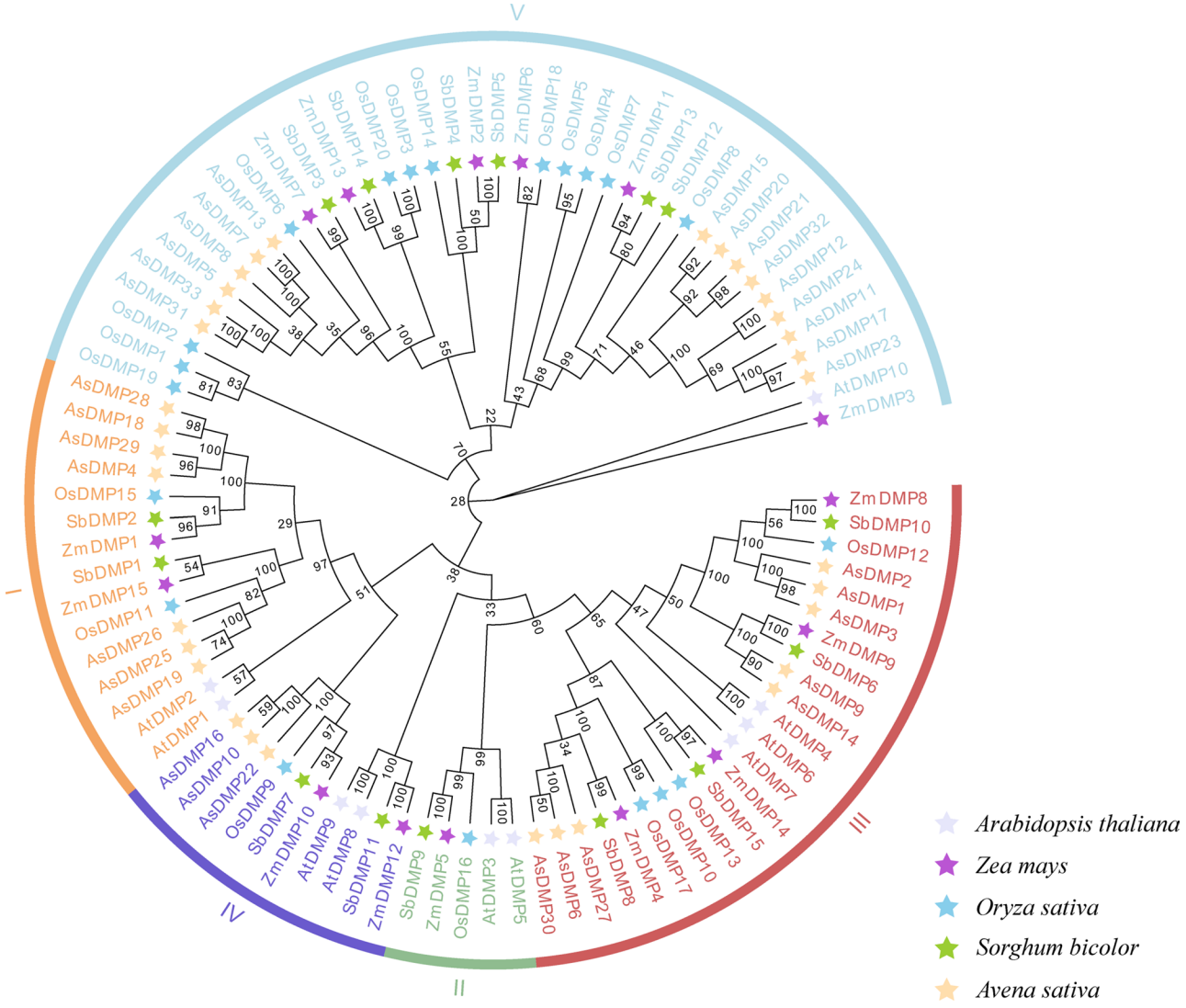

Fig. 3 Unrooted Classification tree representing relationships among *DMP* genes of 5 species. Phylogenetic relationship of the 93 identified *DMP* genes from 5 plant species. DMP protein sequences were aligned with ClustalW, and a phylogenetic tree was constructed with MEGA11 using the neighbor-joining method and 1,000 bootstrap replicates. The members were divided into subfamilies I, II, III, IV and V

temperature, elements involved in defense and stress responses, MYB binding sites (MYBHv1, light response, drought and flavonoid biosynthetic regulation), and elements specific to anaerobic induction and hypoxia response. Growth and development-related regulatory elements include light-responsive elements, meristem expression-related elements, phytochrome-responsive elements, circadian rhythm control elements, cell cycle regulatory elements, and endosperm expression and seed-specific regulatory elements. Other responsive elements include AT-DNA (ATBP-1) binding sites, protein binding sites, activator mediated activation elements, and regulatory elements in zein metabolism.

Among the cis-elements involved in plant growth and development, light-responsive elements are the most abundant. All *AsDMP* genes, except *AsDMP10*, contain light-responsive elements that are widely distributed in their promoter regions. Elements related to endosperm expression are located in the promoter regions of *AsDMP13*, *AsDMP15*, *AsDMP16*, *AsDMP21*, *AsDMP22*, *AsDMP29*, *AsDMP32*, and *AsDMP33*. Seed-specific regulatory elements are located in the promoter regions of *AsDMP5*, *AsDMP6*, *AsDMP7*, *AsDMP8*, *AsDMP11*, *AsDMP12*, *AsDMP13*, *AsDMP15*, *AsDMP17*, *AsDMP20*, *AsDMP21*, *AsDMP23*, *AsDMP24*, *AsDMP27*, *AsDMP29*, *AsDMP31* and *AsDMP33*.

Almost all promoters contain multiple hormone response elements, but these elements are not closely related to their subfamilies (Fig. 9). Among them, cis-acting elements responsive to MeJA were the most abundant in the *AsDMP* gene promoter regions, with 32 of the *AsDMP* gene promoter regions containing at least one

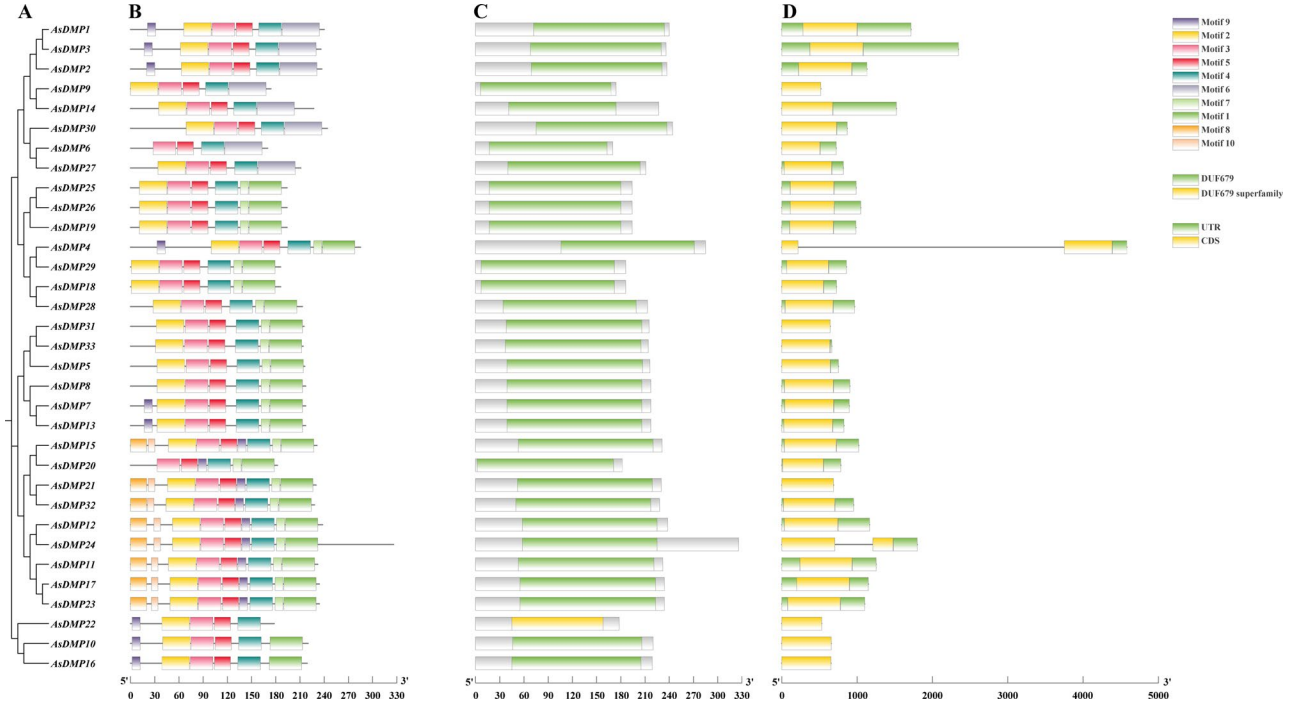

Fig. 4 Phylogenetic tree, conserved motif, conserved domain and gene structure of *AsDMP* gene family. A: Phylogenetic tree of *AsDMP* genes was constructed using MEGA11 and the neighbor-joining (NJ) method; B: Conserved motif of AsDMP proteins was analyzed on the MEME tool, and the results were visualized in TBtools. The motifs, labeled as 1–10 and represented by different colored boxes, demonstrate the conserved patterns in the protein sequences. The scale at the bottom allows for estimation of protein lengths.; C: The conserved domain of the *AsDMP* gene family indicates that they are members of the same gene family; D: The gene structure of *AsDMP* genes was determined, with the coding regions for the AsDMP domain highlighted in yellow. Regions labeled as CDS without the region coding for the AsDMP domain, indicated in green

MeJA response element. ABA-responsive elements are also widely present in the promoter regions of *AsDMP* genes, with 29 AsDMP family members having at least one ABA-responsive element in their promoters. Stress related cis-regulatory elements are also widely distributed in the promoter regions of *AsDMP* genes. Among the cis-acting elements related to abiotic stress, MYB elements are the most prevalent. Most AsDMP members contain one or more MYB elements in their promoters, which are involved in drought response, photo-response and the regulation of flavonoid biosynthesis genes. Twenty *AsDMP* genes are responsive to low temperatures, and thirteen *AsDMP* genes respond to defense and stress. In addition, some cis-acting elements are essential for hypoxia and anaerobic induction. These results indicate that the transcription of *AsDMP* genes may be influenced by multiple environmental factors.

Expression patterns of *DMP* gene in Oat at different tissue and developmental stages

Utilizing *A. sativa* transcriptome data, we analyzed the expression pattern of the *AsDMP* gene across various tissues and developmental stages, including spikes, roots, and leaves during seedling development, and seeds at early, middle and late stages of development (Fig. 10). According to the transcriptome results, *AsDMP5*, *AsDMP6*, *AsDMP31*, and *AsDMP33* were not expressed in any of the above tissues. Among *AsDMP1*, *AsDMP2*, *AsDMP3*, and *AsDMP29*, *AsDMP3* was not expressed in leaves, while *AsDMP29* was not expressed in both ears and leaves but was expressed in other tissues. The expression of *AsDMP1* and *AsDMP3* was initially down-regulated and then up-regulated during the early, middle and late stages of seed development. In contrast, *AsDMP2* was consistently up-regulated, and *AsDMP29* was consistently down-regulated. *AsDMP4* is expressed only during the mid-development of seeds, with no expression at the early or late stages. *AsDMP7* and *AsDMP8* were only expressed in spikes, suggesting a potential role in spike development or fruit formation. *AsDMP27* was expressed solely in roots, indicating a possible involvement in root growth and development. *AsDMP9*, *AsDMP10*, *AsDMP13*, *AsDMP14*, *AsDMP15*, *AsDMP16*, *AsDMP22*, *AsDMP28*, and *AsDMP30* were expressed at various stages of seed development. *AsDMP19*, *AsDMP25*, and *AsDMP26* were expressed in spikes, roots, and leaves, and across all stages of seed development, showing an initial up-regulation followed by down-regulation throughout the seed development period. Interestingly, the expression of *AsDMP25* and *AsDMP26* was the highest among the 33 *AsDMP* genes during the middle stage of seed development. At this stage, the expression levels of these

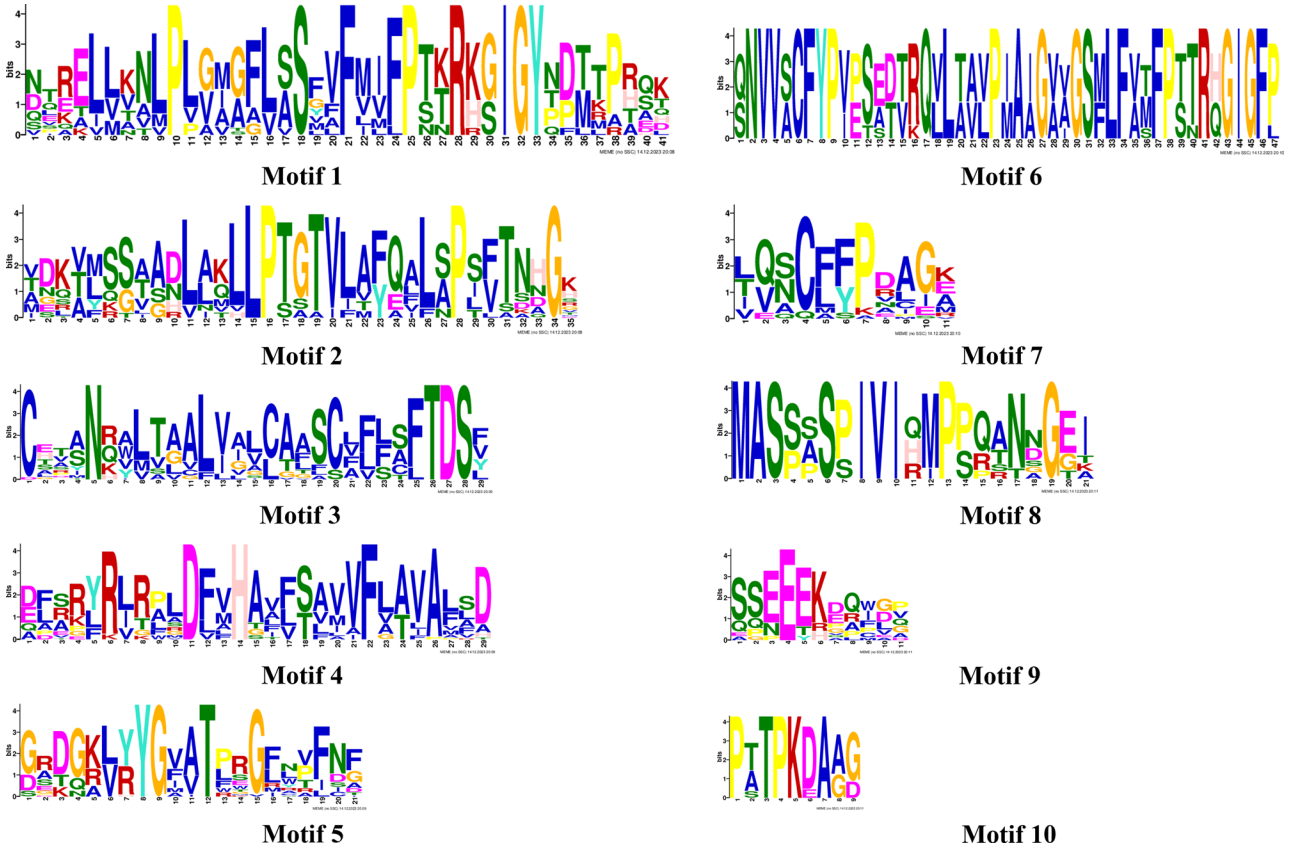

Fig. 5 Conserved domain sequence identification: AsDMP protein

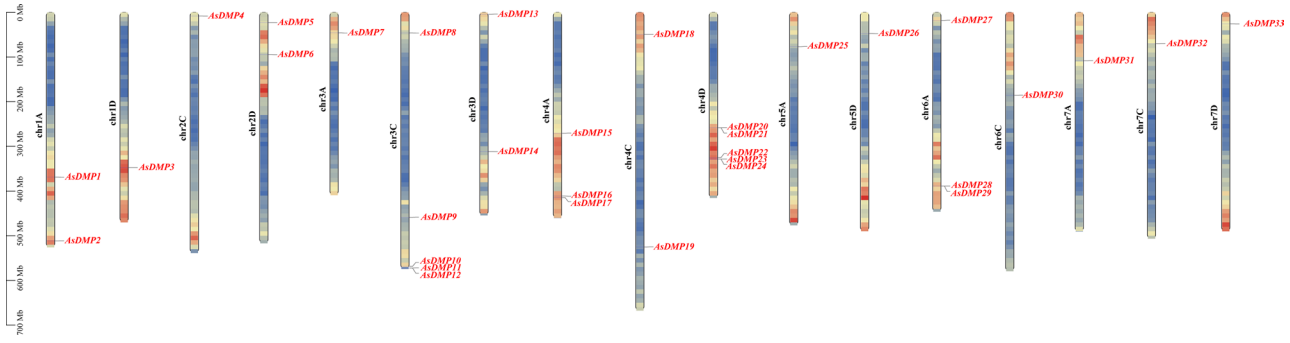

Fig. 6 Chromosomal mapping of *DMP* genes in *A. sativa*. Chromosome numbers are displayed in black font on the side. The *AsDMP* gene is shown in red font on the side. The scale is marked in megabases (Mb)

two genes were 4.2-fold and 2.3-fold higher compared to the early stage, and 9-fold and 4.7-fold higher compared to the late stage. In summary, it is hypothesized that among the 17 genes expressed during seed development, seven genes (*AsDMP1*, *AsDMP2*, *AsDMP3*, *AsDMP19*, *AsDMP25*, *AsDMP26*, *AsDMP29*) are involved throughout the entire seed development process. In contrast, two genes (*AsDMP4* and *AsDMP14*) are specifically active during mid-seed development, while four genes (*AsDMP9*, *AsDMP13*, *AsDMP15*, and *AsDMP30*) are primarily involved in late seed development.

Expression analysis of *DMP* gene in response to high temperature and aging of oat seeds

To further investigate the expression of *AsDMP* genes under natural aging and artificial high-temperature conditions, we selected 10 genes from the *AsDMP* gene family (*AsDMP1*, *AsDMP2*, *AsDMP3*, *AsDMP10*, *AsDMP19*, *AsDMP22*, *AsDMP25*, *AsDMP26*, *AsDMP28*, and *AsDMP29*) for qPCR analysis (Fig. 11). This selection was based on transcriptome analysis and the observed expression changes of *AsDMP* genes during seed development. The qPCR results showed that the expression of *AsDMP1*

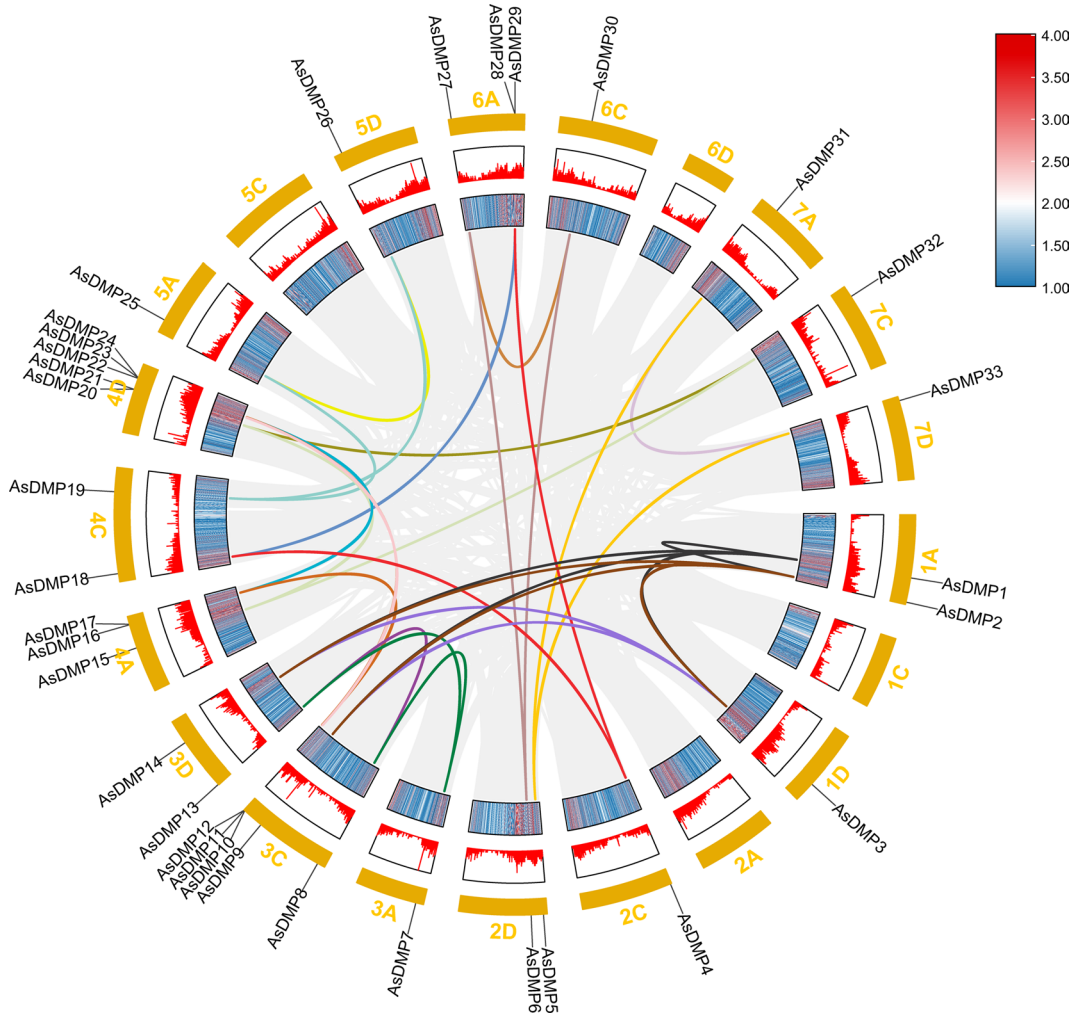

Fig. 7 Chromosomal distribution and duplication events of AsDMP. The segmentally duplicated genes are connected by Colored lines, referring to the 33 genes highlighted in black

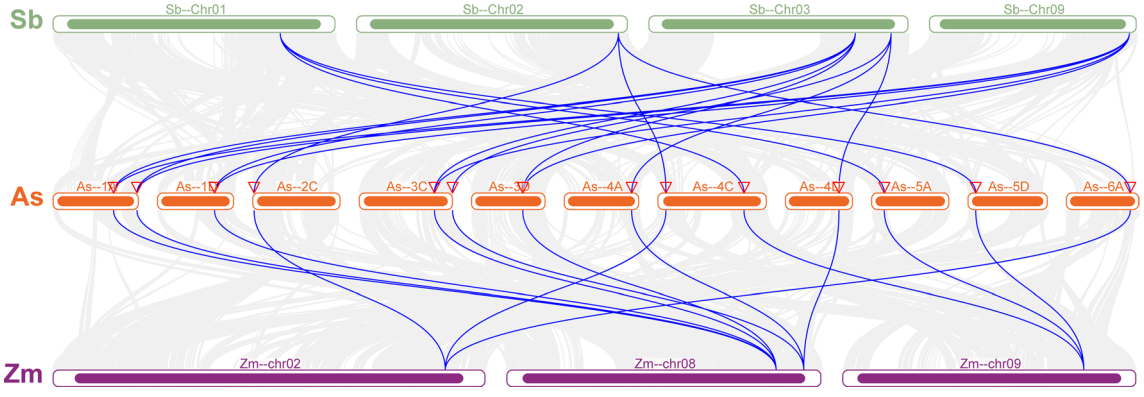

Fig. 8 Syntenic analysis of *DMP* genes in *A. sativa* in comparison with those in two plant species. The blue lines highlight the syntenic *DMP* gene pairs. The specie names with the prefixes "*Sb*", "*As*"and "*Zm*" indicate *S. bicolor*, *A. sativa*, and *Z. mays*

and *AsDMP19* was up-regulated under both natural and artificial aging conditions, while the expression of *AsDMP22* was consistently down-regulated in both aging treatments. This indicates that *AsDMP1* and *AsDMP19* are involved in regulating seed senescence and vigor loss in *A. sativa* under both natural and artificial aging conditions. Conversely, *AsDMP22* contributes to delaying or reducing seed senescence under both natural aging and

Table 3 Ka/Ks values of duplicated *DMP* gene pairs from *A. sativa* species

Gene name	Gene name	Duplication type	Ka	Ks	Ka/Ks
AsDMP1	*AsDMP2*	WGD/segmental	0.027307	0.168114	0.16243
AsDMP1	*AsDMP3*	WGD/segmental	0.01553	0.027602	0.562622
AsDMP1	*AsDMP9*	WGD/segmental	0.219878	NaN	NaN
AsDMP1	*AsDMP14*	WGD/segmental	0.343854	NaN	NaN
AsDMP2	*AsDMP3*	WGD/segmental	0.017651	0.137493	0.128376
AsDMP2	*AsDMP9*	WGD/segmental	0.220654	NaN	NaN
AsDMP2	*AsDMP14*	WGD/segmental	0.345933	NaN	NaN
AsDMP3	*AsDMP9*	WGD/segmental	0.219878	NaN	NaN
AsDMP3	*AsDMP14*	WGD/segmental	0.34523	NaN	NaN
AsDMP4	*AsDMP18*	WGD/segmental	0.037855	0.0854	0.443265
AsDMP4	*AsDMP28*	WGD/segmental	0.046803	0.114023	0.410471
AsDMP5	*AsDMP31*	WGD/segmental	0.037644	0.103329	0.364314
AsDMP5	*AsDMP33*	WGD/segmental	0.029288	0.152551	0.191988
AsDMP6	*AsDMP27*	WGD/segmental	0.043386	0.064124	0.676584
AsDMP6	*AsDMP30*	WGD/segmental	0.038638	0.081658	0.473172
AsDMP7	*AsDMP8*	WGD/segmental	0.035414	0.089984	0.393563
AsDMP7	*AsDMP13*	WGD/segmental	0.003156	0.063771	0.049493
AsDMP8	*AsDMP13*	WGD/segmental	0.033273	0.109512	0.303831
AsDMP10	*AsDMP22*	WGD/segmental	0.059639	0.350143	0.170326
AsDMP11	*AsDMP17*	WGD/segmental	0.032496	0.128036	0.253804
AsDMP11	*AsDMP23*	WGD/segmental	0.037483	0.125291	0.29917
AsDMP15	*AsDMP20*	WGD/segmental	0.031438	0.084768	0.370873
AsDMP15	*AsDMP32*	WGD/segmental	0.0463	0.25935	0.178524
AsDMP17	*AsDMP23*	WGD/segmental	0.016389	0.050315	0.325726
AsDMP18	*AsDMP28*	WGD/segmental	0.012423	0.04846	0.25635
AsDMP19	*AsDMP25*	WGD/segmental	0.011719	0.100388	0.116737
AsDMP19	*AsDMP26*	WGD/segmental	0.011714	0.077808	0.150555
AsDMP20	*AsDMP32*	WGD/segmental	0.052084	0.305306	0.170598
AsDMP25	*AsDMP26*	WGD/segmental	0	0.034058	0
AsDMP27	*AsDMP30*	WGD/segmental	0.069509	0.108539	0.64041
AsDMP31	*AsDMP33*	WGD/segmental	0.012844	0.069892	0.183765
AsDMP11	*AsDMP12*	Tendem	0.106861	0.351275	0.304209
AsDMP20	*AsDMP21*	Tendem	0.060096	0.281151	0.21375
AsDMP23	*AsDMP24*	Tendem	0.11315	0.303374	0.372974
AsDMP28	*AsDMP29*	Tendem	0.026374	0.119097	0.221446

high-temperature artificial aging conditions. It is important to highlight that the expressions of *AsDMP28* and *AsDMP29* genes are observed only under artificial aging treatment, indicating that their expressions are induced under high temperatures but not by natural aging.

Discussion

As a membrane protein unique to green plants, the *DMP* gene family has only been identified in a limited number of studies. Currently, there are no reports on the analysis of *DMP* genes in *A. sativa*. This study used multiple bioinformatics approaches to identify a total of 33 DMP family members in the *A. sativa* genome. The number of *AsDMP* genes identified is more than those in *A. thaliana*, *Z. mays*, *O. sativa*, and *S. bicolor*, but fewer than the number of *DMP* genes found in *G. hirsutum*. As an allohexaploid, the *A. sativa* genome size is 11Gb, whereas the genome sizes of *A. thaliana*, *Z. mays*, *O. sativa*, and *S. bicolor* are 125 Mb, 2300 Mb, 430 Mb, and 709 Mb, respectively. The number of DMPs in *A. sativa* (33 genes) is only 3.3 times that of *A. thaliana* (10 genes), 2.2 times that of *Z. mays* (15 genes), 1.7 times that of *O. sativa* (20 genes), and 2.2 times that of *S. bicolor* (15 genes). In this case, it seems that there is no direct correlation between the number of *DMP* genes and genome size in these plants. This variation is likely due to differences in genome size and gene duplication during plant evolution, *A. sativa* possesses a large genome and a high content of repetitive sequences [35]. This highlights the genomic expansion and complexity of the *DMP* gene family in *A. sativa*.

Determining the physical and chemical properties of *AsDMP* genes, and understanding their protein structure, chromosomal distribution, evolutionary relationships, conserved domains, cis-regulatory elements, and gene expression patterns, is crucial for exploring the functional diversity of the *DMP* gene and enhancing seed viability. The substantial disparities in the physicochemical properties among DMP members, coupled with the existence of divergent conserved motifs, indicate that during the evolutionary process, *DMP* genes have undergone modifications in their intrinsic properties. This evolutionary diversification implies that within the context of *A. sativa* growth and development, different *DMP* genes could potentially be involved in a variety of distinct biological functions, reflecting their adaptability and functional specialization over time. Prediction of the subcellular localization of AsDMP proteins revealed that all proteins were located at the cell membrane, is consistent with the known function of DMP proteins. AsDMP14, AsDMP15, AsDMP19, AsDMP21, AsDMP24, AsDMP25, AsDMP26, and AsDMP32 were found to be localized not only in the cell membrane but also in the extracellular space and chloroplasts. This indicates that DMP are involved in various biological processes and exhibit diverse mechanisms in response to adversity. Differences in subcellular localization also highlight the functional variability among AsDMP members. Similar to other aging-related genes, in addition to inhibiting seed aging and improving seed storage tolerance, these proteins are also involved in various other biological processes. For example, small heat shock proteins (sHSPs) are involved not only in seed aging but also in adversity stress, seed germination, pollen development, and fruit ripening [36, 37]. LOX (lipoxygenase, LOX) regulates plant development and synthesizes signaling substances such as phytodienoic acid, jasmonic acid, and abscisic acid in *O. sativa* [38, 39]. PLD (phospholipase D, PLD) is involved in numerous processes in *A. thaliana* such

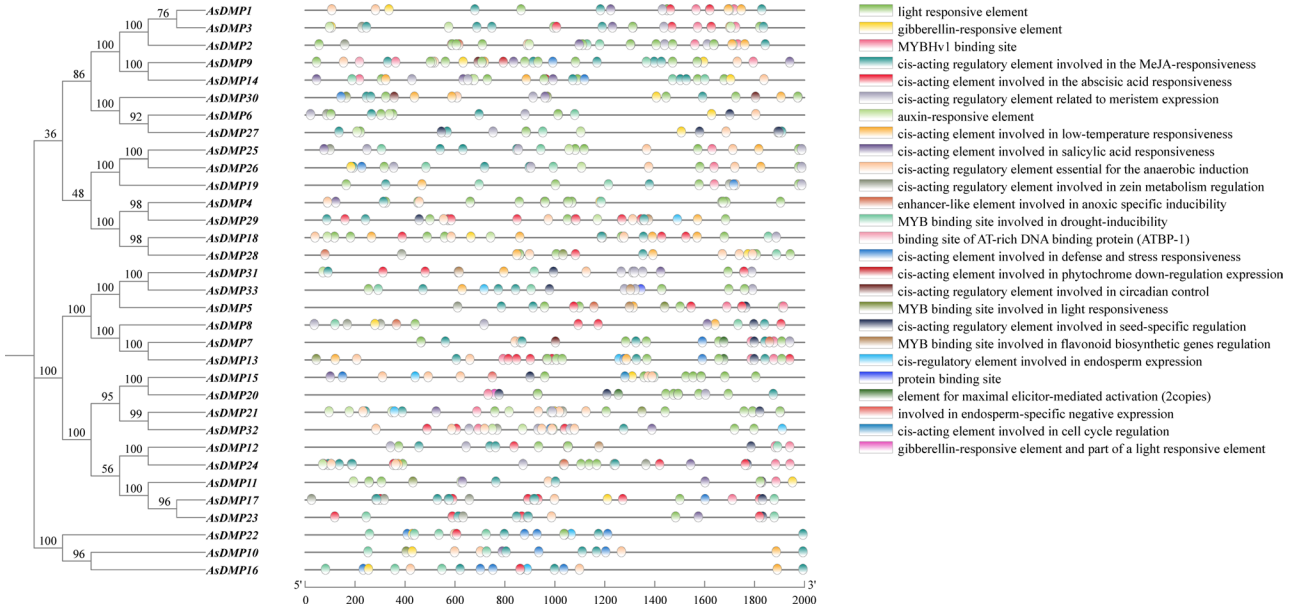

Fig. 9 Cis-regulatory elements in the promoters of *AsDMP* gene families. Different colors represent the different types of cis-elements

as plant growth and development, interactions between phytohormones and abiotic stress signals, defense against fungal pathogens, and stomatal closure [40–45]. This suggests that most aging-related genes are multifunctional. In addition, studies have shown that several biological stresses seem to induce the transcription of *DMP1*. This gene responds moderately to strongly to pathogens such as *Botrytis cinerea* and *Phytophtora infestans*, as well as to both virulent and avirulent strains of *Pseudomonas syringae*. It also reacts to bacterial elicitors such as Flg22, HrpZ and NPP1 [11]. These findings suggest that DMP may be involved in the plant immune system.

Conserved motif analysis showed that three motifs exist in all AsDMPs, while other motifs only exist in specific genes. These unique motifs are a key factor in the functional differentiation of AsDMP proteins. Gene structure analysis revealed that most *AsDMP* genes lack introns, a pattern similar to that observed in the *DMP* gene families of *G. max* and *G. hirsutum* [9, 17]. It is speculated that these genes may have specialized functions. Increased genetic sequencing suggests that ancestral eukaryotes initially possessed intron-rich genes, and that most eukaryotes experienced a loss of introns during the course of evolution [46]. The higher proportion of intron-less genes in *A. sativa* suggests that this species may have also experienced intron loss events during evolution [47].

Protein secondary structure is a key factor in predicting protein function, as it directly influences protein stability [20]. This study found that AsDMP proteins predominantly feature random coil and α-helix structures. Variations in the proportions of α-helix, extended strand, random coil and β-turn lead to differences in the spatial folding of proteins, supporting the functional diversity among DMP members. In the phylogenetic analysis, subfamilies I, III, IV, and V all contain DMP members from the five species, whereas subfamily II includes DMP members from four species excluding *A. sativa*, suggesting that they may have originated from a common ancestor. Tandem duplication and WGD/segmental duplication are key factors in gene doubling, gene functional specificity and diversification. While promoting the expansion of eukaryotic gene families, these processes may also lead to gene functional redundancy [48, 49]. AsDMP has four pairs of genes that have undergone tandem duplication during the evolution of *A. sativa*, while 31 pairs have experienced WGD/segmental duplication events. WGD/segmentation duplication may be the main reason for the expansion of the *AsDMP* gene family, originating from the WGD event in *A. sativa* [35]. This result is consistent with the research on *G. hirsutum DMP* genes [17]. In addition, the Ka/Ks ratio for all duplicated *AsDMP* genes is less than 1, indicating that these genes have undergone strong purifying selection during the evolution of *A. sativa*. This suggests that the functional constraints on *AsDMP* genes mainly come from purifying selection. However, the Ka/Ks ratios for most gene pairs range from 0 to 0.49, indicating that *DMP* gene pairs tend to be conserved throughout evolution [50].

Gene expression is typically regulated by cis-elements located in the upstream promoter region. These cis-elements, located in non-coding DNA upstream of the gene transcription start site, regulate gene expression in response to various environmental conditions or specific tissues. Hormone and stress response elements are the main functional factors detected in the promoter regions

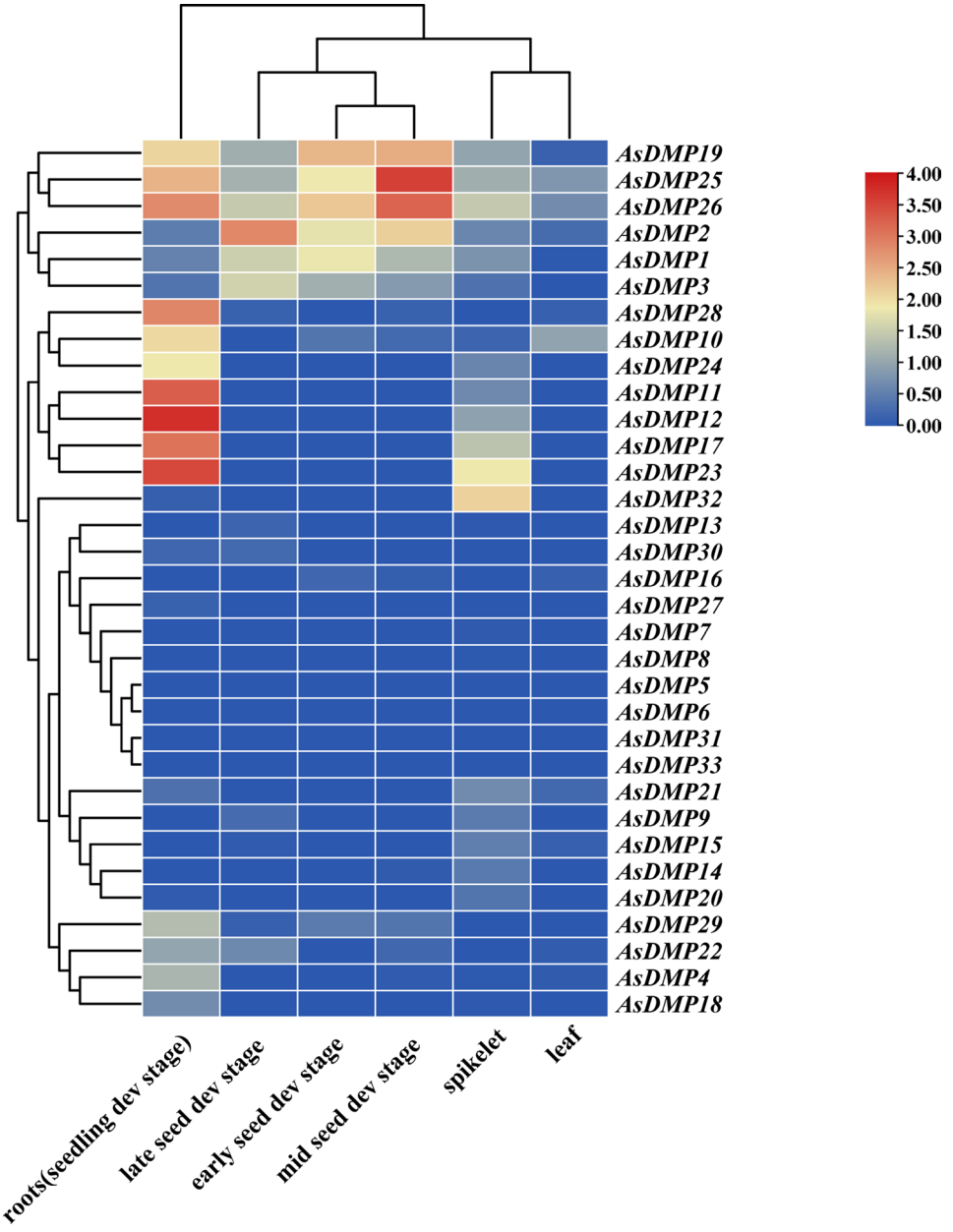

Fig. 10 Analysis of the expression pattern of *AsDMP* gene in different tissues and developmental stages. The expression levels of *AsDMP* genes were log2 transformed. Red or blue indicates the difference in expression levels for each gene

of the *AsDMP* gene, similar to the results for cis-elements in the *DMP* gene of *G. hirsutum* [17]. This study analyzed the expression of *AsDMP* genes under stress during seed aging, but the response of *AsDMP* genes to hormones should also be concerned. The interaction between hormones and redox signaling plays a key role in regulating plant resistance, leaf senescence, seed germination, seed development, and seed longevity [51–53]. Plant growth and environmental adaptation are regulated by various combinations of cis-elements, with only a small set of responsive genes being regulated by a single cis-element [54]. GA, ABA, SA, MeJA, and IAA responsive elements, along with stress-responsive elements, may play an important role in regulating stress resistance and development. In addition, seed-specific regulatory elements were identified in 17 of the *AsDMP* genes, suggesting that these specific genes may play a vital role in regulating seed vigor. These highly diverse cis-regulatory elements in the promoter region of *AsDMP* genes may also reflect the functional divergence at the transcriptional level. Expression profiling is essential for understanding gene functions and identifying candidate genes. Genes that are specifically expressed in various tissues often have distinct functions. We identified 17 *AsDMP*

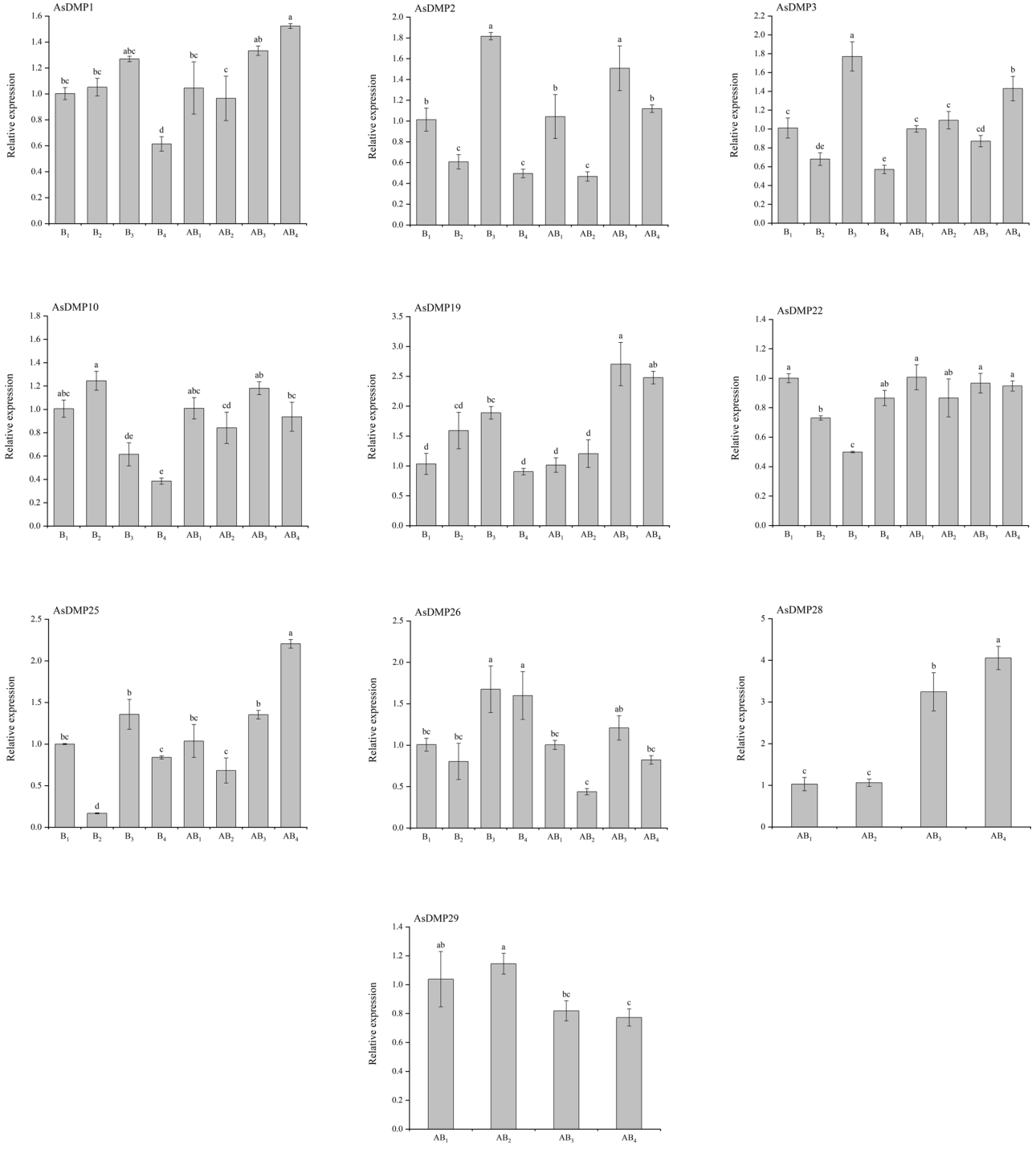

Fig. 11 Relative expression of AsDMP under natural and artificial aging treatments. The data represent the means of three biological replicates ± SEM. B_1: Control check (CK); B_2: Seeds stored naturally for 1 years; B_3: Seeds stored naturally for 2 years; B_4: Seeds stored naturally for 3 years; AB_1: Seeds artificially aged for 24 h; AB_2: Seeds artificially aged for 48 h; AB_3: Seeds artificially aged for 72 h; AB_4: Seeds artificially aged for 96 h. *Note* Different letters represent significant differences ($P < 0.05$)

genes expressing during seed development, suggesting their involvement in the regulation of seed vigor. Overall, *AsDMP* genes showed diverse expression patterns across different tissues and stages of seed development in *A. sativa*, indicating their multiple roles in *A. sativa* development.

Currently, it is widely accepted that seed aging and deterioration are primarily due to lipid peroxidation caused by free radicals [55, 56]. This has been confirmed

in various plant seeds, such as *Zoysia japonica Steud* [57], *Ulmus pumila* [58] and *Jatropha curcas* [59]. It has also been demonstrated in other aging-related genes, such as *sHSP*, that they can enhance tolerance to oxidative stress by protecting photosystem II and increasing peroxidase (POD) activity [60]. In this study, we analyzed the expression of *DMP* genes in *A. sativa* seeds under high temperature and aging stress. The results showed that *AsDMP1* and *AsDMP19* were up-regulated and expressed in response to both natural and artificial aging treatments. The endoplasmic reticulum (ER) contains a variety of antioxidant enzymes, such as peroxidases and GR, which can directly decompose reactive oxygen species (ROS), preventing oxidative damage. However, the expression of the *DMP* gene triggers a series of membrane remodeling events that affect the structure of the ER and vacuoles. These changes lead to the disruption of the entire ER network and vacuolar integrity, causing a massive accumulation of ROS and oxidative damage, which in turn leads to aging [6, 7]. This may be the primary reason for DMP-induced aging. On the other hand, studies have shown that Jasmonic Acid (JA) acts as a positive regulator in the aging process [12, 61]. It was found that after the overexpression of *DMP1*, CYP94B3, which mediates the inactivation and degradation of biologically active Jasmonic Acid-Isoleucine conjugate (JA-Ile), shows enhanced regulatory activity. This could potentially lead to the accumulation of JA-Ile, resulting in aging [11]. However, down-regulation was observed after three years of natural storage. It was hypothesized that this may be due to the extended storage period of naturally aged seeds, which resulted in the accumulation of harmful substances such as ROS and peroxides. These substances can induce membrane lipid peroxidation, damage the internal structure and function of the seeds, cause chromosomal aberrations and DNA damage, and compromise gene integrity, leading to decreased expression of *AsDMP* [62, 63]. In contrast, while *AsDMP22* was down-regulated in both natural and artificial aging treatments, its expression was slightly up-regulated in the artificial aging treatment. It is speculated that high temperature aging treatment leads to increased hydrogen peroxide (H_2O_2) levels in mitochondria and damage to mitochondrial structure. In addition, during mitochondrial aging, changes in ROS scavenging enzymes and antioxidant levels result in ROS accumulation within seed embryos mitochondria, causing oxidative damage and inducing gene expression [64]. Interestingly, the *AsDMP28* and *AsDMP29* genes are expressed only under artificial aging treatments, indicating that their expressions are induced by high temperatures and are less associated with natural seed deterioration.

Phytohormones are key signaling compounds that regulate plant growth, development, and responses to environmental stresses [65]. During plant senescence, abscisic acid, salicylic acid or jasmonic acid alter the expression of aging-related genes, thereby regulating the aging process [10]. Many regulatory elements responsive to plant hormones are found in the promoter regions of *DMP* genes [9, 17]. This suggests that DMP expression may be closely related to hormones. However, the mechanism by which hormones regulate DMP and how *AsDMP* genes influence high-temperature aging require further investigation.

Conclusions

In this study, we identified 33 *AsDMP* members in *A. sativa*, which are unevenly distributed across 17 chromosomes and classified into five subfamilies based on phylogenetic relationships. The study analyzed the basic characteristics, protein structures, subcellular localization, conserved motifs, and gene locations of these DMP members, providing insights into the evolutionary relationships within the *DMP* gene family. Most *AsDMP* genes lack introns, indicating a highly conserved gene structure. While DMP members within the same subfamily exhibit broad similarities, differences in motif composition among some proteins in different subfamilies, or even within the same subfamily, suggest both functional similarities and differences. The qRT-PCR analysis identified *AsDMP* genes (*AsDMP1*, *AsDMP19*, and *AsDMP22*) that may play key roles in regulating *A. sativa* seed senescence and maintaining redox homeostasis. These genes are potential candidates for germplasm breeding studies aimed at enhancing *A. sativa* anti-aging. However, this study only provides a preliminary characterization of the *DMP* genes in *A. sativa*. Further functional validation is needed to fully understand the roles of these genes in different biological processes and to elucidate the exact mechanism by which *DMP* genes regulate seed senescence. Despite this, this study provides valuable insights into factors affecting seed vigor and presents new opportunities for developing aging-resistant *A. sativa* varieties.

Materials and methods

Identification of DMP protein family members

The genome sequences of *Z. mays* (version 1.1), *O. sativa* (version 7.0), and *S. bicolor* (Version 3.1) were obtained from phytozome (https://phytozome-next.jgi.doe.gov/). The *A. sativa* (version 1.1) genome sequence as well as annotation files were downloaded from the Ensembl Plants database (https://plants.ensembl.org/index.html), while the published *A. thaliana* AtDMP proteins were retrieved from the *A. thaliana* Information Resource (TAIR, version 10, http://www.arabidopsis.org). The amino acid sequences of *AtDMPs* were used as query sequences in a blastp search to identify candidate sequences in *A. sativa*. Subsequently, Interproscan 5

(http://www.ebi.ac.uk/interpro/) and CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) were used to search for the DMP domain (IPR007770, pfam05078) in the candidate sequences, leading to the identification of the DMP sequences [66, 67]. ExPAsy ProtParam (https://web.expasy.org/protparam/) and ExPAsy ProtScale (https://web.expasy.org/protscale/) were used to analyze the physical and chemical properties and hydrophilicity of the AsDMPs proteins [68]. The subcellular localization of each gene was predicted using the CELLO v2.5 server [69]. The number of transmembrane domains in AsDMP proteins was predicted using the Deep TMHMM tool (https://dtu.biolib.com/DeepTMHMM) [70]. Signal peptide amino acid sequence of AsDMP were forecasted using SignaIP-6.0 (https://services.healthtech.dtu.dk/services/SignalP-6.0/) [71].

Prediction of protein structure of Oat *DMP* gene family

The secondary structure of proteins was predicted using the SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_ automat. pl? page=npsa%20_sopma.html) [72]. The tertiary structure of proteins was predicted using the SWEISS-MODEL tool (https://swissmodel.expasy.org) [73].

Phylogenetic analysis of *DMP*gene

We selected *Z. mays*, *O. sativa*, and *S. bicolor*, which are in the same grass family as *A. sativa*, along with the model plant *A. thaliana*, to construct an unrooted phylogenetic tree. This tree aims to explore the phylogenetic relationships and classification of *AsDMP* genes in *A. sativa*. Based on the reported *DMP* gene IDs for *Z. mays*, *O. sativa* and *S. bicolor*, the corresponding DMP sequences were extracted from their respective genomic sequences [17]. The obtained AsDMP sequences were compared with the amino acid sequences of *A. thaliana*, *Z. mays*, *O. sativa* and *S. bicolor* using ClustalW in MEGA 11 software. A phylogenetic tree was constructed using neighbor-joining (NJ) method in MEGA 11 with 1000 bootstrap replicates [74]. Utilizing EvolView v3 for the visualization and beautification of phylogenetic trees [75].

Analysis of the conserved protein motifs and gene structure

Conserved motifs were predicted using the MEME (Multiple Expectation Maximization for Motif Elicitation, version 5.5.5) tool (http://meme-suite.org/tools/meme) [76], with the number of output motifs set to 10 and other parameters at their defaults settings. The conserved structural domains in AsDMP sequences were identified using Batch CD-Search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) [77]. The distribution of the motifs, along with the phylogenetic tree and gene structures, was visualized using TBtools software [78].

Chromosomal localization and collinearity of oat *DMPs* gene

The Gene Density Profile tool in TBtools was used to determine the gene density across *A. sativa* chromosomes, and Gene Location Visualize from GTF/GFF was employed to visualize the chromosomal locations of *AsDMP* genes. To analyze the interspecific collinearity between the *DMP* gene families of *A. sativa*, *Z. mays* and *S. bicolor*, One Step MCScanX in TBtools was utilized, and the results were visualized using the Multiple Synteny Plot program [78].

Calculation of selection pressure for duplicated gene pairs

Duplicated *DMP* gene pairs were used to calculate nonsynonymous (Ka) and synonymous substitution rates (Ks) using the Ka/Ks calculator in the TBtools software [78]. A Ka/Ks ratio>1 indicates positive selection, a ratio=1 indicates neutral selection, and a ratio<1 suggests negative or purifying selection. The selection pressure of each duplicated *DMP* gene pair was then estimated [17].

Analysis of DMPs promoter regions and differentially expressed genes of RNA-Seq data

We used TBtools software to extract the 2000 bp DNA sequence upstream of the *AsDMP* genes from the *A. sativa* genome. Cis-regulatory elements related to phytohormones, plants growth and development and abiotic stress in promotor regions of *DMP* genes were predicted and analyzed using the PlantCARE website (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) for prediction and analysis of [79]. RNA-Seq data for analyzing expression patterns in different tissues or developmental stages were obtained from the Plant Genomics & Phenomics Research Data Repository (https://doi.org/10.5447/ipk/2022/2) [80]. The heat map, along with the phylogenetic tree and cis-elements, was generated using TBtools software [78].

qRT-PCR of AsDMPs

The materials tested were *A. sativa* "Baiyan No.7" varieties, naturally stored for 0, 1, 2 and 3 years at an average annual temperature of approximately 10 °C. Seeds harvested in 2023 were subjected to artificial aging under high temperature and high humidity conditions. Initially, seed moisture content was adjusted to 10%~14% and placed in an aging chamber set at 45 °C with a relative humidity of 95%. Aging durations were 24, 48, 72, and 96 h. After aging treatments, seeds are dried until their moisture content returned to the original state and then stored at 4 °C [81]. Unaged seeds served as the control check (CK).

Table 4 Sequences of primers used in qRT-PCR

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)
AsDMP1	CAACGCAGCACGAATCAG	TCGTCTACGAG GATCTGGTC
AsDMP2	TCGTCACCGTCATGGTCTT	ACCGGGTAGA AGCATGACAC
AsDMP3	GGTAAGGTTCCTCGACCTC	GAAGCATGAC ACCACGTTC
AsDMP10	ATGAACCTTGTCCTGAGTGG	AGATGGCGA AAGTCAGGATG
AsDMP19	CACGGTCTTCATGTTCCAGT	CGCTGAGGAC CTTGTTGTAT
AsDMP22	ATTCTGACCTTCGCCATCTT	GGTCAGGACG TGGTTGATG
AsDMP25	CCTTCTCATCCTTCACCGAC	AAGACCAAGA GCGAGAGC
AsDMP26	TCTCGCTCTTGGTGTTCG	ACATCATGAAG GCGTAGCTG
AsDMP28	GTCAGGTACGGCATCGTAA	AACACGATCAC GGAGAAGAG
AsDMP29	CCCTGAGGGATTTCTCAA AGTA	CGAACACGATC ACGGAGAA
AsActin(KP257585.1)	CATTGGTATGGAAGCTGCTG	CACTGAGCAC AATGTTACCG

The Total RNA extraction kit (TIANGEN, DP419, China) was used to extract RNA from *A. sativa* seeds. RNA quality was assessed by 1% agarose gel electrophoresis, while RNA concentration and purity were determined by the Nanopro 2010/2020 ultra-micro ultraviolet spectrophotometer (Beijing, China). Reverse transcription of RNA was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, RR047A, China).

The specific primers for *AsDMPs* used in the qRT-PCR analysis are detailed in Table 4. qRT-PCR analysis was conducted with the TIANGEN fluorescence quantitative kit (FP206-02). The reaction mix was prepared in a total volume of 20 μL, consisting of 5 μL template, 1.6 μL primer, 3.4 μL ddH_2O, and 10 μL of 2×SuperReal PreMix Plus. The qRT-PCR conditions were as follows: pre-denaturation at 95℃ for 15 min, followed by 40 amplification cycles including denaturation at 95℃ for 10 s and annealing at 58℃ for 30 s. The solution curve analysis was performed with the following steps: 1 s at 95℃, 15 s at 65℃, and 1 s at 95℃. *AsActin* (*KP257585.1*) was sed as the internal reference gene. Each experiment was independently repeated three times, and the relative expression levels of *AsDMP* genes were measured using the $2^{-\Delta\Delta Ct}$ method [82].

Statistical analysis

One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics 23.0 software, with data presented as means±standard errors. Mapping was conducted using Origin 2021 software (OriginLab Corporation, 2021).

Abbreviations

DMP	Domain of unknown function 679 membrane protein
dPCD	Development-programmed cell death
BFN1	Bifunctional nuclease1
PASPA3	Putative aspartic protease A3
RNS3	Ribonuclease3
CEP1	Cystein endopepditase1
EXI1	Exitus1
GR	Glutathione reductase
CK	Control check
NaN	Not a number
Ka/Ks	The ratio of the number of nonsynonymous substitutions per nonsynonymous site (Ka) to the number of synonymous substitutions per synonymous site (Ks)
ABA	Abscisic acid
sHSP	Small heat shock protein
LOX	Lipoxygenase
PLD	Phospholipase D
POD	Peroxidase
JA	Jasmonic acid
ROS	Reactive oxygen species
H_2O_2	Hydrogen peroxide

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Author contributions

Y.M, H.L., G.Z., J.W., K.N. and X.Z. conceived and designed the project; J.W. provided the experimental materials; Y.M. conducted the experiments and wrote the first draft; H.L. and R.Z. reviewed and revised the first draft, and H.L. provided financial support for the experiments; R.Y. conducted the experiment instruction. All authors edited and approved the final manuscript.

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Data availability

The genome sequences of *Zea mays* (version 1.1), *Oryza sativa* (version 7.0), and *Sorghum bicolor* (Version 3.1) were acquired from phytozome (https://phytozome-next.jgi.doe.gov/). The *Avena sativa* genome sequence as well as annotation files were downloaded from the Ensembl Plants database (https://plants.ensembl.org/index.html). The published *Arabidopsis* AtDMP protein sequence was downloaded from the TAIR Web site (http://www.arabidopsis.org). RNA-Seq data were obtained from the Plant Genomics & Phenomics Research Data Repository (https://doi.org/10.5447/ipk/2022/2) to analyse expression patterns in different tissues or at different developmental periods in the same tissue. In this study, *Avena sativa* seeds were obtained from Gansu Agricultural University.

Declarations

Ethics approval and consent to participate

In this study, *Avena sativa* seeds were obtained and collected from Gansu Agricultural University. Professor Huan Liu has gained the permission from Gansu Agricultural University to perform a breeding trial.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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