Expression Profiling of the Dof Gene Family Under Abiotic Stresses in Spinach

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Abstract

DNA-binding with one finger (Dof) transcription factors are plant-specific transcription factors involved in numerous pathways of plant development, especially in respond to abiotic stresses. Although genome-wide analysis of this gene family has been performed in many species, *Dof* genes in spinach have not been thoroughly analyzed. We performed a genome-wide analysis and characterization of *Dof* gene family in spinach. Twenty two *Dof* genes were identified and classified into four groups with nine subgroups, which was further corroborated by gene structure and motif analyses. Ka/Ks analysis revealed that *SoDofs* were subjected to purify selection. Using *Cis*-acting elements analysis, *SoDofs* were involved in plant growth and development, plant hormones and stress responses. Expression profiling demonstrated that *SoDofs* expressed in leaf and inflorescence, and responded to cold, heat, and drought stresses. *SoDof22* expressed the highest level in male flowers and under cold stress. These results provided a genome-wide analysis of *SoDof* genes, their gender- and tissue-specific expression, and response to abiotic stresses. The knowledge and resources gained from these analyses will benefit spinach improvement.

Introduction

Spinach (*Spinacia oleracea* L.) is an annual or biennial diploid species belonging to the Amaranthaceae family in the order Caryophyllales[1]. The annual worldwide gross production of spinach in 2016 was about 26 million tonnes (FAOSTAT; http://faostat3.fao.org). Spinach is a dietary source of Ca, Cu, Fe, K, Mg, Mn, P, Zn, folate, vitamins and dietary fiber[2], providing its great potential for medical economy [3,4]. However, like most crops, the development and production of spinach is hampered by biotic stresses from diseases, pests and weed infestations and abiotic stresses such as salinity, drought, and heat[5]. Drought, heat and cold conditions are the common abiotic stresses for most crops in the natural environment. Climate change causes increasing temperature and a network of events triggering the response of plants and animals on Earth[6,7]. Although it seems that creatures on earth gradually developed local thermal adaptation, mean temperatures and daily temperature fluctuations are still supposed to impact the healthy condition of each creature[8]. Spinach has cold tolerant but heat sensitive characteristics so that the plant may suffer from heat stress during its entire life with influencing the growth of spinach plant and significantly decrease yield and quality[9]. Winter sweet treatment (WST), termed the cold enrichment technique, has been established for cultivating high-quality leafy spinach during winter[10]. At that time (early December), the average daily temperature is generally below 5°C. But staying at a low temperature for a long time would also damage spinach by reactive oxygen species (ROS)[11]. Although drought stress has no direct effects on the leaf nutrition quality, some physiological indexes for spinach will decreased, such as leaf area, fresh and dry weight, leaf relative water content and specific leaf area, so that the shape of plant might be changed[12].

The DNA-binding with one finger (Dof) domain proteins are plant-specific transcription factors which contain a highly conserved 52 amino acid DNA-binding domain at the N-terminal presumably including a single Cys2/Cys2 zinc finger structure[13]. It was projected that Cys2/Cys2 zinc finger specifically binds a conserved sequence with 5′-(T/A)AAAG-3′ in gene promoters[14]. At the C-terminal of the Dof proteins, there is a transcription regulation domain with diverse functions involving interaction with a variety of regulatory proteins and activation of gene expression[15]. Indeed, previous studies corroborated its functional role in plant growth and development, such as in flowering control[16,17], maturation[18], seed development[19] and germination[20,21]. Specifically, mutant dag1 (encoding a Dof transcription factor in Arabidopsis) seeds are induced to germinate by much lower red light fluence rates[22]; the *COG1* gene (encoding a Dof protein in Arabidopsis) functions as a negative regulator in phytochrome signaling pathways[23]; *CDFs* (CYCLING DOF FACTORS, a Dof-type transcriptional repressors) directly suppress the expression of CONSTANS (*CO*), which could prevent the expression of photoperiodic gene, the perception of day-length and the floral transition in Arabidopsis[24]. Moreover, there were evidenced that Dof factors also participated in phytohormone and stress responses, such as the *TDDF1* (encoding a Dof protein in tomato) which could improve drought, salt, various hormones stress as well as resistance to late blight[25]; *ThZFP1* and *ThDof1.4* that could improve salt and osmotic stress tolerance by increase the proline level and ROS scavenging capability[26]. Therefore, Dof gene family plays an essential role in the life cycle of plants.

In recent years, with the sequencing of genome, the identification of Dof genes were widely researched in various plant species, such as Arabidopsis, Rice[27], Soybean[28], Maize[29], Sorghum[30], Sugarcane[31] and so on. The draft genome of spinach was reported in 2017[1], however, few gene families were analyzed for the genome. The functions of members of Dof genes remain unknown in spinach. In this study, we identified 22 Dof genes, showed the structure and motifs and classified the group of Dof genes in spinach. In addition, duplication events and *cis*-element on their promoters were predicted. Functional prediction was performed based on gene expression analysis in different tissues and in responses to different abiotic stresses. The results will provide a foundation for gene cloning and functional characterization of Dofs in spinach.

Materials And Methods

Identification of *SoDof* gene family members in the spinach genome

In order to identify the Dof gene family members in *Spinacia oleracea* L., all proteins from the spinach genome were scanned by HMMER-3.2[32] using the Hidden Markov Model (HMM) corresponding to the HMM profile of the Dof domain (PF02701). The spinach genome data came from SpinachBase (http://www.spinachbase.org/?q=download). Then predicted proteins were confirmed by performing NCBI Conserved Domain Database (CDD)[33]、Pfam[34] and SMART[35] to make sure the presence of the conserved Dof domain in the existence of putative *SoDof* members in the spinach genome. Similarly, Arabidopsis *Dof* genes had been identified by scanning Arabidopsis database (ftp://ftp.ensemblgenomes.org/pub/plants/release-42/fasta/arabidopsis_thaliana/) using HMM and CDD. We performed the ExPASy server[36] to detect the theoretical pI and molecular weight of those candidate *SoDof* genes.

Mutiple sequences alignment and phylogenetic characterization

For a phylogenetic analysis of the Dof gene family, multiple sequence alignments were conducted on the amino acid sequences of Dof protein from Spinach and Arabidopsis by Mafft[37] with default settings. After that, FastTree[38] software was used to construct phylogenetic tree between Spinach and Arabidopsis with the Neighbour-Joining (NJ) method and 1000 bootstraps. Alignment of multiple *SoDofs* was preformed by DNAMAN.

Chromosomal locations and duplication time

The distribution information for each *SoDof* genes on their own chromosome was obtained from their annotation information in SpinachBase. Then the MG2C (http://mg2c.iask.in/mg2c_v2.1/) was used to map the chromosomal locations for each *SoDof* genes with default settings. To estimate the synonymous and non-synonymous substitution, we calculated the value of Ka and Ks. ClustalW was performed to align the nucleotide sequence of *SoDof* genes. Then the Ka and Ks values were used to estimate by DnaSp. The time (million years ago, Mya) of segmental duplication events for each *SoDof* gene was estimated using a formula, $T=Ks/2\lambda$ which assumed λ of $7.0e^{-9}$ synonymous/substitution site/year for spinach[1].

Gene structure analysis and conserved motif identification

The exon-intron organizations of the genes were determined using the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) through a comparison of predicted coding sequences with phylogenetic tree and Dof motifs. The motifs of the Dof protein sequences were statistically identified by the MEME program (http://meme-suite.org/) with the motif length set to 6-100 and the maximum number of motifs was set to 25. Then TBtools[39] was employed to create the motif structure with phylogenetic tree.

Promoter regions analysis of SoDofs cis-elements

To investigate *cis*-elements in promoter sequences of *Dof* coding genes in spinach, the upstream sequences (2000 bp) of each *SoDof* gene were extracted from spinach genome according to the GFF3 (general feature format) file. Then the retrieved sequences were submitted to a search by the PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [40] for predicting the *cis*-elements which might be involved in regulation of *SoDof* genes expression.

Sample collection and preparation

Spinach 9A0073 was obtained from CAAS (China Academy of Agricultural Sciences) and was selected as the experimental material. Seeds were sown in plots, and seedlings grew in an artificial climate chamber with a photoperiod of 16 h light / 8 h dark, the temperature was controlled at 24℃ and the humidity was controlled at about 60%. After cultivation for three weeks, spinach seedlings with consistent growth were selected and prepared for environmental stress treatment. Abiotic stresses were performed by adding 20% (mass fraction) PEG 4000 to simulate the drought condition and adjusting the temperature of the artificial climate box to simulate high temperature stress (40℃) and low temperature stress (4℃). Under stress conditions, the spinach functional leaves were sampled at 0, 2, 4, 7, 12, 24 h after treatment. The plants with non-treatment were collected for their roots, functional leaves and stems which stayed in vegetative growth stage, as well as their male flowers and female flowers. All samples were immediately frozen in liquid nitrogen and stored at -80℃.

RNA extraction and quantitative real-time PCR analysis

Total RNA from different samples was extracted using the Trizol reagent. The quality and concentration of RNA were tested on 1.0% agar gel electrophoresis and the NanoDrop 2000 (Thermo Fisher Scientific, USA). The total RNA was reverse transcribed into cDNA with its 200 ng per microliter final work concentration using Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biotechnology, China) according to the manufacturer's instruction. For qRT-PCR, the specific primers were designed by IDT (https://sg.idtdna.com/pages) and the sequences of all primers used in the experiment are listed in Supplementary Table S2. The qRT-PCR was conducted by a machine with SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate Biotechnology, China) in accordance with the manufacturer's protocol. Experiments were repeated three times with technical and biological replications for each sample. The relative gene expression level was calculated by the $2-\Delta\Delta CT$ method of Graphpad.

Results

Identification, distribution and duplication time of SoDofs genes

To identify the Dof gene family members in Spinach, all proteins from the spinach genome were scanned by using HMMER-3.2 and 22 genes were predicted as Dof gene family members in spinach. These Dof candidate genes were named as *SoDof*1-*SoDof*22 (Table 1). Then the predicted proteins were further confirmed to contain the conserved Dof domain. Similarly, 36 *Dof* genes had been identified in the Arabidopsis database. The full-length of the coding sequence (CDS) ranged from 492 bp to 1485 bp with an average length of 1060 bp. The quantity of aa (amino acids) for *SoDof* varied from 163 (*SoDof12*) to 494 (*SoDof13*) aa, with average protein length of ~352 aa. The molecular weights (MW) fluctuated between 18.5 Kilodalton (kDa) (*SoDof12*) and 54.5 kDa (*SoDof13*), and the theoretical isoelectric points (pI) ranged from 4.6 (*SoDof20*) to 8.92 (*SoDof9*). Over half *SoDofs* were alkaline with all members in Group B, and Group D1 contained most members with a wide wave in MWs (Table 1).

Table 1
Spinach Dof genes and their related information

Gene name	Gene ID	Chromosome	Location	Gene DNA(bp)	CDS(bp)	Protein length(aa)	Molecular weight	Theoretical pI	Dof domain	Int
SoDof1	Spo01218	chr2	58115820..58118612 forward	2793	1104	367	40642.53	8.52	57-114	1
SoDof2	Spo26525	chr4	115910084..115910743 reverse	660	660	219	23339.72	8.47	23-79	0
SoDof3	Spo14528	chr3	51468026..51469123 forward	1098	1098	365	39514.46	7.32	41-96	0
SoDof4	Spo15329	chr5	13015823..13016842 forward	1020	1020	339	37310.74	5.59	52-108	0
SoDof5	Spo26037	chr6	40210301..40212930 forward	2630	1197	398	44408.07	6.25	58-115	1
SoDof6	Spo25524	SpoScf_02134	33891..35945 reverse	2055	1287	428	46606.00	8.80	90-146	1
SoDof7	Spo19252	chr5	6739988..6741368 reverse	1381	1110	369	39234.09	6.93	47-104	1
SoDof8	Spo19232	SpoScf_01574	110099..110860 reverse	762	762	253	25482.25	8.12	28-83	0
SoDof9	Spo13986	SpoScf_01503	63276..64439 reverse	1164	1165	387	41004.88	8.92	79-135	0
SoDof10	Spo20892	Super_scaffold_114	1245494..1248131 reverse	2638	1326	441	46968.23	8.21	95-150	1
SoDof11	Spo08108	chr5	10912882..10916291 forward	3410	1344	447	49445.56	5.39	108-164	1
SoDof12	Spo04353	SpoScf_01506	92311..92802 forward	492	492	163	18468.93	8.87	44-99	0
SoDof13	Spo05430	SpoScf_01199	340472..345369 forward	4898	1485	494	54499.48	5.63	154-210	1
SoDof14	Spo16539	SpoScf_00408	13249..16754 forward	3506	1059	352	38506.78	6.46	99-155	1
SoDof15	Spo26832	chr6	26503975..26505054 reverse	1080	1080	359	40449.97	6.23	28-82	0
SoDof16	Spo22565	chr1	19149992..19151942 reverse	1951	1098	365	39747.75	8.50	84-138	1
SoDof17	Spo22229	SpoScf_01420	149590..151164 forward	1575	1101	366	40015.00	8.51	87-141	1
SoDof18	Spo07164	SpoScf_08285	1203..2777 forward	1575	1101	366	40027.05	8.51	87-141	1
SoDof19	Spo25703	Super_scaffold_205	553984..554928 reverse	945	945	314	35306.63	8.53	58-111	0
SoDof20	Spo00332	Chr4	83899644..83900468 reverse	825	825	274	30538.30	4.60	34-88	0
SoDof21	Spo10686	chr1	41630415..41632583 forward	2169	1305	434	47592.39	5.74	149-205	1
SoDof22	Spo16511	SpoScf_00982	142499..143254 forward	756	756	251	27368.16	7.60	44-98	0

To better understand the distribution of *SoDof* genes on the Spinach chromosome, we performed MG2C to draft the chromosomal map. The 22 putative Dof genes were found to be distributed in 6 chromosomes, and unplaced contigs (Fig.1). Only 50% *SoDofs* genes were anchored in chromosomes. The largest number of *SoDof* members was located in chromosome 5, which contains *SoDof* 7, 11 and 4. Compared with the gap of *SoDof* on other chromosomes, these three genes were closer with each other, especially *SoDof*11 and *SoDof*4. There were 2 *SoDof* genes in chromosomes 1, 4, and 6, respectively. *SoDof*1 and *SoDof*3 were located in chromosomes 2 and 3, respectively.

Calculating the value of Ka and Ks aims to identify duplication event for each *SoDof* gene. The duplication of *SoDof* genes originated from about 5.66 Mya (Ks=0.793) to 41.27 Mya (Ks=5.778) with an average of 16.12 Mya (Table S1). All values of Ka/Ks were below 1 and even almost all of them were below 0.5. Especially, the Ka/Ks values for five segmental duplication were extremely low (below 0.1) (Fig.2).

Multiple sequence alignment, phylogenetic analysis and classification

Multiple sequence alignment showed a Dof conserved motif of 52 amino acid located in 22 *SoDof* genes, with a single Cys2/Cys2 zinc finger structure at the N-terminal (Fig.3). Phylogenetic tree was constructed between 22 *SoDof* genes and 36 *Dofs* in Arabidopsis (Fig.4). A total of 22 *SoDof* TFs from spinach were classified into four main groups (Groups A to D), which could be divided into multiple subgroups, A, B1, B2, C1, C2.1, C2.2, C3, D1 and D2. The quantity of

SoDofs in Group B, Group C and Group D was similar with a total number of 18. Specifically, Group B (contained the most number among all groups) could be divided into subgroup B1 and subgroup B2 with *SoDof10*, *SoDof16*, *SoDof17*, *SoDof18* in subgroup B1 and *SoDof3*, *SoDof6, SoDof19* in subgroup B2 (Fig.4). Subgroup D1 had the second largest number of *SofDofs* (*SoDof11*, *SoDof12*, *SoDof13*, *SoDof14*, *SoDof21*). *SoDof2*, *SoDof4* and *SoDof9* belonged to Group A (Fig. 4).

Gene structure and motif analysis of SoDof genes

Candidate *SoDof* genes were analyzed using Gene Structure Display Sever to investigate the characterization of exon-intron structure. Remarkably, there were one or no intron occurred in *SoDofs* (Fig.5). *SoDofs* showed closed position in the phylogenetic tree, displayed similar intron-exon distribution, indicating the similar function within subgroup. For example, there were only one exon appeared in Group A.

To further reveal the diversification of *SoDof* genes, we performed the MEME program to detect motif patterns, and 25 distinct motifs were identified (Fig.6). The schematic distribution of the 25 motifs showed that only the Dof region (motif1) was highly conserved in all *SoDof* proteins. Notably, *SoDofs* shared similar conserved motif composition. Motif 10 and 7 were highly conserved in Group B. And in subgroup B1, motif 4 jointly related to the N-terminal Dof domain. Interestingly, motif 9 and 11 were prominently conserved in the subgroup D1 (contained the most *SoDof* members among all subgroups). Specifically, motif 9 presented at the N-terminal and motif 11 jointed the N-terminal Dof region.

Cis-regulatory element analysis

PlantCARE was used to analyse the *cis*-regulatory element for each *SoDof* gene by retrieving the 2kb upstream sequence of each candidate, except for *SoDof18* because of lack of 2kb upstream sequence on its scaffold location (Supplementary Data). Dof gene family in spinach had TATA-box, CAAT-box and typical eukaryotic switch elements. *SoDof* genes may also be controlled by many phytohormones, such as methyl jasmonate (MeJA), gibberellins (GA), ethylene, auxin, and salicylic acid (SA). We also detected many other important *cis*-elements on Dof gene family that involve in plant growth and development. For example, there were a large amount of elements associated with physiological process, such as light responsiveness, circadian control, endosperm expression, meristem and flower meristem expression, root specific and seed-specific regulation. Some elements, participated in some small molecule pathway, also had been found, such as zein metabolism regulation, maximal elicitor-mediated activation and flavonoid biosynthetic genes regulation. Additionally, eight *cis*-elements (WUN-motif, STRE, TC-rich repeats e.g.) were also predicted, which were related to defense and stress responsiveness.

Tissue-specific expression analysis of SoDof genes

We isolated RNA samples from organs, roots, stems, leaves, male flowers, and female flowers, and detected expression of all *SoDof* genes in spinach using qRT-PCR. A heatmap to visualize a expression profile of the *SoDof* genes was generated, revealing nine *SoDofs* that exhibited their highest transcript level in reproductive organs and eight *SoDofs* in vegetative leaves (Fig.7A). Only two *SoDofs* (*SoDof1* and *SoDof5*) expressed in roots and stems, respectively. Notably, *SoDof10* and *SoDof15* had extremely high expression in leaves; *SoDof22* showed high expression in male flowers (Fig.7B). Comparing with the expression in leaves or inflorescences, the transcript level of these three genes in other tissues was neglectable, indicating that their expression were tissue-specific such as in leaves and flowers. There were three homologous genes (*SoDof16*, *SoDof17* and *SoDof18*) with same mRNA sequence, and their expression patterns were not analyzed.

Expression patterns of SoDof genes under abiotic stresses

To investigate the different stress responsiveness and expression pattern for each *SoDof* gene within different gender of spinach, we treated female plants, male plants and plants at vegetative stage by three types of abiotic stress (low temperature 4℃, high temperature 40℃ and drought 20%PEG4000). Then the spinach functional leaves were collected at 0h, 2h, 4h, 7h, 12h, 24h after treatment and detected by qRT-PCR.

Over half *SoDof* genes in female plants were up regulated under low temperature (Fig.8A). The greatest increase in expression occurred in *SoDof*22 at female plants (Fig.8B). The *SoDof*22 was also up-regulated and expressed most compared to other *SoDofs* in plants at vegetative stage, and its extreme expression reached to the top at 7h and then went down. However, in male plants, the expression of *SoDof3* reached the highest level (Fig.8B). In vegetative plants, 84% *SoDof* genes (more than those in male or female plants) were up-regulated and its highest expression appeared at 7h (Fig.8A). There were the most number of *SoDofs* (*SoDof*5, *SoDof*6 and *SoDof*9) down-regulated in male plants, indicating that SoDofs in males showed more negative response under 4℃.

Under high temperature, most *SoDofs* was up-regulated and only five *SoDofs* (*SoDof*4, *SoDof*5, *SoDof*6, *SoDof*11 and *SoDof*21) were down-regulated in female plants. In plants at vegetative stage, the down-regulated *SoDofs* were *SoDof*1, *SoDof*4, SoDof6, *SoDof*11 and *SoDof*21. Additionally, the expression of *SoDof*6, *SoDof*11 and *SoDof*21 were also suppressed in male plants (Fig.8C). The expression of *SoDof*21 and *SoDof6* in vegetative plants was constrained until 24h when the expression was almost equal to that before treatment (Fig.8C). In addition, over half *SoDofs* showed the highest transcript level at 24h in plant at vegetative stage, and over half *SoDofs* showed the highest transcript level at 7h in male plants.

To investigate the expression profile for each *SoDofs* when they suffered from the drought condition, we simulated the drought condition by using 20% (mass fraction) PEG 4000. *SoDof*21 was down-regulated (Fig.8C) and *SoDof*15 (Fig.8B) expressed at the highest level in three types of spinach. All *SoDofs* were up-regulated in females and vegetative plants except *SoDof*21. In male plants, five *SoDofs*, *SoDof*5, *SoDof*6, *SoDof*11, *SoDof*20 and *SoDof*21, exhibited suppressed expression, and the expression of all *SoDofs* are lower than in female and vegetative plants.

Discussion

The *Dof* gene family is a plant-specific family of transcription factors. Since the discovery of the first *Dof* gene in maize[32], its members in other species have been uncovered and its function in the growth and development has been characterized. We identified 22 *SoDof* genes in spinach genome and constructed a phylogenetic tree to divide them into four categories (A, B, C and D) (Fig. 4). The quantity of *SoDofs* is lower than that of Arabidopsis (36)[27], tomato (34)[33], wheat (96)[34], rice (30)[27], potato (35)[35], soybean (78)[28], and sugarcane (29)[31]. This is because spinach separated with Arabidopsis just after the ancient whole genome triplicationand there was no whole genome replication in spinach genome[1].

From our analysis of the spinach genome[1], only half of the *Dof* genes were assembled in chromosomes. Their distribution were relatively even, but there were three *Dof* genes clustered on one end of the chromosome 5 (Fig. 1). Although the spinach genome has no recent whole genome duplication, tandem gene duplications have led to the formation of specific *Dof* genes clustered in specific part of chromosomes. It is the main effect on gene family expansion[36]. The exon-intron divergence is a supporting evidence to determine the evolutionary relationship of plants[37]. The intron-exon analysis showed that there were no more than two introns in each *Dof* gene (Fig. 5). The intron-exon structure in each subgroup is conserved except for that in the subgroup D1. The distribution of motifs is indicative of evolutionary relationship[34]. The protein sequence analysis of the 22 *SoDof* genes revealed that only Dof motifs of these 22 protein sequences are conserved (Fig. 6). The Dof proteins in the same subgroup contain relatively conserved motif structures, which might be related to the evolution of the exon-intron organization by the loss or acquisition of introns.

To figure out the potential roles of *SoDofs*, we analyzed the expression profiles of 19 *SoDof* genes in different spinach tissues. The expression analysis revealed that all of the *SoDofs* express in spinach. 42% *SoDofs* showed a dominant expression in leaves and 47% in reproductive organs (Fig. 7A). In grapevine, eleven of twenty-five *Dof* gene expressed in inflorescences[38] (similar to the number of *SoDofs*). Over half of *Dof* genes were expressed in vascular system in spinach, as in Arabidopsis[39]. Among them, there are six *SoDofs* (*SoDof*4, *SoDof*11, *SoDof*19, *SoDof*20, *SoDof*21 and *SoDof*22) that expressed at a high level in flowers, indicating that they might be involved in the development of reproductive organs, especially for *SoDof*22 (Fig. 7B). *SoDof*22 is orthologous to AT4G21050, which is involved in regenerated shoot numbers[40]. These *Dof* genes may be involved in the growth and development of spinach reproductive organs, and directly or indirectly affect the development of the female and male flowers of spinach. In the expression profile for abiotic stress, the expression of *SoDofs* in male plants is lower than that in female plants and the plants at vegetative stage. *SoDof*22, *SoDof*3 and *SoDof*15 showed the highest level in expression after treatment under low temperature, high temperature and drought condition (Fig. 8B). As previous studies shown, *Dof* genes participate in responding to various stresses. In tomato, *SlCDF1-5* genes were induced in response to osmotic, salt, heat, and low-temperature stresses. Overexpressing *SlCDF1* or *SlCDF3* in Arabidopsis showed increased drought and salt tolerance[41]. In Brassica, the *BnCDF1* gene was induced in response to low temperatures, and overexpressing *BnCDF1* in Arabidopsis could increase freezing tolerance[42]. In watermelon, nine selected *Dof* genes showed differential expression under salt stress and ABA treatments[43]. In Chinese cabbage, most *Dof* genes were up-regulated quickly under salt, drought, heat and cold stresses[44]. Therefore, we postulate that *SoDof*22, *SoDof*3 and *SoDof*15 have an important role in responding to heat, cold and drought stresses. Notably, overexpressing *BnCDF1* in Arabidopsis also delays flowering time by reducing the expression of *CO* and *FT*[42]. Interestingly, *SoDof*22 contains high expression level both in inflorescence and under cold stress, suggesting that the role of *SoDof22* might be similar to *BnCDF1* within the interplay between environmental conditions and flowering time.

The promoter of *SoDof*22 contains a LTR *cis*-element responding to low-temperature and the promoter of *SoDof*15 contains a MBS *cis*-element participated in drought inducibility[45] (Supplementary Data). The response of its *cis*-element leads to the increase in expression after treating low temperature or PEG4000. *SoDof*5, *SoDof*11 and *SoDof*21 were down-regulated by treating abiotic stress. The quantity of down-regulated *SoDofs* under high temperature treatment was higher than that under other treatments. Under low temperature and drought condition, the number of down-regulated *SoDofs* in male plants is the highest. *SoDof*6, *SoDof*9 and *SoDof*11 are more responsive in male plants. These findings clarified the roles of *Dof* gene family members in response to these three abiotic stresses.

Declarations

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Author contributions

R.M. and H.Y. conceived the project and designed experiments. H.Y., Y.M. and Y.L. performed the qRT-PCR experiments. H.Y. and Y.M.a draw the figures. H.Y. and J.Y. discussed the results. H.Y. wrote the manuscript and R.M. revised it.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

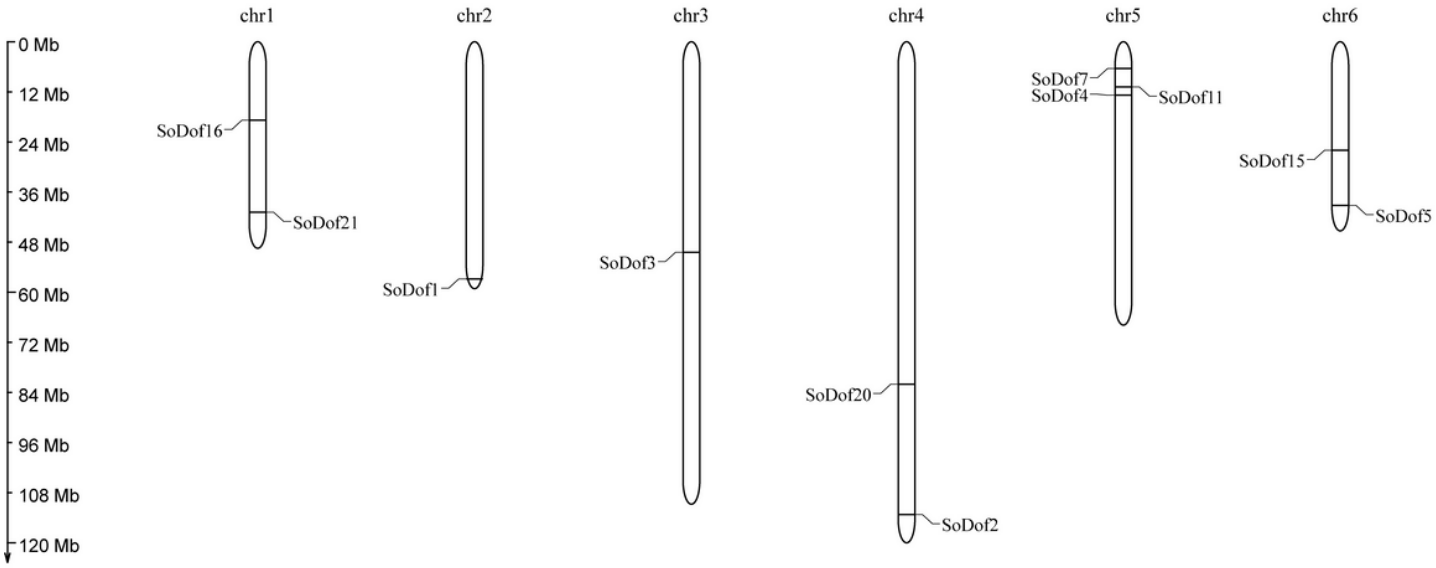

Figure 1

Chromsomal location of SoDof genes. The size of a chromosome is indicated by its relative length.

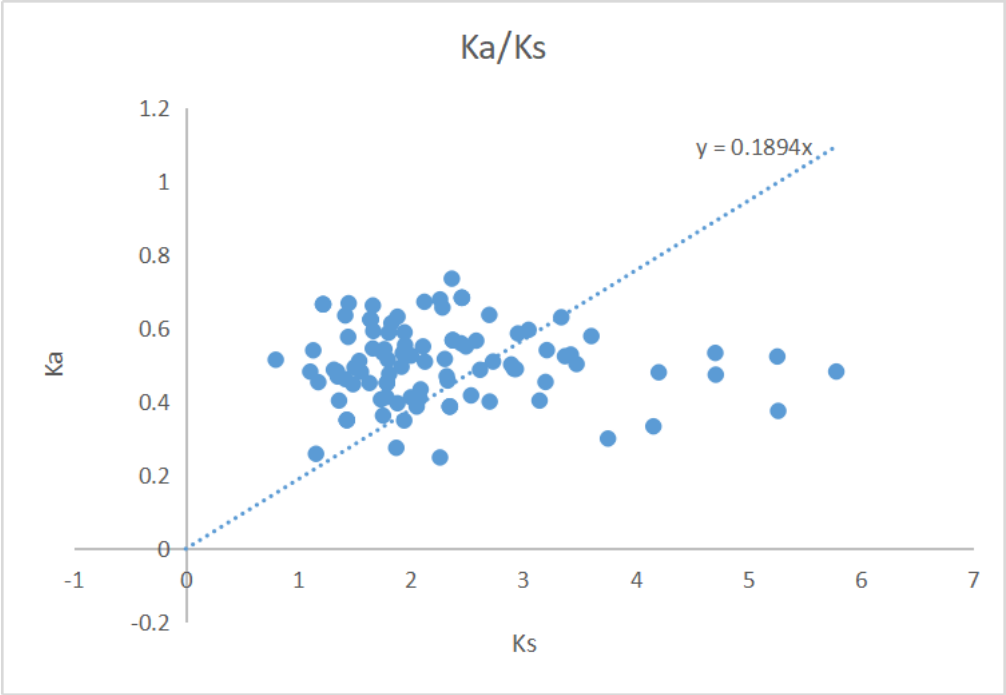

Figure 2

The Ka/Ks value of SoDofs. The details Ka/Ks information are shown in Table S1

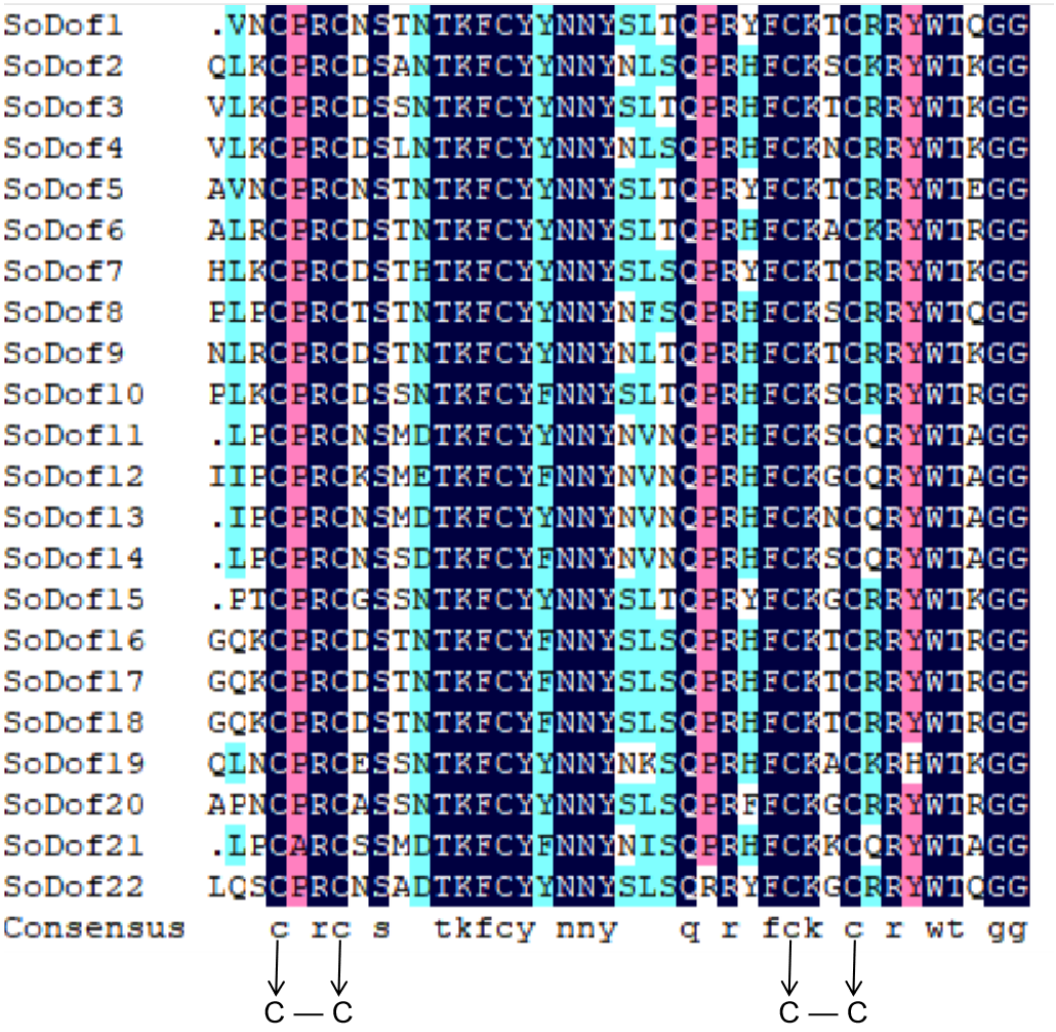

Figure 3

Alignment of multiple SoDofs and conserved amino acid sequences.

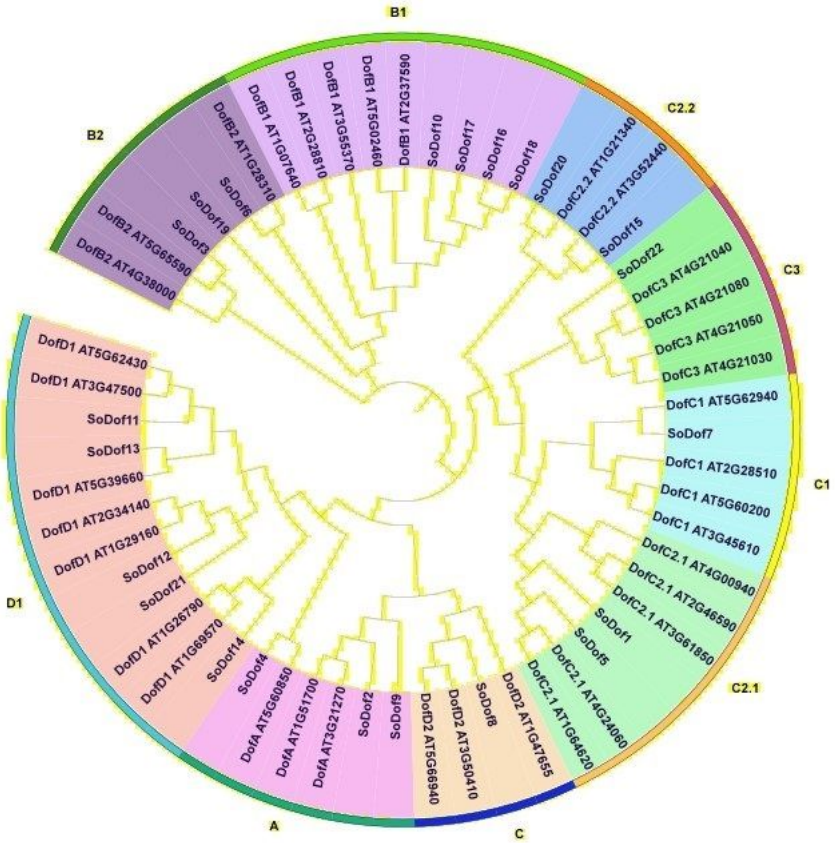

Figure 4

Phylogenetic tree of Dof proteins in Spinach and Arabidopsis.

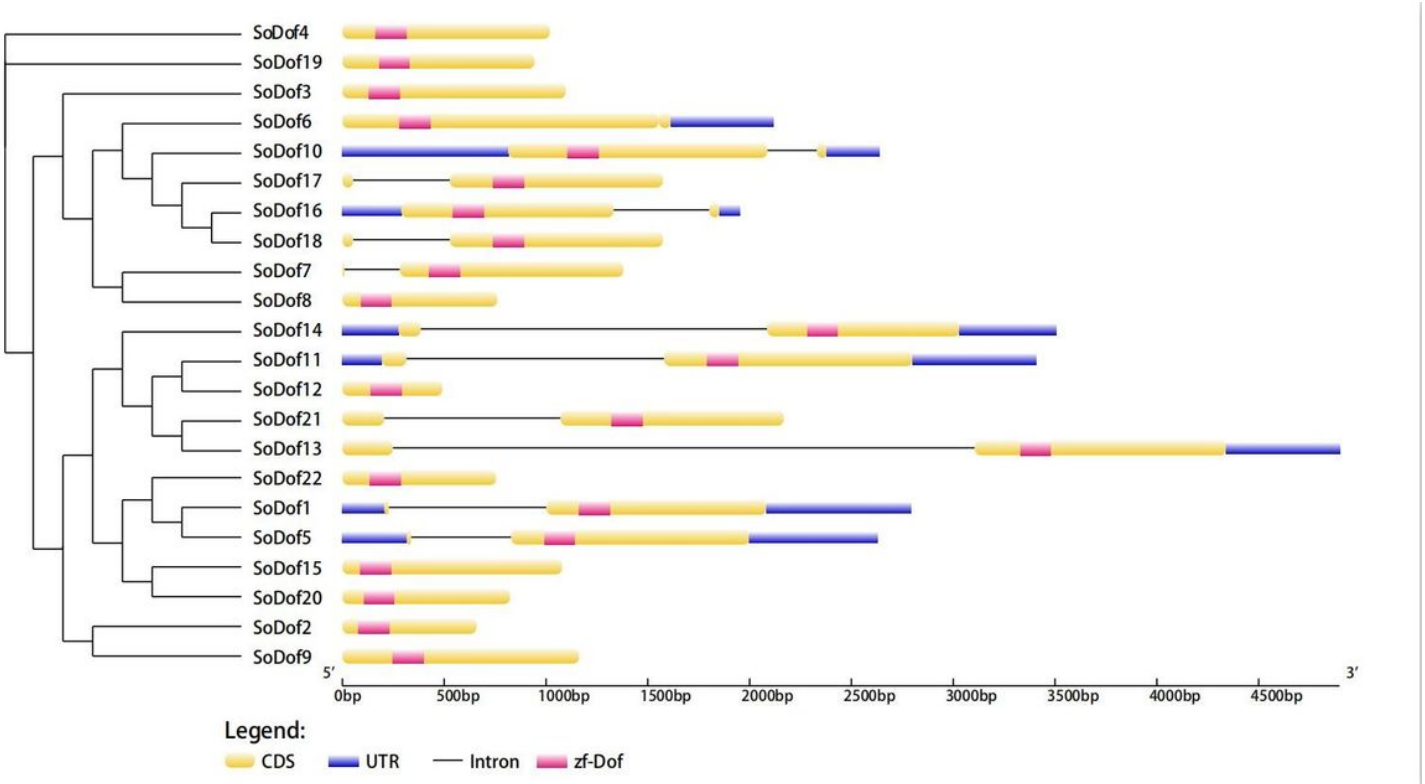

Figure 5

The exon-intron structure of Dof genes in Spinach

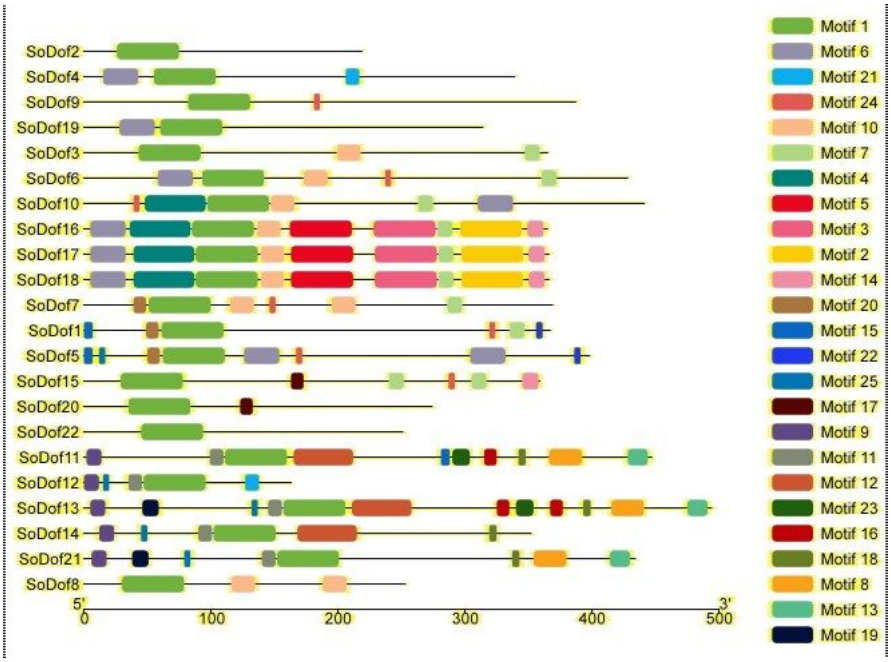

Figure 6

The schematic distribution of motifs for Dof genes in Spinach

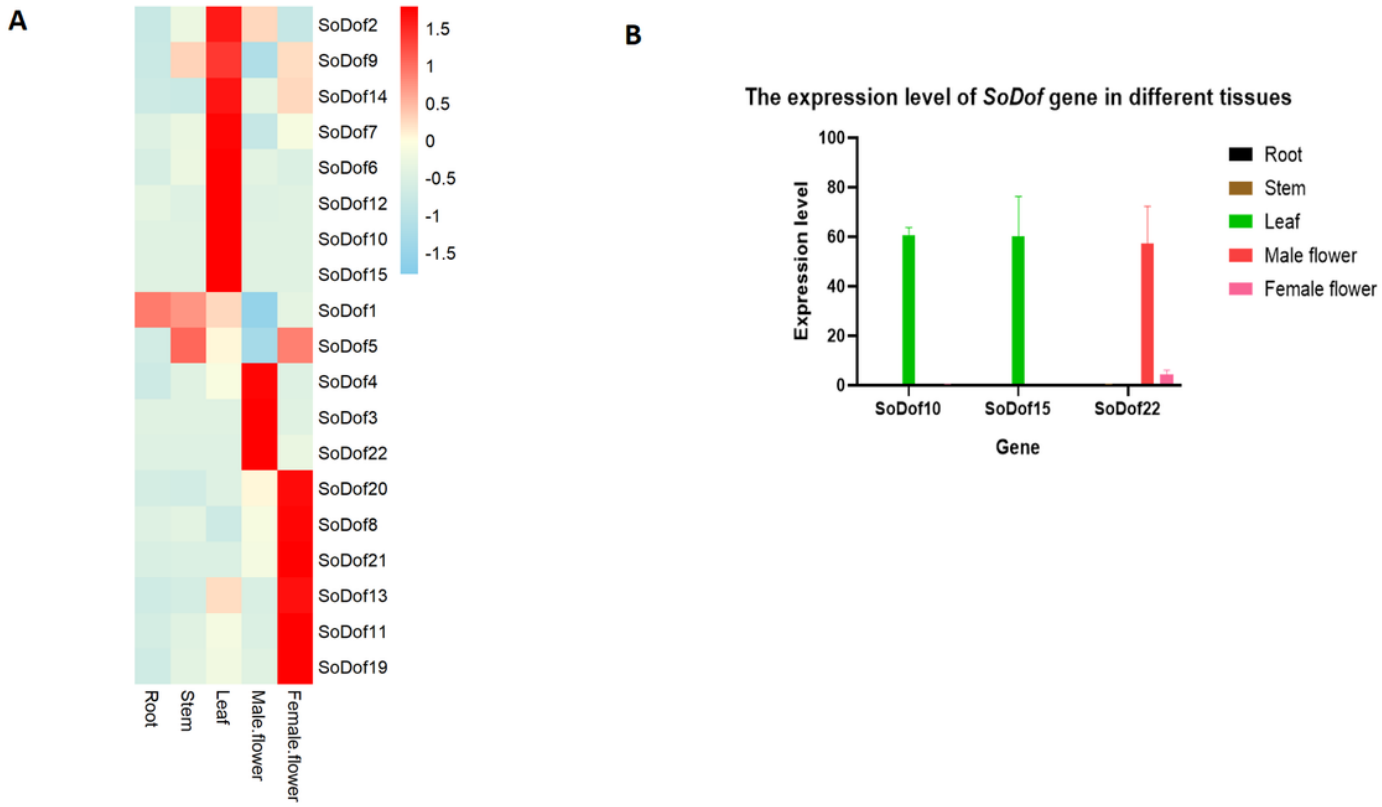

Figure 7

The tissue-specific expression of Dof genes in Spinach by qRT-PCR. (A). Expression level of SoDofs. The color scheme used to present expression level is sky-blue/red: light-yellow boxes indicate low variation in gene expression, sky-blue indicate a fold decrease, and red boxes indicate a fold increase in relation to mean value. (B). The expression level of SoDofs in different tissues.

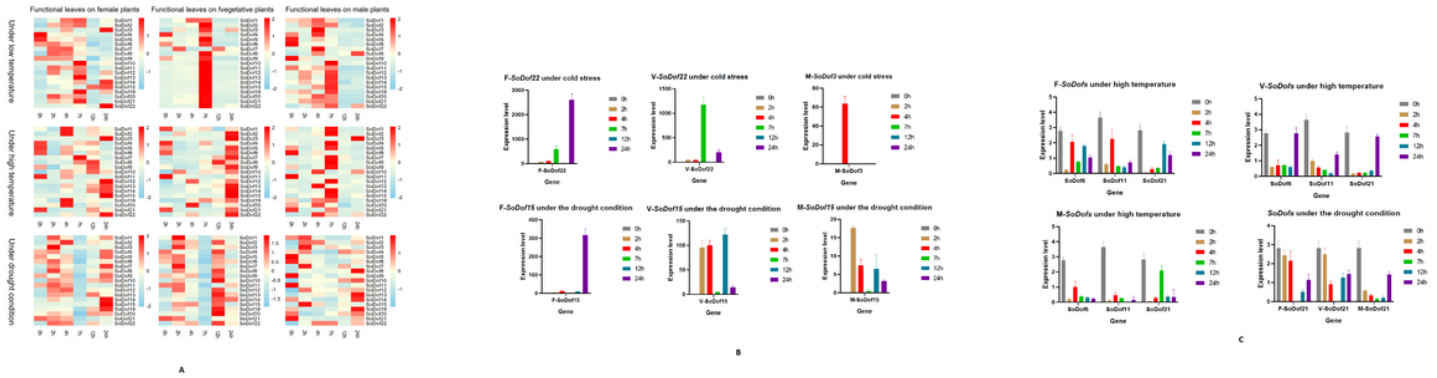

Figure 8

The expression pattern of SoDof genes under stresses. (A). The expression pattern of all SoDof genes under cold stress, heat stress and drought stress. The color scheme used to present expression level is sky-blue/red: light-yellow boxes indicate low variation in gene expression, sky-blue indicate a fold decrease, and red boxes indicate a fold increase in relation to mean value. (B). The expression level of SoDofs with the obvious change. F-SoDof means the SoDof gene in female plants; V-SoDof means the SoDof gene in vegetative plants; M-SoDof means the SoDof gene in male plants. (C). The expression level of down-regulated SoDofs.

Supplementary Files

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

- SupplementaryData.xlsx
- Supplementarytable.docx