Genome-wide identification of the oat DMP gene family and its expression analysis in response to seed aging

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Abstract

Background:

The Domain of unknown function 679 membrane proteins (DMPs) family, as a green plant-specific membrane protein, plays an important role in plant reproductive development, stress response and aging. To identify the *DMP* gene members of oat (*AsDMP*) and to investigate their family structural features and tissue expression profile characteristics, a study was conducted. Based on the whole genome and transcriptome data, in this investigation, we have scrutinized the physicochemical properties, gene structure, cisacting elements, phylogenetic relationships, conserved structural (CS) domains, CS motifs and expression patterns of the *AsDMP* family of oat.

Results

The *DMP* family genes of oat were found distributed across 17 chromosomal scaffolds with 33 members. We could divide the *AsDMP* genes into five subfamilies based on phylogenetic relationships. The gene structure suggests that oats may have also undergone an intron loss event during evolution. Covariance analysis suggests that genome-wide duplication/segmental duplication may be the major contributor to the expansion of the *AsDMP* gene family. Ka/Ks selective pressure analysis of oat *DMP* gene family, suggests that *DMP* gene pairs tend to be conserved over evolutionary time. The upstream promoter of these genes containing several cis-acting elements indicates a plausible role in abiotic stress and hormone induction. Gene expression pattern according to transcriptome data revealed participation of the *DMP* genes in tissue and organ development. In this study, *AsDMP* genes (*AsDMP1*, *AsDMP19*, and *AsDMP22*) were identified as potentially regulating oat seed senescence, and can be used as candidate genes for seed longevity and anti-aging germplasm breeding studies in oat. The study provides valuable information on the regulatory mechanism of the *AsDMP* gene family in the aging process of oat germplasm, and also provides theoretical support for further function investigation in the oat *DMP* gene and the molecular mechanism of seed anti-aging.

Conclusions

In this study, we found that the *AsDMP* gene is involved in the aging process of oat seeds, which is the first report on the potential role of *DMP* genes in oat seeds.

1. Introduction

Membrane proteins play a crucial role in various biological processes, including cell proliferation and differentiation, signal transduction and recognition, as well as material transport [1–4]. Members of the DMPs (Domain of unknown function 679 membrane proteins) family are a group of membrane proteins that are specific to green plants [5]. This uncharacterized plant-specific gene family member typically contains four transmembrane domains with cytoplasmic amino and carboxyl termini. It is predicted to play a role 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 *DMP* gene family members have been identified. These members exhibit distinct expression patterns in various tissues and organs. Among them, AtDMP1, AtDMP2, AtDMP3, AtDMP4 and AtDMP7 are involved in different types of programmed cell death, such as organ senescence, silique dehiscence and abscission of floral organs and siliques; while AtDMP8 and AtDMP9 promote gamete fusion during double fertilization [5, 8, 9]. AtDMP1 is reported to be up-regulated during both developmental senescence and darkness-induced senescence, and the expression of DMP1 increases from the beginning to late senescence, indicating that the function of DMP1 is consistent in developmental senescence and induced senescence, and it is involved in throughout the senescence process; whereas AtDMP3 and AtDMP4 are upregulated during leaf senescence, indicating overlapping functions during senescence [9, 10]. Studies have shown that both DMP1 T-DNA insertion mutants and DMP1 overexpressing plants age earlier than the wild type, and DMP1 aging-specific transcriptional activation is regulated by WRKY transcription factors[11]. Mutations in two W-boxes (homologous binding sites for WRKY proteins) in the DMP1 promoter cause loss of DMP1 expression during aging [11, 12]. In addition, DMP1 directly or indirectly participates in membrane division during endoplasmic reticulum decomposition during leaf aging, membrane fusion during root vacuole formation [7], and dual targeting of vacuole membrane and plasma membrane [13]. The DMP gene has been extensively studied for haploid induction. The haploid induction system of polyploid mothers of *Arabidopsis thaliana*, maize (*Zea mays*), *Brassica napus* and tobacco (*Nicotiana tabacum*) was established [14–16]. The ability of DMP gene to induce haploid in polyploid monodicotyledonous crops was further confirmed. A systematic analysis of the DMP family in cotton (*Gossypium hirsutum*) identified 58 DMPs, and the analysis of these DMPs expression patterns revealed their possible involvement in key biological processes, such as plant aging, reproductive development, and stress response [17]. It is reported that development-programmed cell death (dPCD) genes include BIFUNCTIONAL NUCLEASE1 (BFN1), PUTATIVE ASPARTIC PROTEASE A3 (PASPA3), RIBONUCLEASE3 (RNS3), CYSTEIN ENDOPEPDITASE 1(CEP1), DUF679 MEMBRANE PROTEIN4(DMP4), and EXITUS1(EXI1). During stigma senescence of *Arabidopsis thaliana*, AtDMP4, together with BFN1, RNS3, EXI1, CEP1 and PASPA3, promote senescence and dPCD process [18], and DMP4 is the only protein interacting with DMP1 among DMP proteins [11].

Seed "aging" or "deterioration" is a very common phenomenon in the storage process of agricultural and pasture seeds, which often occurs with the extension of seed storage time, resulting in the irreversible changes of seed viability, vitality and germination ability [19, 20]. Natural aging and artificial accelerated aging are two ways of seed aging. Natural aging refers to the process of gradually losing the vitality of seeds after maturity under natural conditions. Artificial accelerated aging refers to the process of aging seeds through artificial control, which causes the rapid loss of seed vitality. It is an effective way to study seed deterioration, so it is widely used in the study of storage resistance of seeds [21, 22].

As an important multipurpose grass crop with excellent nutritional quality and high economic value, oats play a prominent role in livestock production [23]. At the same time, it owns significance in reducing the grazing pressure on grassland, recovering from grassland degradation, and ecological protection, and is

regarded as an irreplaceable unique food and fodder crop in areas with fragile ecological conditions [24]. Aging of oat seeds is not only related to seed and seedling growth as well as yield and quality, but also has an impact on the conservation, utilization and development of germplasm resources. Therefore, reducing the economic losses caused by quality decline due to aging and deterioration of oat seeds is of great significance in agricultural production. DMP proteins, as a membrane protein found almost exclusively in green plants, have been systematically analyzed in Arabidopsis [6], cotton [17], and soybean [9]. In addition, DMP was separately characterized in several species including rice(*Oryza sativa*) (20), grape (*Vitis vinifera*) (7), maize (15), sorghum(*Sorghum bicolor*) (15), and pineapple (*Ananas comosus*) (9), revealing the involvement of DMP in a variety of biological processes in plants including the promotion of gamete fusion during double fertilization, induction of maternal haploid production, plant senescence, reproductive development, and stress response [17]. For the time being, most of the studies on *DMP* genes have focused on haploid breeding, while fewer studies have been conducted on aging. The existence of *DMP* gene family members in oat in response to aging stress is not known, and studies on the expression and function of *DMP* genes in oat seeds during aging are even less reported. In this study, we identified 33 *DMP* gene family members from the oat genome and analyzed their phylogenetic relationships. In addition, we used bioinformatics tools to comprehensively analyze the physicochemical properties, protein structure, subcellular localization, conserved motifs, and chromosomal location of the oat DMP family members, and characterized the expression patterns of the genes, which will provide theoretical support for further investigation of the molecular mechanism of the oat *DMP* genes and for breeding research.

2. Results

Identification of the DMP proteins in oat

Thirty-three DMP sequences were identified and renamed as *AsDMP1* to *AsDMP33* according to their positions on the chromosome (Table 1). In addition, the physicochemical properties of these oat DMP genes were determined, including amino acid sequence length (aa), isoelectric point(PI), protein molecular weight (kDa), instability index, fat index, and subcellular localisation. All 33 DMP genes encode amino acid lengths ranging from 169 (*AsDMP6*) to 325 (*AsDMP24*) aa; Theoretical equivalence points range from 5.26 (*AsDMP28*) to 9.04 (*AsDMP33*); Molecular weight range 17.88(*AsDMP6*) to 35.50(*AsDMP24*) kDa; The predicted fat index range was 76.04 to 107.62. Fat index indicates thermal stability of proteins. Hydrophilicity and hydrophobicity of proteins is one of the most important factors affecting the structural stability of proteins. All AsDMP proteins were analysed and found to be hydrophilic (Fig. 1), indicating their transmembrane nature. In addition, all AsDMP proteins had transmembrane structural domains ranging from 3 to 4 in number, and none of the AsDMP proteins possessed a signal peptide, suggesting that all AsDMP proteins are transmembrane unsecreted proteins. Predictions of the subcellular localisation of DMP proteins indicate that all proteins are localised to the plasma membrane, which is consistent with the function of DMP. In addition, AsDMP14, AsDMP19, AsDMP24, AsDMP25, AsDMP26 and AsDMP32 were localised extracellularly, as well as AsDMP15 and AsDMP21 in chloroplasts, and these results suggest that the DMPs also regulate biological processes inside and outside the plasma membrane.

Table 1
The characterization of oat *DMP* genes in this study

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

Protein structure prediction of oat DMP family members

The main protein secondary structures are α-helix, β-folding, β-turning, irregularly coiled and extended chains. The predicted secondary structure based on the amino acid sequence of oat AsDMP was found to consist mainly of α-helices, β-turns, irregularly coiled and extended chains (Table 2). In addition, α-helix and random curl account for a large proportion in the secondary structure of all AsDMPs members, and it can be determined that the amino acid secondary structure of AsDMPs is mainly composed of α-spiral and random coil. By predicting the tertiary structures of oat DMP family members' proteins, the tertiary

structures of AsDMP1, AsDMP2 and AsDMP3 proteins, the tertiary structures of AsDMP4, AsDMP18, AsDMP20, AsDMP28 and AsDMP29 proteins, the tertiary structures of AsDMP5, AsDMP7, AsDMP8, AsDMP13 and AsDMP31 proteins, the tertiary structures of AsDMP10, AsDMP16, and AsDMP22 proteins, the tertiary structures of AsDMP11, AsDMP12, AsDMP15, AsDMP21, AsDMP23, and AsDMP24 proteins, the AsDMP19, AsDMP25, and AsDMP26 tertiary structures of AsDMP19, AsDMP25 and AsDMP26 proteins, and the tertiary structures of AsDMP27 and AsDMP30 proteins were similar to a high degree, presumably with some functional similarity, but showed different morphologies from each other and differed from the tertiary structures of the rest of the family members, suggesting a diversity of tertiary structures of the AsDMP family members (Fig. 2).

Table 2
Prediction of secondary structure of *DMP* gene family proteins in oats(%)

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

Phylogenetic analysis of DMP gene family in oat

To analyze the phylogenetic relationships of DMP family members among different species, we used the MEGA 11 eighbor-joining (NJ) method to construct a phylogenetic evolutionary tree based on 33 DMP members in oat and Arabidopsis, maize, rice and sorghum. The phylogenetic tree showed that the plant DMP gene family can be classified into five subfamilies, subfamilies I, II, III, IV and V (Fig. 3). Subclade V has the highest number of DMPs, containing 40 genes, of

which 15 are AsDMPs. This was followed by subfamilies III, I, IV, and II, which contained 23, 15, 10, and 5 genes, respectively, of which 8, 7, 3, and 0 were AsDMPs, respectively. The oat DMP gene underwent significant expansion compared to other plants and the branching clustering pattern of the oat DMP protein was similar to that previously reported for cotton [17].

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

To further clarify the diversity and conservation of the oat *DMP* gene family during evolution, the conserved motifs, conserved domains and gene structures of *AsDMP* were analysed based on phylogeny (Fig. 4). A total of 10 Motifs were predicted through the MEME website (Fig. 4B; Fig. 5), with the number of motifs ranging from 4 to 9 for all DMP proteins, and all DMP members had Motif 3, Motif 4, and Motif 5, suggesting that these three motifs are highly conserved among the 33 *AsDMP* members. The basal composition and distribution of DMP members in the same subfamily are essentially the same, which may prove their functional similarity. It is noteworthy that there are differences in the Motif composition of some proteins in different or the same subfamily. For example, Motif 8 and Motif 10 are present for only some of the proteins in subfamily V, indicating their functional variability. In addition, certain motifs are absent in certain subfamily members, and some specific motifs are present only in specific genes. For example, Motif 1 and Motif 7 are missing in all *AsDMP* members in subfamily III, and Motif 7 is missing in subfamily IV. Motif 6 is present only in subfamily III, and Motif 8, Motif 9, and Motif 10 are also present in only some members. Whether the presence or absence of these specific motifs confers unique functional roles on the *DMP* genes requires further study, and the variation in subfamily motif composition may be due to their functional diversity. According to gene structure, no intron structure was found in other oat *DMP* members except *AsDMP4* of subfamily I and *AsDMP24* of subfamily V (Fig. 4D), indicating that although DMP family members may perform similar functions, there are still differences among individuals.

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

In order to better understand the distribution mechanism of *DMPs* gene in oat chromosomes, the chromosome map of 33 *DMP* genes in oat was constructed according to the genome sequence of oat (Fig. 6), and the 33 identified *AsDMP* genes were distributed on 17 chromosomes. Five genes were distributed on each of chromosomes chr 3C and chr 4D, three genes on chr 4A and chr 6A, two genes on chr 1A, chr 2D, chr 3D, and chr 4C, and one gene on each of the remaining chromosomes. Tandem replication occurs on chr 3C, chr 4D, and chr 6A.

Gene family evolution mainly includes whole genome replication, fragment replication and tandem replication [25]. Most plants underwent an ancient genome-wide replication event, or polyploidy, resulting in the duplication of all genes in a region [26]. This large-scale chromosomal doubling event resulted in the retention of a large number of chromosomal doubling fragments in the genome [27]. Tandem repeats occur on the same chromosome and are adjacent to each other, often with similar sequences and similar functional clusters [28]. Fragment duplications are duplicated genes that located far apart or on different chromosomes. Gene duplication events are the main cause of gene family expansion and doubling [29, 30].

Through homology analysis of oat *DMP* genes, we visualized the relationships among oat *DMP* genes to elucidate the expansion mechanism of oat *DMP* gene family (Fig. 7). Among the 35 homologous gene pairs, 4 gene pairs were identified to have tandem replication events (*AsDMP11*/ *AsDMP12*, *AsDMP20*/ *AsDMP21*, *AsDMP23*/ *AsDMP24*, *AsDMP28*/ *AsDMP29*) (Fig. 6). Thirty-one pairs of genes underwent genome-wide replication or fragment replication. Therefore, we hypothesized that the major causes of gene amplification during *DMP* gene evolution were whole-genome duplication events or fragment duplication events.

In order to further understand the evolutionary relationship of *AsDMP* genes, we constructed collinearity maps of DMP families in oats, sorghum and maize (Fig. 8). 14 *AsDMP* genes were found to be colinear with at least two or more homologous genes, 19 pairs of colinear relationships between 14 *AsDMPs* and 5 *SbDMPs*, 14 pairs of colinear relationships between 14 *AsDMPs* and 4 *ZmDMPs*, and the 14 *AsDMP* genes with colinear relationships in sorghum and maize were the same, suggesting that poaceae family showed higher conservation between them. It 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 judging the ratio of the number of nonsynonymous substitutions per nonsynonymous site (Ka) to the number of synonymous substitutions per synonymous site (Ks) (Ka/Ks)(Table 3). Not a Number (NaN) means that these pairs of genes have almost synonymous mutations at sites where synonymous mutations can occur, indicating that the sequence divergence is quite large and the evolutionary distance is very far. We found that since the Ks value of *AsDMP-1*/ *AsDMP-9*, *AsDMP-1*/ *AsDMP-14*, *AsDMP-2*/ *AsDMP-9*, *AsDMP-2*/ *AsDMP-14*, *AsDMP-3*/ *AsDMP-9*, *AsDMP-3*/ *AsDMP-14* is NaN, The Ka/Ks is NaN. Among other gene pairs with segmental duplication, 22 gene pairs have Ka/Ks ratios less than 0.5, 3 gene pairs have Ka/Ks ratios between 0.5 and 1.0, and Ka/Ks are all less than 1, and 4 tandemly repeated genes The Ka/Ks ratio is also less than 1, indicating that these *AsDMP* genes were subject to purifying selection during the evolution process and functional differentiation occurred after whole-genome duplication/segment duplication.

Table 3
Ka/Ks values of duplicated *DMP* gene pairs from oat species

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

Cis-element analysis of AsDMP gene promotors

Gene expression was usually regulated by cis-elements in its upstream promoter sequence. Exploring the cis-regulatory elements contained in the promoter region of the oat *DMP* gene will help to understand the regulatory mechanism of the *DMP* gene and speculate on its potential functions. We use the PlantCARE database to conduct predictive analysis of promoter cis-regulatory elements. Based on the role and function of cis-regulatory elements, they are divided into the following categories: Hormone responsive elements 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 low temperature, defense and stress response elements, MYB binding sites (MYBHv1, light response, drought and flavonoid biosynthetic gene regulation), anaerobic induction and hypoxia-specific induction response elements. 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 of zein metabolism.

Among the cis-elements involved in plant growth and development, light-responsive elements are the most abundant, with all except *AsDMP10* having light-responsive elements and widely distributed in the promoter region. Elements related to endosperm expression are located in the *AsDMP13*, *AsDMP15*, *AsDMP16*, *AsDMP21*, *AsDMP22*, *AsDMP29*, *AsDMP32*, and *AsDMP33* promoter regions; The seed-specific regulatory elements involved 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*. The regulatory elements involved in endosperm expression and seed-specific regulatory elements may be involved in the regulation of plant endosperm and seed development, suggesting that these *DMP* genes may play an important role in plant reproductive development.

Almost all promoters contain several hormone response elements, but the hormone response elements are not closely related to their subfamilies (Fig. 9). Among them, cis-acting elements involved in MeJA response were most abundant in the *AsDMP* gene promoter region, and 32 *AsDMP* gene promoter regions contained at least one MeJA response element. ABA-responsive elements are also widely present in the promoter region of *AsDMP* gene, with 29 AsDMP family members containing at least one ABA responsive element in their promoters. Stress related cis-regulatory elements are also widely distributed in the promoter region of *AsDMP* gene. Among the cis-acting elements of abiotic stress, MYB elements are the majority. Most AsDMP members contain one or more MYB elements in their promoters, which are involved in drought, photoresponse and the regulatory response of flavonoid biosynthesis genes. There are 20 *AsDMP* genes that respond to low temperature, and 13 *AsDMP* genes that respond to defense and stress. In addition, there are some cis-acting elements that are necessary for hypoxia and anaerobic induction. These results indicate that the transcription of oat *AsDMP* gene may be affected by multiple environmental factors.

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

Use of oat transcriptome data to analyse the expression pattern of the *AsDMP* gene in different tissues or at different developmental periods in the same tissue, including spikes, roots and leaves during seedling development, and seeds at the early, middle and late stages of development (Fig. 10). According to the transcriptome results, we found that *AsDMP5*, *AsDMP6*, *AsDMP31*, and *AsDMP33* were not expressed in the above tissues. In *AsDMP1*, *AsDMP2*, *AsDMP3* and *AsDMP29*, *AsDMP3* was not expressed in leaves, and *AsDMP29* was not expressed in ears and leaves, but was expressed in other tissues. And the expression of *AsDMP1* and *AsDMP3* was first down-regulated and then up-regulated in the early, middle and late stages of seed development, while *AsDMP2* was consistently up-regulated and *AsDMP29* was consistently down-regulated; AsDMP4 is expressed in seeds only at mid-development and not at early or late developmental stages; *AsDMP7* and *AsDMP8* were only expressed in spikes, suggesting that they may be related to spike development or fruit formation; *AsDMP27* was expressed only in roots, and it was hypothesised that it might be involved in root growth and development. *AsDMP9*, *AsDMP10*, *AsDMP13*, *AsDMP14*, *AsDMP15*, *AsDMP16*, *AsDMP22*, *AsDMP28*, and *AsDMP30* were each expressed during seed development; *AsDMP19*, *AsDMP25*, and *AsDMP26* were expressed in spikes, roots, and leaves as well as in the early, middle, and late stages of seed development, and were first up-regulated and then down-regulated with the period of seed development. Notably, the expression of *AsDMP25* and *AsDMP26* was the highest among the 33 *AsDMP* genes in the middle stage of seed development, when the expression of these two genes was 4.2-fold and 2.3-fold higher than that in the early stage and 9-fold and 4.7-fold higher than that in the late stage. In summary, it is hypothesised that of the 17 genes expressed during seed development, 7 genes (*AsDMP1*, *AsDMP2*, *AsDMP3*, *AsDMP19*, *AsDMP25*, *AsDMP26*, *AsDMP29*) are involved in the whole process of seed development; 2 genes (*AsDMP4*, *AsDMP14*) are involved in mid-seed development; and 4 genes (*AsDMP9*, *AsDMP13*, *AsDMP15*, *AsDMP30*) are involved in late seed development.

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

In order to further analyze the expression of oat *DMP* genes under natural aging and artificial high temperature aging, based on transcriptome analysis and according to the expression changes of *AsDMP* genes during seed development period, we selected 10 genes in the *AsDMP* gene family (*AsDMP1*, *AsDMP2*, *AsDMP3*, *AsDMP10*, *AsDMP19*, *AsDMP22*, *AsDMP25*, *AsDMP26*, *AsDMP28*, *AsDMP29*) were analyzed by qPCR (Fig. 11). The qPCR results showed that the expression of *AsDMP1* and *AsDMP19* was up-regulated in both natural aging treatment and artificial aging treatment, while the expression of *AsDMP22* was down-regulated in both aging ways. It shows that the two genes *AsDMP1* and *AsDMP19* are involved in regulating the senescence and vigor reduction of oat seeds under natural aging and artificial aging, while the *AsDMP22* gene plays a positive role in delaying or hindering the senescence of oat seeds under natural aging conditions and artificial high-temperature aging. It is worth noting that the *AsDMP28* and *AsDMP29* genes are only expressed under artificial aging treatment, indicating that the expression of *AsDMP28* and *AsDMP29* is induced under high temperature treatment, but not under natural aging.

3. Discussion

This study used bioinformatics methods to identify a total of 33 DMP family members in the oat genome. The number of *DMP* genes is different from that of Arabidopsis, cotton, rice, corn, etc. This is probably due to genome size and gene duplication during plant evolution caused by differences, oat owns large genome and high content of repetitive sequences [31]. The physical and chemical properties of the oat *DMP* genes were determined, and understanding its protein structure, chromosomal distribution, evolutionary relationships, conserved domains, cis-regulatory elements and gene expression patterns is crucial to exploring the functional diversity of the *DMP* gene and improving seed viability. The physical and chemical properties of DMP members differ greatly, and there are certain differences in conserved motifs, suggesting that DMP may have different biological functions in the growth and development of oats. Prediction of the subcellular localisation of the AsDMP proteins revealed that all proteins were localised to the cell membrane, which is consistent with the function of the DMP proteins. In addition, AsDMP14, AsDMP15, AsDMP19, AsDMP21, AsDMP24, AsDMP25, AsDMP26, and AsDMP32 are located in extracellular and chloroplasts, respectively, in addition to being localised in the cell membrane, suggesting that DMP proteins are also involved in other

biological processes. Differences in subcellular localisation also illustrate the variability in function among AsDMP members. As in the case of some other aging-related genes, in addition to having the ability to inhibit seed aging and improve seed storage tolerance, they are also involved in other biological processes. For example, small heat shock proteins(sHSPs) is involved in adversity stress, seed germination, pollen development and fruit ripening in addition to seed aging [32, 33]; Lipoxygenase(LOX) has the function of regulating plant development and synthesizing signaling substances such as phytodienoic acid, jasmonic acid, and abscisic acid [34]; Phospholipase D(PLD) is involved in many processes such as plant growth and development, interaction of plant hormones and abiotic stress signals, defense against fungal pathogen infection, stomatal closure, etc. [35, 36]. It suggests that most aging-related genes are multifunctional. In addition, studies have shown that several biological stresses seem to induce DMP1 transcription. DMP1 moderately to strongly responded to Botrytis cinerea, phytophtora infestans, virulent and avirulent pseudomonas syringae strains and several bacterial elicitors such as Flg22, HrpZ and NPP1 [11]. Thus, DMP1 might be involved in the plant immune system.

The results of conserved motif analysis showed that three motifs exist in all AsDMPs, while other motifs only exist in specific genes. These unique motifs are an important reason for the functional differentiation of AsDMP proteins. Gene structure analysis shows that most *AsDMP* genes do not contain introns, which is similar to the results of soybean and cotton *DMP* gene families[9, 17]. It is speculated that they may have special functions. Increased genetic sequencing suggests that each eukaryotic ancestor possessed intron-rich genes and that most eukaryotes experienced intron loss during the evolution process[37]. The higher proportion of genes without introns in oats suggests that oats may also have experienced intron loss events during evolution [38].

The proportions of α-helices, extended chains, irregular coils and β-turns in the secondary structure of the oat *DMP* gene family protein are different, resulting in differences in the spatial folding of the protein, which provides support for functional differences among DMP members. In phylogenetic analysis, oat DMP protein was combined with DMP members of 4 species, including Arabidopsis, rice, maize and sorghum, to construct evolutionary tree, and it was found that DMP members could be divided into 5 subfamilies, which were different from the evolutionary analysis of Arabidopsis, and the differences in evolutionary analysis may be related to the differences in division methods. The number of AsDMP family members in the five subfamilies varies greatly, with more than 45% of AsDMP members (15) belonging to subfamily V. Tandem duplication and whole-genome duplication/segment duplication are one of the important reasons for gene doubling, gene functional specificity and diversification. While promoting the expansion of eukaryotic gene families, they may also lead to gene functional redundancy [39, 40]. The high degree of expansion of the *DMP* gene family mainly comes from several whole-genome duplications. Only 3 pairs of genes in the 4 cotton species have undergone tandem duplication, and the remaining 134 pairs of genes have experienced whole-genome duplication/segment duplication [17]. AsDMP has 4 pairs of genes that have undergone tandem duplication during the evolution of oats, and 31 pairs of genes have undergone segmental duplication events. Whole-genome duplication/segmentation duplication may be the main reason for the expansion of the *AsDMP* gene family, which originated from the whole-genome duplication event in oats [31]. In addition, the Ka/Ks ratio of all repeated *AsDMP* genes is less than 1, indicating that the *AsDMP* genes have been subject to strong purifying selection during the evolution of oats, indicating that the functional limitations of oat DMP mainly come from purifying selection. However, the Ka/Ks of most gene pairs ranged from 0 to 0.49, indicating that *DMP* gene pairs tend to be conserved during evolution [41].

Gene expression is usually regulated by cis-elements in its upstream promoter region. These cis-elements located in non-coding DNA upstream of the gene transcription start site regulate the stress expression or tissue-specific expression behavior of genes under different environments. Therefore, the analysis of cis-elements involved in the regulation of the *AsDMP* gene can help to understand the regulatory mechanism of the *AsDMP* gene and predict its potential function. There are many cis-elements in the promoter region of the *AsDMP* gene that are related to growth, development, stress and phytohormone response, most of which are involved in abiotic stress and hormone signalling, which is similar to the predicted cis-acting elements of the cotton *DMP* gene [17].

At present, it is generally believed that the reason of seed aging and deterioration is lipid peroxidation caused by free radicals [42, 43], and it has been confirmed in other plant seeds, such as *Zoysia japonica Steud* [44], *Ulmus pumila* [45] and *Jatropha curcas* [46]. It has also been demonstrated in other aging-related genes, such as *sHSP*, that it can improve tolerance to oxidative stress by protecting photosystem II and increasing peroxidase (POD) activity [47]. In this study, we analysed the expression of *DMP* genes in oat seeds under high temperature and aging stress, and the trend of changes showed that *AsDMP1* and *AsDMP19* were up-regulated and expressed under natural and artificial aging treatments. DMP induces a series of membrane fusions and divisions that impact on changes in the structure of the endoplasmic reticulum and vesicles, which in some cells lead to rupture of the entire endoplasmic reticulum and vesicles, cell death, and consequent seed aging [6, 7]. On the other hand, many studies have shown that jasmonic acid (JA) is a positive regulator of the senescence process [12, 48]. By investigating the effects of DMP overexpression, it was found that CYP94B3, which mediates the inactivation and catabolism of biologically active jasmonic acid (JA-Ile), exhibits strong regulatory effects, which may lead to the accumulation of JA-Ile, which can lead to ageing [11]. However, the down-regulation was observed at 3 years of natural storage. It was hypothesised that this might be due to the longer storage period of naturally aged seeds, which resulted in the accumulation of harmful substances such as reactive oxygen species (ROS) and peroxides in the seeds, inducing membrane lipid peroxidation, causing damage to the internal structure and function of the seeds, chromosomal aberrations and DNA damage, and destroying the integrity of the genes, which would lead to the decrease in the expression of *AsDMP* [49, 50]. In contrast, *AsDMP22* was down-regulated in the natural and artificial aging treatments, but the gene expression was slightly up-regulated in the artificial aging treatment. It is speculated that high temperature aging treatment causes the increase of hydrogen peroxide (H_2O_2) content in mitochondria and the damage of mitochondrial structure. In addition, during the aging process of mitochondria, accompanied by changes in the contents of ROS scavenging enzymes and antioxidant substances, ROS accumulates in the mitochondria of seed embryos, resulting in oxidative damage and inducing gene expression [51]. It is worth noting that the *AsDMP28* and *AsDMP29* genes are only expressed under artificial aging treatment, indicating that the expression of *AsDMP28* and *AsDMP29* is induced by high temperature and has little to do with natural deterioration.

Phytohormones are known to be key signaling compounds in regulating plant growth, development, and responses to environmental stresses [52]. During the senescence period of plants, abscisic acid, salicylic acid or jasmonic acid will change the expression of aging-related genes in plants, thereby regulating the aging process [10]. Many regulatory elements that respond to plant hormones are found in the promoter region of the *DMP* gene [9, 17]. These conditions

mean that the expression of DMP may be closely related to hormones, and The mechanism on hormones regulate the expression of DMP and the *AsDMP* gene participates in high-temperature aging response both needs further exploration.

4. Conclusions

In this study, we identified 33 *AsDMP* members in oats. The 33 genes are unevenly distributed on 17 chromosomes and divided into 5 subfamilies based on phylogenetic relationships. The study analyzed the basic characteristics, protein structure, subcellular localization, conserved motifs and gene locations of DMP members, providing a basis for the evolutionary relationship of the DMP gene family. Most *AsDMP* genes lack introns, indicating that the *AsDMP* gene structure is highly conserved. DMP members in the same subfamily share broad similarities, yet there are differences in the Motif composition of some proteins in different subfamilies or in the same subfamily, suggesting both functional similarities and differences. qRT-PCR analysis identified *AsDMP* genes (*AsDMP1*, *AsDMP19*, and *AsDMP22*) as potentially regulating oat seed senescence and can be used as candidate genes for anti-aging germplasm breeding research in oats. However, the current study only provides preliminary *AsDMP* gene characterization in oat seeds, and further functional validation is needed to understand the role of *AsDMP* genes in different biological processes.

5. Materials and methods

Identification of DMP protein family members

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 oat genome sequence as well as annotation files were downloaded from the Ensembl Plants database (https://plants.ensembl.org/index.html), while the published Arabidopsis DMP proteins were retrieved from the Arabidopsis Information Resource (TAIR, version 10, http://www.arabidopsis.org). The amino acid sequences of *AtDMPs* were used as query sequences, and the blastp program was used to search for oat candidate sequences. Subsequently, the 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, and eventually the DMP sequences were identified [53, 54]. ExPAsy - ProtParam (https://web.expasy.org/protparam/) and ExPAsy ProtScale AsDMPs protein (https://web.expasy.org/protscale/) were used for analysis of the physical and chemical properties and hydrophilicity [55]. The subcellular localization prediction was conducted using Subcellular localization prediction of each gene was predicated by the CELLO v2.5 server [56]. The numbers of transmembrane domains in oat DMP proteins were predicted using the DeepTMHMM tool (https://dtu.biolib.com/DeepTMHMM) [57]. Using SignalP – 6.0(https://services.healthtech.dtu.dk/services/SignalP-6.0/) forecast AsDMP signal peptide amino acid sequence [58].

Prediction of protein structure of Oat DMP gene family

Predicting the secondary structure of proteins using the SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa%20_sopma.html) [59]. Predicting the tertiary structure of proteins using the SWEISS MODEL tool (https://swissmodel.expasy.org) [60].

Phylogenetic analysis of DMP gene

Based on the reported DMP gene ID numbers of maize, rice and sorghum, the corresponding DMP sequences were extracted from their respective genome protein sequences [17]. The obtained AsDMP sequences were compared with the amino acid sequences of *Arabidopsis*, maize, rice and sorghum using ClustalW in MEGA 11 software. The phylogenetic evolutionary tree was constructed using neighbor-joining (NJ) in MEGA 11 (Bootstrap = 1000) [61].

Analysis of the conserved protein motifs and gene structure

The conserved motifs were predicted by the MEME (Multiple Expectation Maximization for Motif Elicitation) tool (http://meme-suite.org/tools/meme) [62]. Using MEME suite, the motifs were searched with these parameters: the number of output domains is 10, and other parameters are system defaults. Conserved structural domain information of oat DMP sequences obtained using Batch CD-Search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) [63]. TBtools software was employed to visualize the distribution of the motif along with the phylogenetic tree and gene structures [64].

Chromosomal localization and collinearity of oat DMPs gene

Gene Density Profile in TBtools was used to obtain the gene density of oat chromosomes, and Gene Location Visualize from GTF/GFF was used to visualise the chromosomal location of *AsDMP* genes; One Step MCScanX in TBtools was used to obtain the covariance between oat, maize and sorghum DMP family interspecific covariates using One Step MCScanX in TBtools, and visualise the covariate results using the Multiple Synteny Plot program [64].

Calculation of selection pressure for duplicated gene pairs

Duplicate DMP gene pairs were used to calculate non-synonymous (Ka) and synonymous substitution rates (Ks) by the Ka/Ks calculator of the TBtools software[64]. Based on the Ka/Ks ratio, Ka/Ks ratio > 1 indicates positive selection, Ka/Ks = 1 indicates neutral selection, while a ratio < 1 indicates negative or purifying selection. Finally, the selection pressure of each duplicated DMP gene pair was estimated [17].

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

We used TBtools software to take the DNA sequence 2000bp upstream of AsDMP from the oat genome sequence. We used the PlantCARE website (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) for prediction and analysis of cis-regulatory elements related to phytohormones, plants

growth and development and abiotic stress in promotor regions DMP genes [65]. 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. The heat map, along with the phylogenetic tree and cis-elements, was generated through the TBtool software [64].

qRT-PCR of AsDMPs

The materials for the test were germplasm materials of ruderal oat "Baiyan 2" stored naturally for 0, 1, 2 and 3 years, and the average annual temperature of natural storage was kept at about 10℃. The seeds harvested in 2023 were artificially aged at high temperature and high humidity. Firstly, the seed moisture content is adjusted to 10%~14% and placed in an artificial aging chamber. The temperature is set at 45℃ with a relative humidity of 95%. Aging durations include 24h, 48h, 72h, and 96h. After aging, the seeds are removed for drying until their moisture content returns to its original state. Finally, they are stored at a temperature of 4℃ [66]. Unaged seeds were used as control check (CK).

Total RNA extraction kit (TIANGEN, DP419, China) was used to extract RNA from oat seeds. RNA quality was detected by 1% agarose gel electrophoresis, and RNA concentration and purity were determined by Nanopro 2010/2020 ultra-micro ultraviolet spectrophotometer (Beijing, China). RNA reverse transcription was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser(Perfect Real Time) Kit (TaKaRa, RR047A, China).

qRT-PCR specific primers for *AsDMPs* are shown in Table 4. qRT-PCR analysis was performed using TIANGEN fluorescence quantitative kit (FP206-02). The 20 μL reaction system was as follows: Template 5 μL, primer 1.6 μL, ddH2O 3.4 μL, 2×SuperReal PreMix Plus 10 μL. The conditions of fluorescence quantitative PCR were: Predenaturation at 95℃ for 15 min, 40 cycles of PCR amplification, including denaturation at 95℃ for 10 s and annealing at 58℃ for 30 s. The solution curve analysis was retained as default, which lasted for 1s at 95℃, 15s at 65℃, and 1s at 95℃. The internal reference gene was *AsActin* (*KP257585.1*). The experiments were independently repeated three times, and $2^{-\Delta\Delta Ct}$ method was used to measure relative expression levels of *AsDMP* genes [67].

Table 4
qRT-PCR primers of *DMP* genes

Name	Forward primer (5'-3')	Reverse primer (5'-3')
AsDMP1	CAACGCAGCACGAATCAG	TCGTCTACGAGGATCTGGTC
AsDMP2	TCGTCACCGTCATGGTCTT	ACCGGGTAGAAGCATGACAC
AsDMP3	GGTAAGGTTCCTCGACCTC	GAAGCATGACACCACGTTC
AsDMP10	ATGAACCTTGTCCTGAGTGG	AGATGGCGAAAGTCAGGATG
AsDMP19	CACGGTCTTCATGTTCCAGT	CGCTGAGGACCTTGTTGTAT
AsDMP22	ATTCTGACCTTCGCCATCTT	GGTCAGGACGTGGTTGATG
AsDMP25	CCTTCTCATCCTTCACCGAC	AAGACCAAGAGCGAGAGC
AsDMP26	TCTCGCTCTTGGTGTTCG	ACATCATGAAGGCGTAGCTG
AsDMP28	GTCAGGTACGGCATCGTAA	AACACGATCACGGAGAAGAG
AsDMP29	CCCTGAGGGATTTCTCAAAGTA	CGAACACGATCACGGAGAA
AsActin(KP257585.1)	CATTGGTATGGAAGCTGCTG	CACTGAGCACAATGTTACCG

Statistical analysis

One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics 23.0 software, and data are presented as means and standard errors. Mapping using origin 2021 software.

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

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

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. Avena nuda seeds were collected with permission by Baicheng Academy of Agricultural Sciences in Jilin Province in accordance with institutional and national guidelines.

Consent for publication

Not applicable.

Availability of data and materials

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 oat 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.

Competing interests

The authors declare that they have no competing interests.

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Authors' 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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Figures

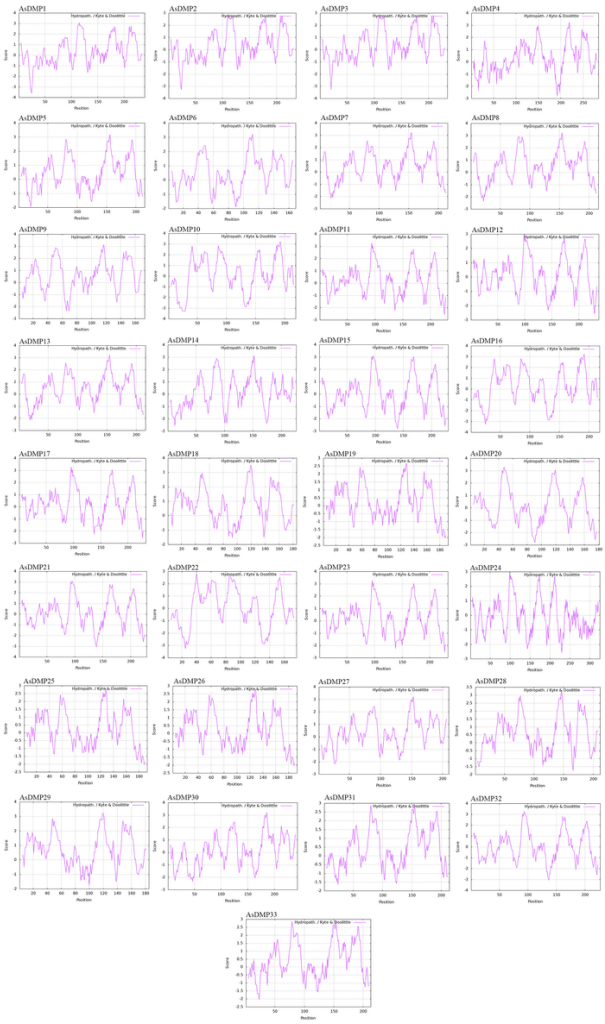


Figure 1

Prediction of AsDMP protein hydrophilicity and hydrophobicity

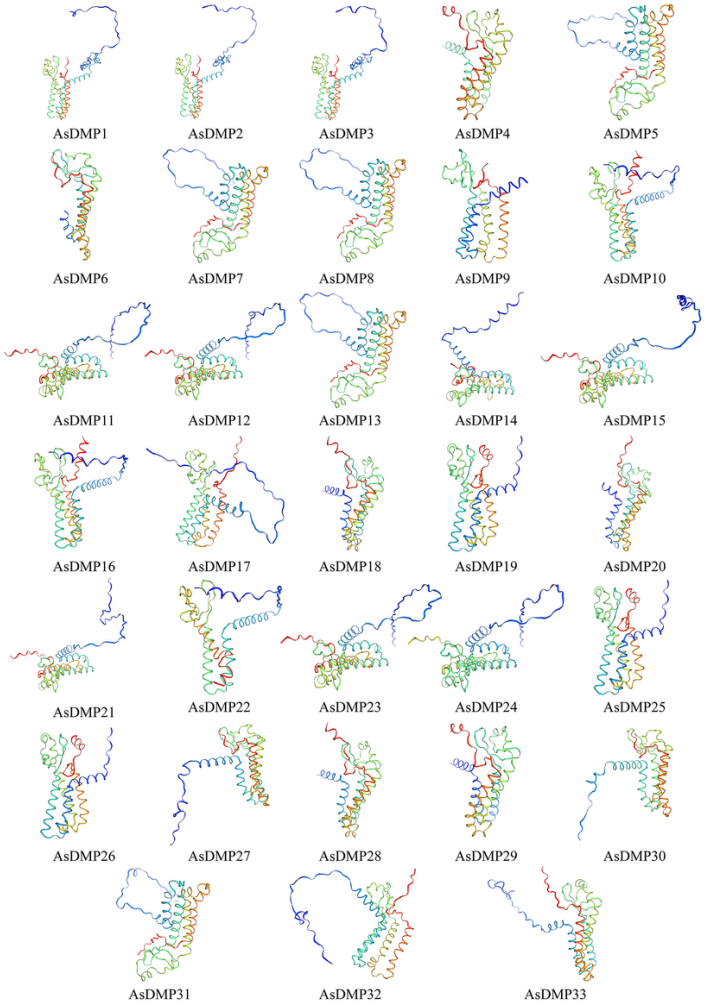


Figure 2

Tertiary structure of oats DMP protein

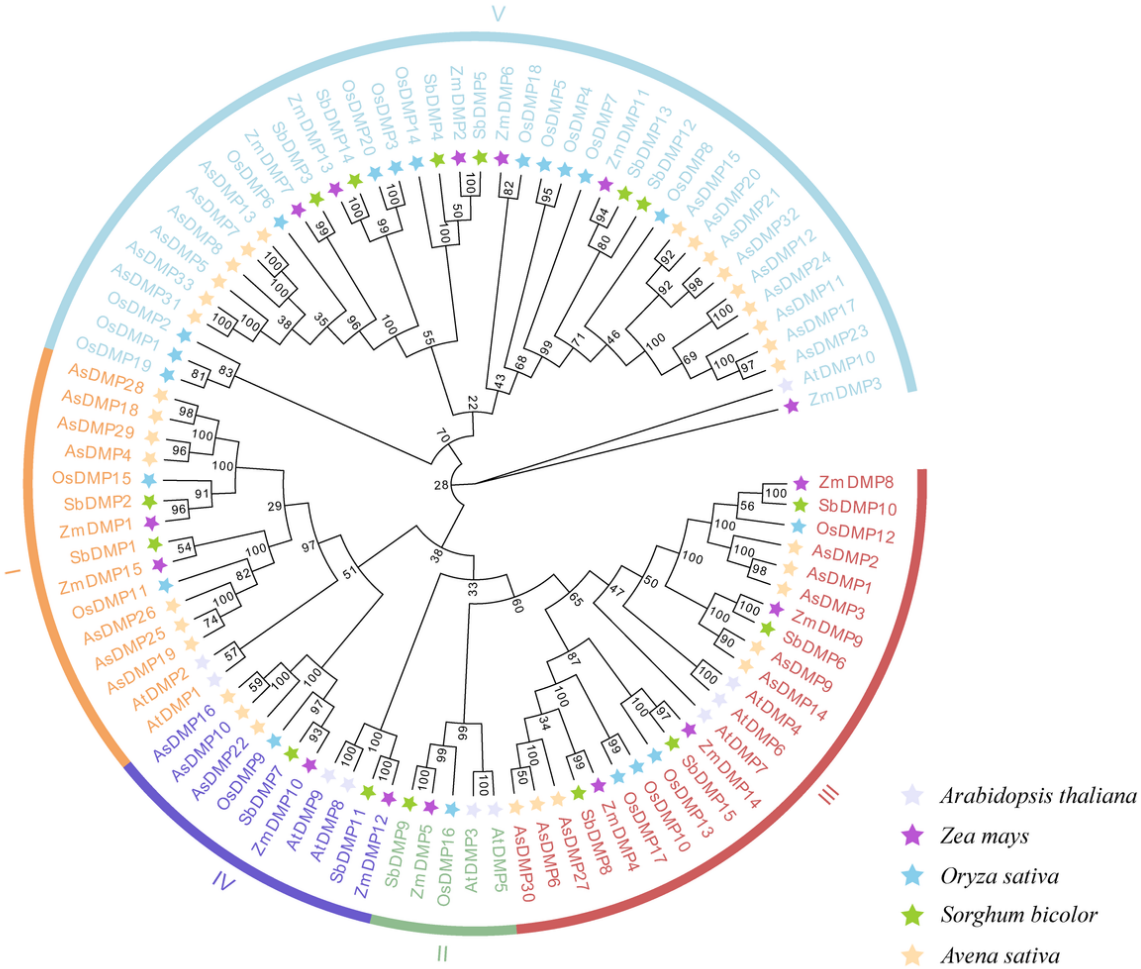


Figure 3

Tertiary structure of oats DMP protein

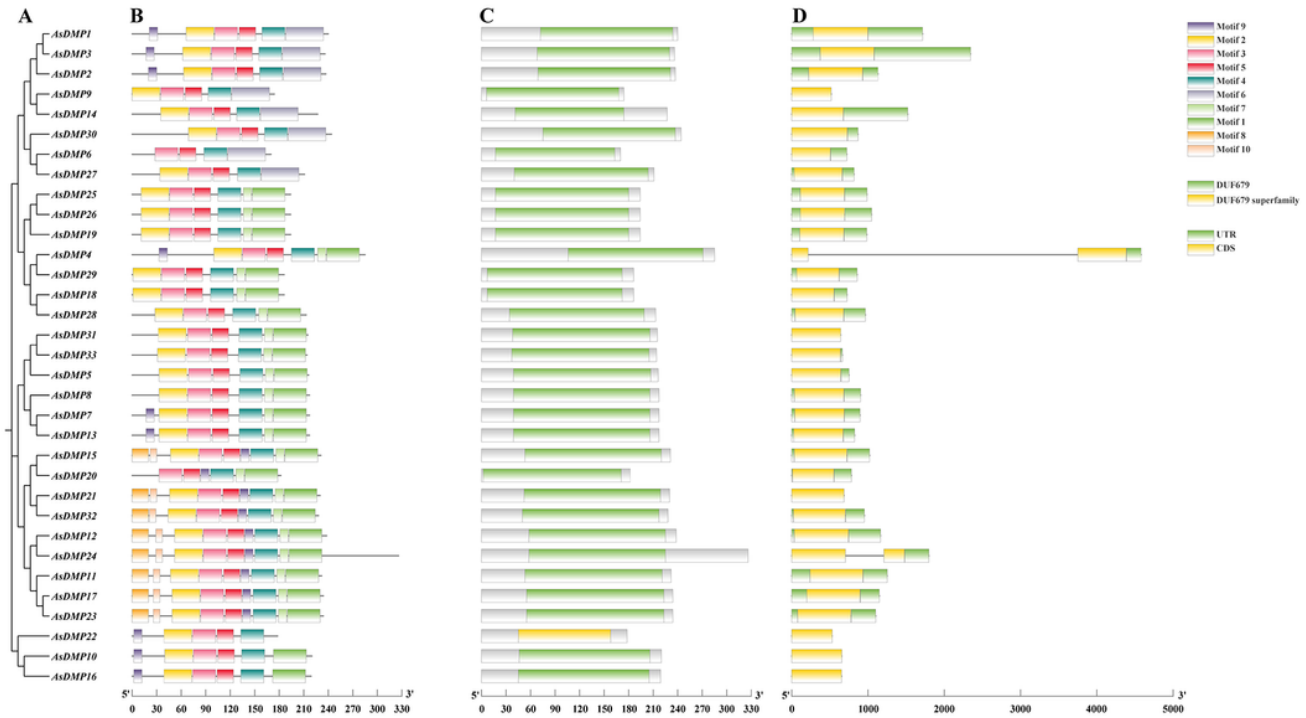
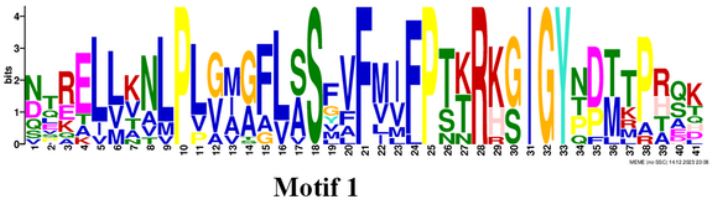
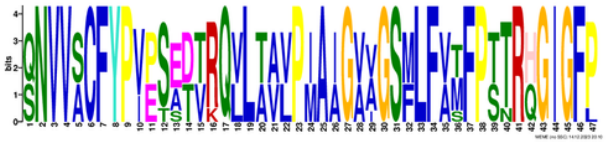


Figure 4

Phylogenetic tree, conserved motif, conserved domain and gene structure of Oat *DMP* gene family. A: Phylogenetic tree of the *DMP* gene family of oats; B: Oat *DMP*gene family conserves motif order; C: The oat *DMP* gene family conserves domain; D: Gene structure of Oat *DMP* gene family.

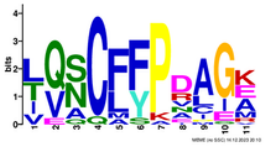


Motif 1



Motif 6

Motif 2



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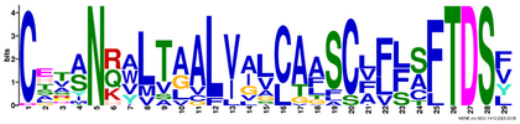
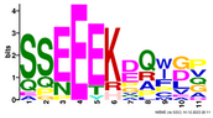


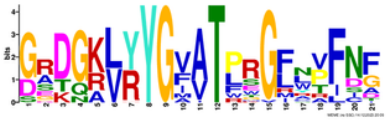
Motif 3

Motif 8

Motif 4



Motif 9



Motif 5

Motif 10

Figure 5

Sequence logos of conserved domains

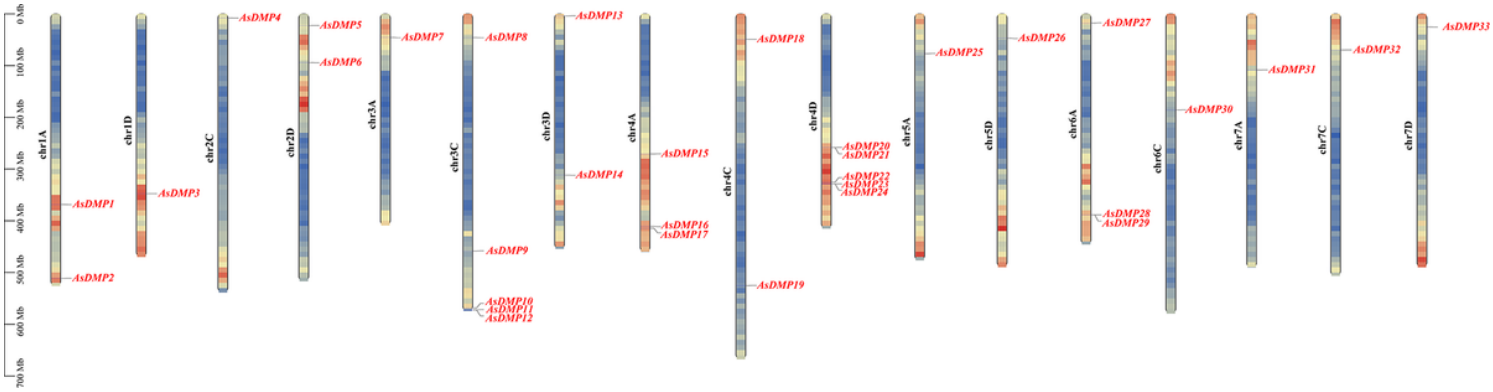


Figure 6

Chromosome localization of *DMPs* gene in Oat

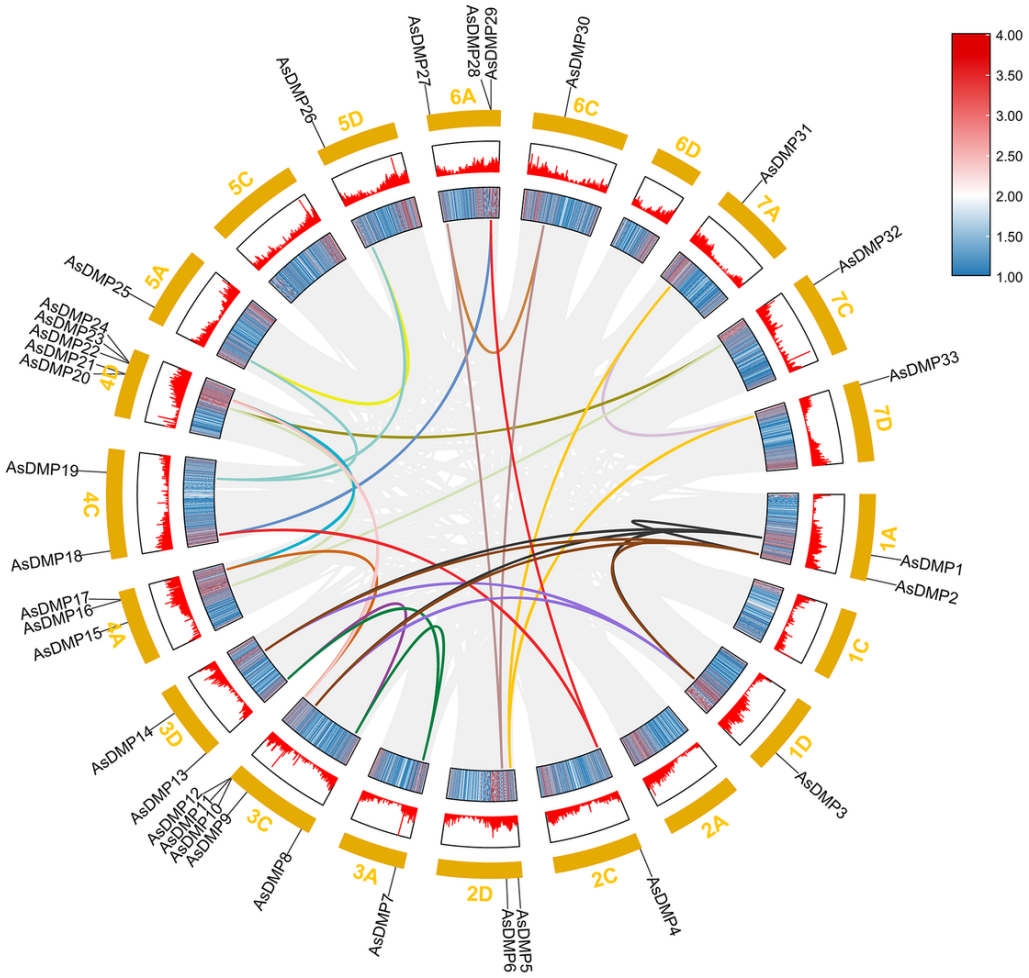


Figure 7

Homologous relationship between *DMPs*genes in Oat

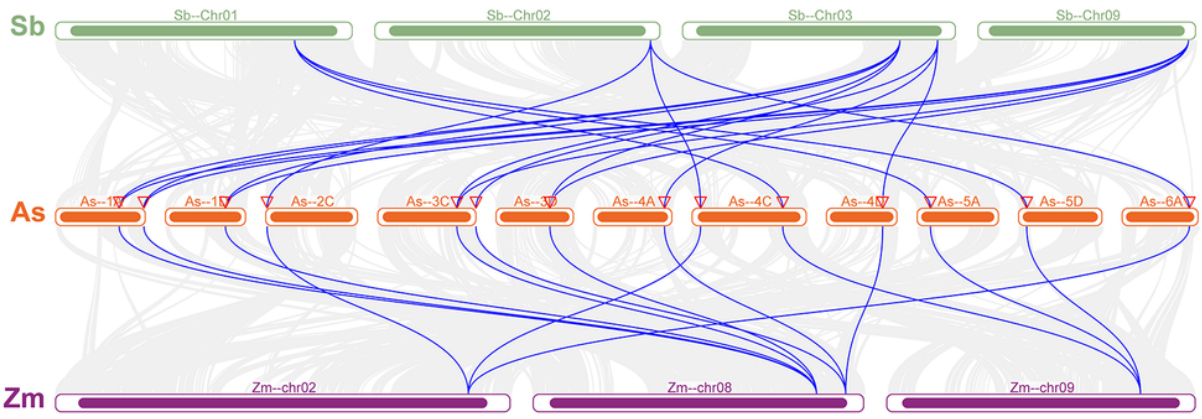


Figure 8

Homology analysis of *DMP* gene between Oat and two plants. The blue lines highlight the syntenic *DMP* gene pairs. The specie names with the prefixes "Sb", "As"and "Zm" indicate sorghum, oats, and corn.

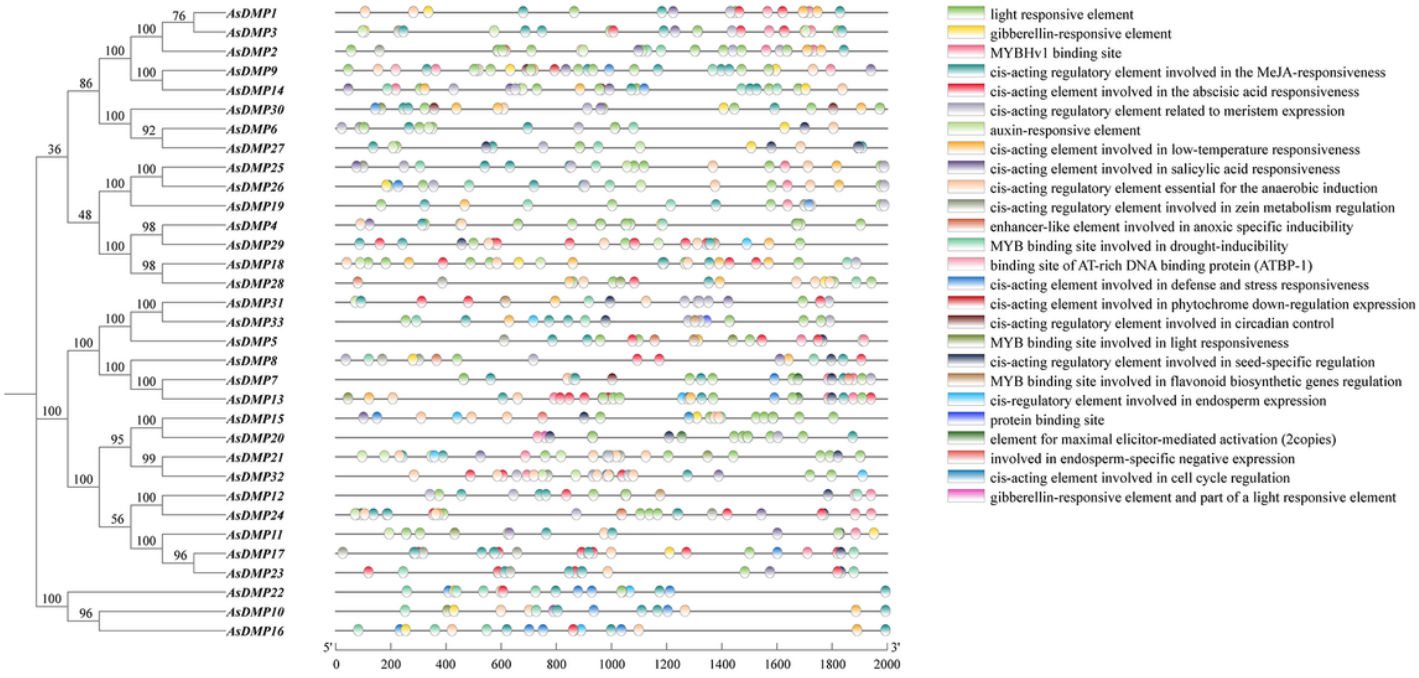


Figure 9

Analysis of *DMP* genes promoter

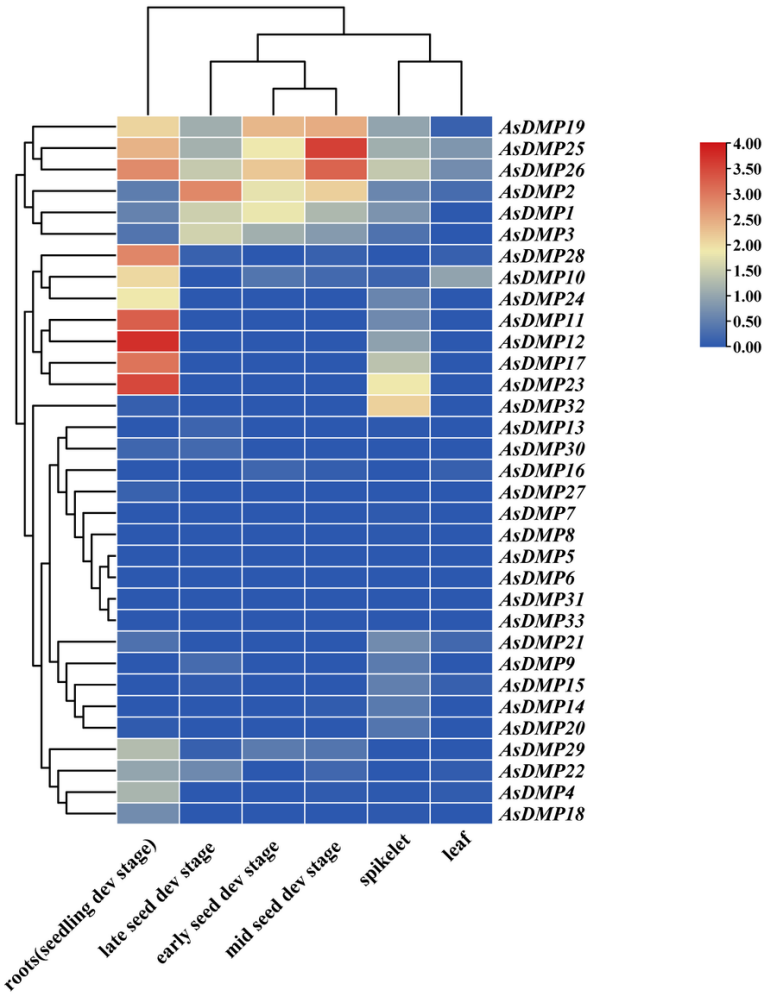


Figure 10

Expression patterns of *DMP* gene in different tissues and developmental stages

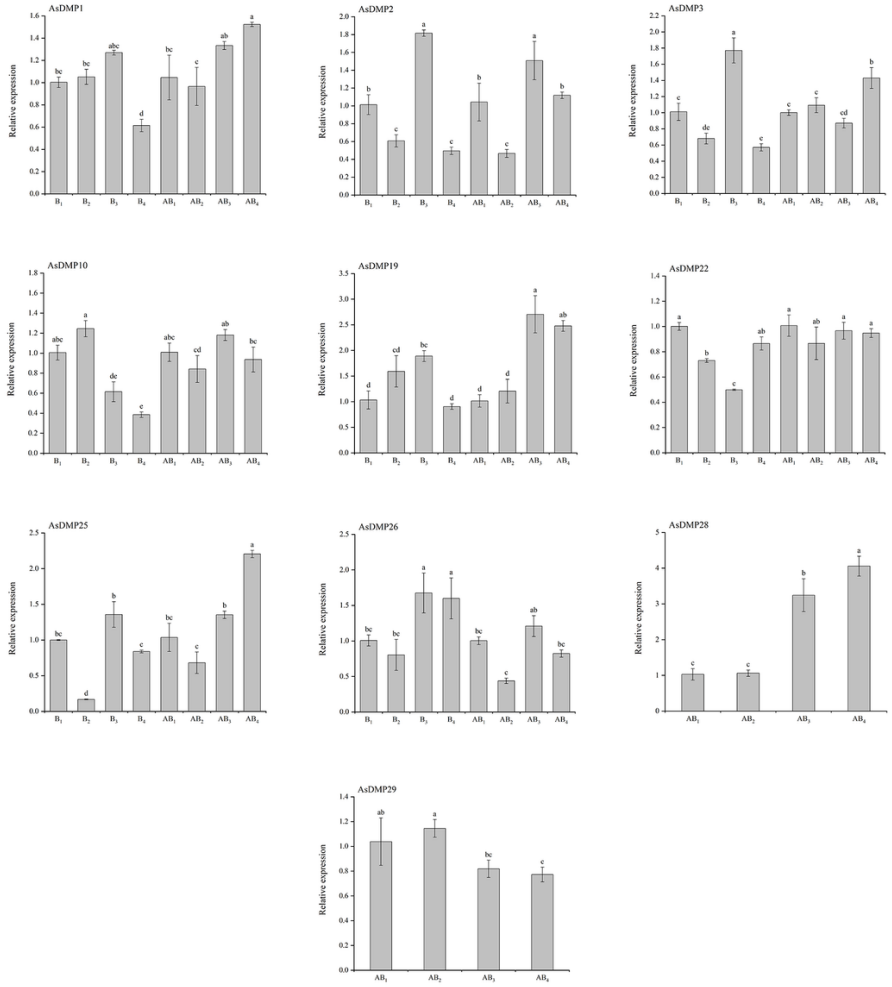


Figure 11

Relative expression of *AsDMP* under natural aging and artificial aging. 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 hours; AB_2: Seeds artificially aged for 48 hours; AB_3: Seeds artificially aged for 72 hours; AB_4: Seeds artificially aged for 96 hours. Note: Different letters represent significant differences(P<0.05).