Genome-Wide Analysis of the *Dof* Gene Family in Durian Reveals Fruit Ripening-Associated and Cultivar-Dependent Dof Transcription Factors

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Supplementary material for this submission is available as follows:

Supplementary Table S1

Supplementary Table S2

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Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

Supplementary Figure S4

Supplementary Figure S5

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Supplementary Table S1. Sequence similarities among DzDof sequences. Numbers indicate percent homologies at the amino acid level. The protein sequences of 24 DzDofs were used as input queries to find sequence similarities using the web-based tool Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/), using its default parameters. The highest and lowest similarities are highlighted in yellow.

		DzDof 1.1	DzDof 3.7	DzDof 5.7	DzDof 2.2	DzDof 3.6	DzDof 2.4	DzDof 5.1	DzcDof 2	DzcDof 3	DzcDof 1	DzDof 1.5	DzDof 1.4	DzDof 1.2	DzDof 3.5	DzDof 4.5	DzDof 5.3	DzDof 5.4	DzDof 3.1	DzDof 1.6	DzDof 3.4	DzDof 2.5	DzDof 4.6	DzDof 2.1	DzDof 5.6
1	**DzDof1.1**	**100**																							
2	**DzDof3.7**	**85.58**	**100**																						
3	**DzDof5.7**	**38.26**	**37.79**	**100**																					
4	**DzDof2.2**	**34.71**	**34.85**	**34.15**	**100**																				
5	**DzDof3.6**	**31.56**	**30.15**	**32.06**	**55.48**	**100**																			
6	**DzDof2.4**	**31.56**	**30.15**	**34.22**	**56.41**	**56.97**	**100**																		
7	**DzDof5.1**	**31.68**	**29.50**	**33.46**	**53.23**	**56.13**	**89.85**	**100**																	
8	**DzcDof2**	**26.64**	**24.61**	**28.23**	**25.48**	**23.76**	**25.18**	**24.91**	**100**																
9	**DzcDof3**	**27.82**	**26.53**	**28.86**	**28.63**	**26.59**	**25.84**	**25.19**	**58.63**	**100**															
10	**DzcDof1**	**26.61**	**24.80**	**24.72**	**25.46**	**23.94**	**25.44**	**24.74**	**46.79**	**43.42**	**100**														
11	**DzDof1.5**	**34.51**	**33.33**	**31.76**	**33.81**	**33.56**	**31.97**	**31.29**	**40.26**	**39.60**	**40.70**	**100**													
12	**DzDof1.4**	**29.91**	**28.91**	**29.90**	**31.63**	**30.59**	**29.82**	**29.86**	**25.35**	**28.14**	**24.56**	**35.83**	**100**												
13	**DzDof1.2**	**23.66**	**25.79**	**27.63**	**27.23**	**28.32**	**28.63**	**27.88**	**25.50**	**25.51**	**22.82**	**31.51**	**29.55**	**100**											
14	**DzDof3.5**	**28.99**	**29.76**	**27.57**	**31.05**	**28.28**	**35.35**	**34.36**	**27.03**	**28.96**	**26.32**	**33.59**	**34.34**	**38.02**	**100**										
15	**DzDof4.5**	**33.66**	**34.65**	**30.43**	**33.95**	**31.53**	**32.74**	**30.80**	**30.20**	**29.15**	**25.00**	**34.75**	**32.09**	**27.97**	**29.91**	**100**									
16	**DzDof5.3**	**32.56**	**32.55**	**31.80**	**35.87**	**32.33**	**34.62**	**33.62**	**30.81**	**28.37**	**25.22**	**33.33**	**31.25**	**28.74**	**29.57**	**87.86**	**100**								
17	**DzDof5.4**	**33.33**	**33.33**	**31.52**	**34.95**	**35.57**	**31.47**	**30.85**	**27.32**	**27.80**	**23.01**	**38.79**	**33.78**	**29.47**	**29.41**	**32.79**	**33.33**	**100**							
18	**DzDof3.1**	**36.09**	**35.93**	**36.69**	**36.14**	**33.92**	**35.88**	**35.43**	**32.05**	**31.61**	**28.57**	**43.30**	**33.15**	**32.78**	**31.07**	**33.80**	**34.40**	**35.68**	**100**						
19	**DzDof1.6**	**32.75**	**35.12**	**31.32**	**30.90**	**30.60**	**29.28**	**30.11**	**31.55**	**31.33**	**27.23**	**42.31**	**29.28**	**28.72**	**31.07**	**31.28**	**29.22**	**32.73**	**41.01**	**100**					
20	**DzDof3.4**	**32.00**	**32.56**	**32.18**	**36.47**	**32.95**	**34.10**	**33.15**	**31.93**	**29.52**	**25.82**	**39.22**	**32.39**	**31.15**	**30.86**	**28.64**	**26.76**	**36.57**	**43.93**	**47.16**	**100**				
21	**DzDof2.5**	**35.96**	**32.84**	**32.98**	**36.87**	**34.74**	**35.79**	**35.23**	**30.69**	**32.31**	**28.43**	**33.06**	**29.36**	**31.60**	**33.17**	**35.24**	**34.55**	**32.86**	**37.10**	**32.80**	**33.51**	**100**			
22	**DzDof4.6**	**32.68**	**31.53**	**34.21**	**40.46**	**38.38**	**39.46**	**37.97**	**29.95**	**30.53**	**29.23**	**32.00**	**30.52**	**30.19**	**34.15**	**32.85**	**32.72**	**33.65**	**38.04**	**33.33**	**33.70**	**64.93**	**100**		
23	**DzDof2.1**	**28.44**	**30.62**	**35.61**	**33.67**	**33.17**	**32.18**	**31.22**	**32.62**	**32.61**	**26.44**	**38.53**	**34.50**	**35.27**	**31.86**	**35.24**	**34.04**	**35.02**	**39.18**	**36.41**	**31.77**	**37.77**	**39.30**	**100**	
24	**DzDof5.6**	**26.58**	**28.31**	**31.12**	**31.58**	**30.05**	**30.99**	**32.41**	**25.12**	**26.24**	**25.70**	**32.17**	**30.00**	**30.63**	**32.84**	**32.75**	**33.05**	**32.46**	**33.99**	**30.50**	**30.15**	**34.90**	**36.00**	**38.85**	**100**

Supplementary Table S2. List of primers for *DzDofs* used in this study.

Gene name	Corresponding gene ID	Forward primer (5'-3')	Reverse primer (5'-3')
DzcDof1	XM_022890912.1	CCCGGAAATGTCACCGTCTG	AGTGTTCAGACCAGAAAACAACAC
DzcDof2	XM_022884508.1	CCAACTTCGTTAGCTTAGCCAC	ACAGGAACTCTTCTTCTGTTCTCC
DzcDof3	XM_022882662.1	ATTGGAGCTGCTGAAAAGGGC	GGCACCATCAGCCACAATTAAC
DzDof1.1	XM_022885861.1	CTGATCTTCCAGCAACTAATGGTG	AAGGCATATACAGCTAGCCAACA
DzDof1.2	XM_022897927.1	TTCCCACATGAATTGGCTAGT	TAGAACCAACCCAGTAACGGT
DzDof1.4	XM_022864763.1	GTCTCTACCGATCTCAACATGCC	GAACACCCAAATCAAGAAAAGAGC
DzDof1.5	XM_022921573.1	CGAGACTTTGGAGCAAAACCC	GACCCATTGACATCACAGGA
DzDof1.6	XM_022916439.1	TGCAGCAGAACACTCGTGA	GGTGGTTTAATTGGCAACGCC
DzDof2.1	XM_022896728.1	GCTGACCAATAAGGCAGTTCC	CCGAAGTCCTGCAACTAACCA
DzDof2.2	XM_022874863.1	GCAGACTAAACAAAGATTGGCAG	CACACACAACCGTAGAACAAATC
DzDof2.4	XM_022888412.1	TGTCAAGGCAATATTTGGGGG	ATGATTGCTAGTGGATGAAGAGC
DzDof2.5	XM_022903860.1	TGGGTTTGGTTTCAAAAGGCTG	CAACACTGACAGAAGCAGTCC
DzDof3.1	XM_022865170.1	TCCGGGATTAGGAGAGAGCAAT	GAAAACTTGAAGAACCTGGAGTG
DzDof3.4	XM_022918231.1	GGTGGCGATTACTTTTCTTGGT	GGAACATGGAAAACACAATGAACC
DzDof3.5	XM_022889354.1	CTGTGGTCGAATACTCACTCCT	TACCAAACAATAGATTTGGATCGTG
DzDof3.6	XM_022908803.1	CGAATGAATTGCTTGTCACTCCA	TCATGTCAGATCAAACTCTCTGC
DzDof3.7	XM_022895569.1	TGTGGCTGTAGGGGTAGCA	CCATCCTGGTACACTTTCACAT
DzDof4.5	XM_022921611.1	ATGTGGGTGTTCGTTCCTGT	CGCACCAACAAAGCAGAAGT
DzDof4.6	XM_022877869.1	TTGGGGTAAAAATGTAGGAGAGGAG	TGACGCATATGGTTGCTCTTTC
DzDof5.1	XM_022891906.1	AACCTCCAGACACCTTATCCA	GCGCAATGCATATCATCAGTAGG
DzDof5.3	XM_022868588.1	TTTCTTCAGCCACTGCTGTACT	GCTTTTTGCATACACAAGGCAA
DzDof5.4	XM_022920120.1	TGGCAAGGGAGTGGAGATCA	ACGGGATATAGAGGGTAGGATG
DzDof5.6	XM_022881477.1	AAGGGGAAACTGTGTGGTGAAT	CTTTTGCCCAAGACACCCAATC
DzDof5.7	XM_022885261.1	GGTAGACCCTAAAAGGAAGATATGG	CAAGGTCGACATGTTGATATCTGA

Supplementary Table S3. List of primers used for expression analysis of auxin and ethylene biosynthetic genes in durian pulp.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Auxin biosynthetic genes		
DzTAA1	TTGAATATGGACAGCGCCTCA	AAGTTGCAGTAGTCTGGCGA
DzYUCCA4	TAACCCTCCAAAACCGCAGAA	TTCCCAAAGTACCCGACCAAG
Ethylene biosynthetic genes		
ACC synthase (*DzACS*)	CAGAAATCATTGGCCTGGTAG	CTGAGTTGGAGCTGAAATGG
ACC oxidase (*DzACO*)	TGGAGAAAGAAGCAGAGGAG	AAGTTGTTTCCCTGGCTTTC
Elongation factor 1 alpha (DzEF-1α)	GAAACCTTCTCTGCGTACC	CTCCACACTCTTGATGACAC

Supplementary Table S4. List of primers used for transient expression of *DzDof2.2* in *Nicotiana benthamiana* and expression analysis of auxin biosynthetic genes in *N. benthamiana leaves*

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
For gene cloning		
DzDof2.2	AAAAAGCAGGCTATGGTTTTCTCATCTGTTCCAG	AGAAAGCTGGGTCTATAAGAGATTATTAGTACTAGAAGAGTTGAGACC
For expression analysis of auxin biosynthetic genes		
NbTAA1	ATACTGGCGAAAGATGGGCAAC	CTGGCTTGATCCTGTCCCAAC
NbYUCCA3	AAGCTGATAGTCTGTAATGAGGGG	TTGGGATTTCTCTGGAGAGGG
Elongation factor 1 alpha (NbEF-1α)	TGAGGGACATGCGTCAAACT	GGAGCGGATACCAGTCATACA

Underlined sequences represent bases of the *att*B1 or *att*B2 site on the 5' end of each primer (for BP reaction).

Supplementary Fig. S1. Exon-intron structures of *DzDofs*. Green bars indicate exons. No introns were found for durian Dofs. DzcDof is durian cyclic Dof.

Supplementary Fig. S2. Multilevel consensus sequences identified by MEME. Protein sequences of DzDofs were used to identify conserved motifs among durian DzDof proteins. Ten conserved motifs were identified. Motif 1 represents the conserved Dof domain always observed at the N-terminus.

Supplementary Fig. S3. Schematic distribution of conserved motifs in durian DzDofs. Ten conserved motifs, identified by MEME, are represented in different colours. Motif 1 represents the conserved Dof domain. Several subgroups were distinguished based on their motif distributions, which was consistent with the phylogenetic analysis.

Supplementary Fig. S4. Tissue-specific expression profiles of *DzDofs*. The Illumina RNA-seq data were used to analyse the expression levels of *DzDofs* in different tissues, including root, stem, leaf, and pulp. The higher expression for each gene was presented in red; otherwise, blue was used. Data were sum normalized, log transformed and autoscaled.

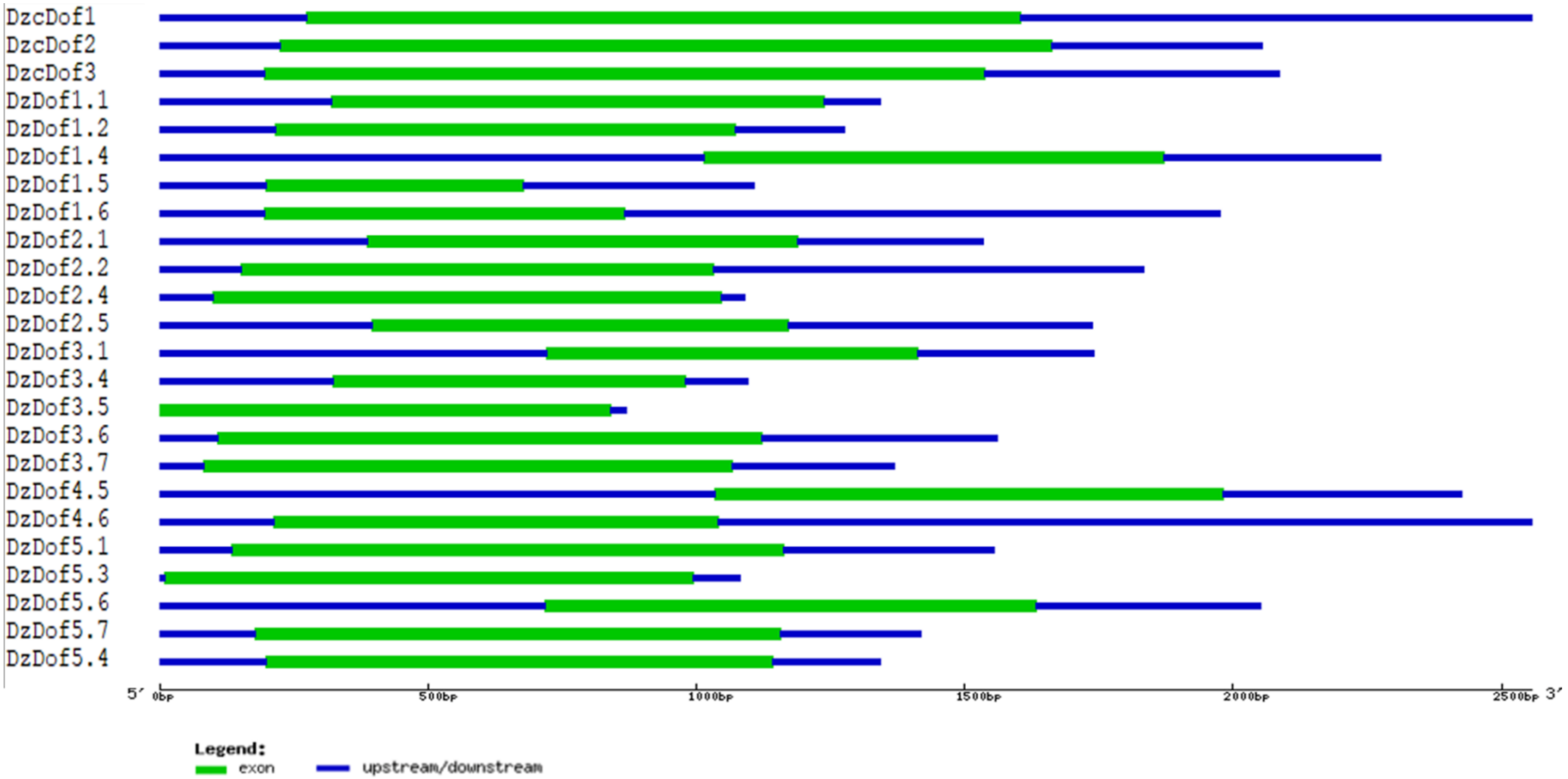
Supplementary Fig. S5. Comparison of the expression levels of ten potential cultivar-dependent *DzDofs* between the Monthong and Chanee cultivars. Expression levels of ten potential cultivar-dependent *DzDofs* during ripening were compared at three different stages (unripe, midripe, and ripe) between the Monthong and Chanee cultivars. For each *DzDof*, a comparison was made between the two cultivars at each stage. Three independent biological replicates were used. Three technical replicates were also performed for each biological replicate. An asterisk (∗) above the bars indicates a significant difference between the two cultivars (Student’s *t*-test, $P < 0.05$).

Supplementary Fig. S6. Expression levels of ethylene biosynthetic genes in durian pulp. The expression levels of ACC synthase (*DzACS*) and ACC oxidase (*DzACO*) were measured at three different stages (unripe, midripe, and ripe) during post-harvest ripening of Monthong and Chanee cultivars. Comparisons were made between the two cultivars at each stage. Three independent biological replicates were used. Three technical replicates were also performed for each biological replicate. An asterisk (∗) above the bars indicates a significant difference between cultivars (Student’s *t*-test, $P < 0.05$). Comparisons were also made for one cultivar at different stages. Bars with different letters (lower case for Monthong and upper case for Chanee) above them differed significantly according to Duncan’s multiple range test ($P < 0.05$).

Supplementary Fig. S7. Nucleotide sequences of promoter regions of candidate auxin biosynthetic genes from durian (*DzTAA1* and *DzYUCCA4*). Dof binding sites (AAAG and CTTT) are highlighted in yellow. The translational start site (ATG) is underlined.

Supplementary Fig. S8. Nucleotide sequences of promoter regions of candidate auxin biosynthetic genes from *N. benthamiana* (*NbTAA1* and *NbYUCCA3*). Dof binding sites (AAAG and CTTT) are highlighted in yellow. The translational start site (ATG) is underlined.

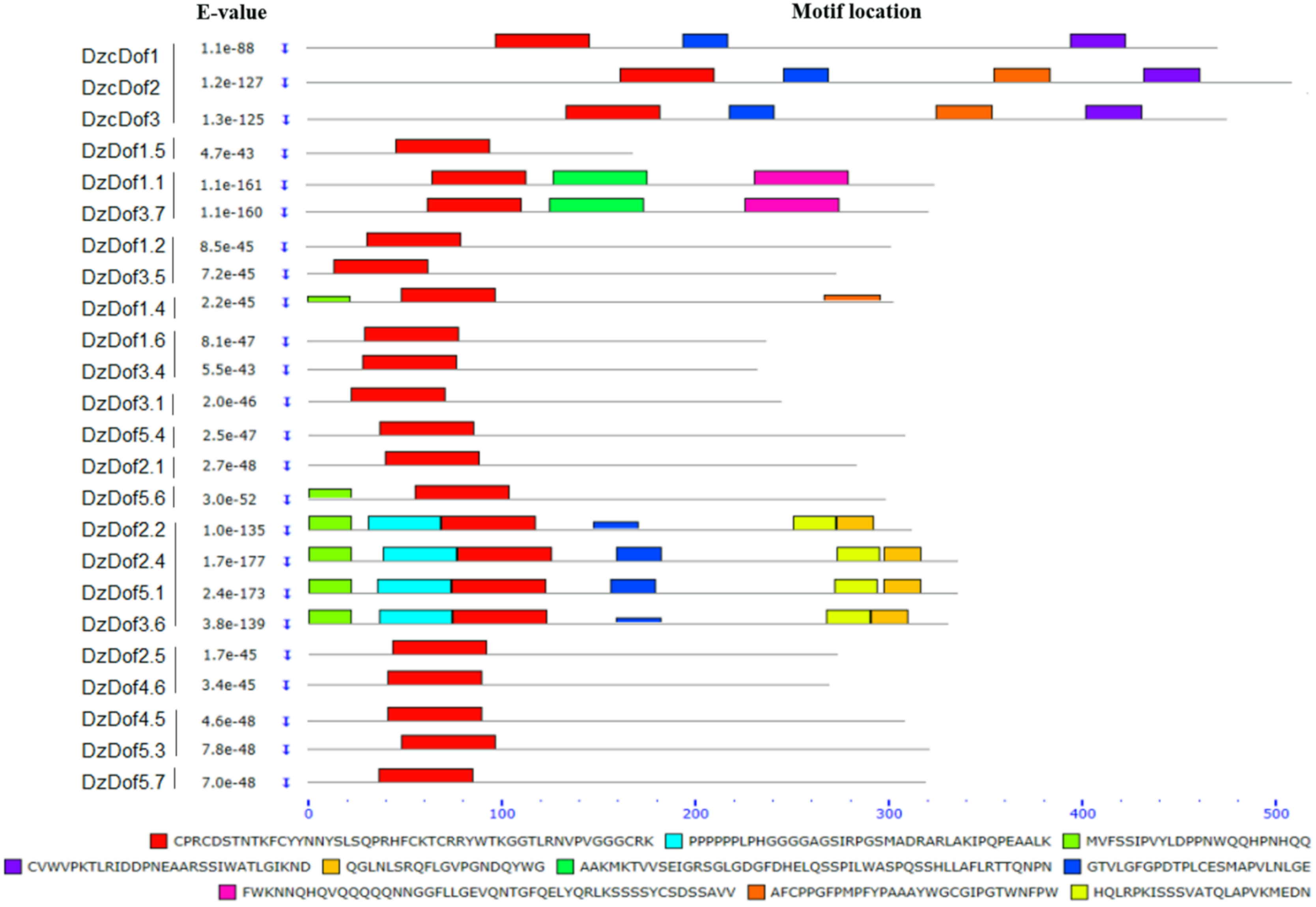
Supplementary Fig. S1



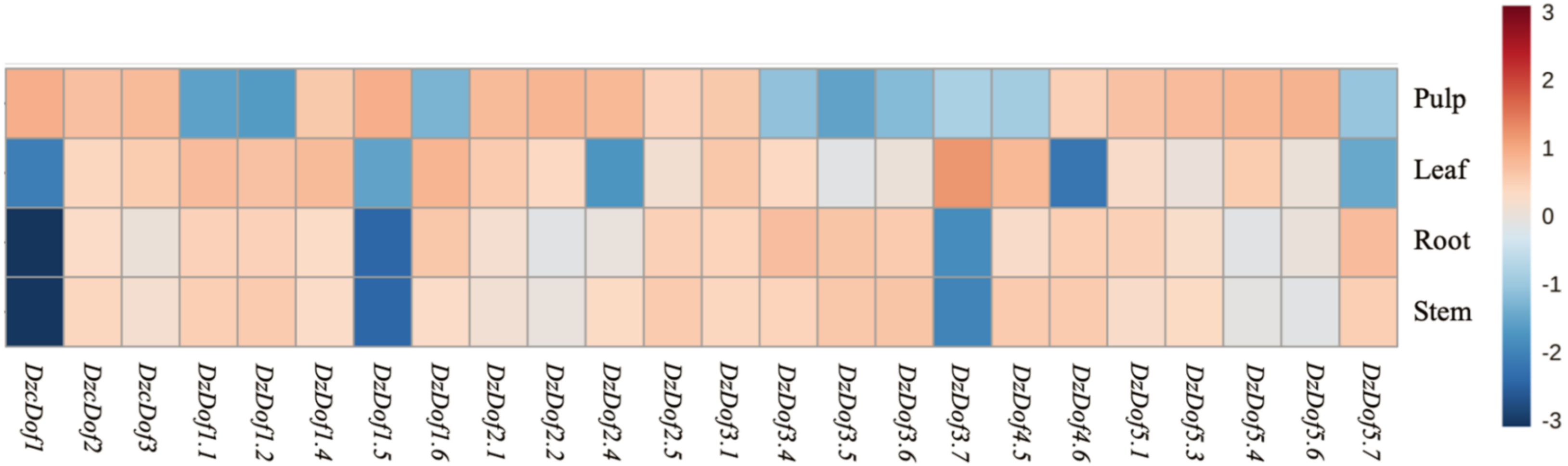
Supplementary Fig. S2

	Logo	Amino acid sequence	Length (aa)
1.		CPRCDSTNTKFCYYNNYSLSQPRHFCKTCRRYWTKGGTLRNVPVGGGCRK	50
2.		PPPPPPLPHGGGGAGSIRPGSMADRARLAKIPQPEAALK	39
3.		MVFSSIPVYLDPPNWQQHPNHQQ	23
4.		CVWVPKTLRIDDPNEAARSSIWATLGIKND	30
5.		QGLNLSRQFLGVPGNDQYWG	20
6.		AAKMKTVVSEIGRSGLGDGFDHELQSSPILWASPQSSHLLAFLRTTQNPN	50
7.		GTVLGFGPDTPLCESMAPVLNLGE	24
8.		FWKNNQHQVQQQQQNNGGFLLGEVQNTGFQELYQRLKSSSSYCSDSSAVV	50
9.		AFCPPGFPMPFYPAAAYWGCGIPGTWNFPW	30
10.		HQLRPKISSSVATQLAPVKMEDN	23

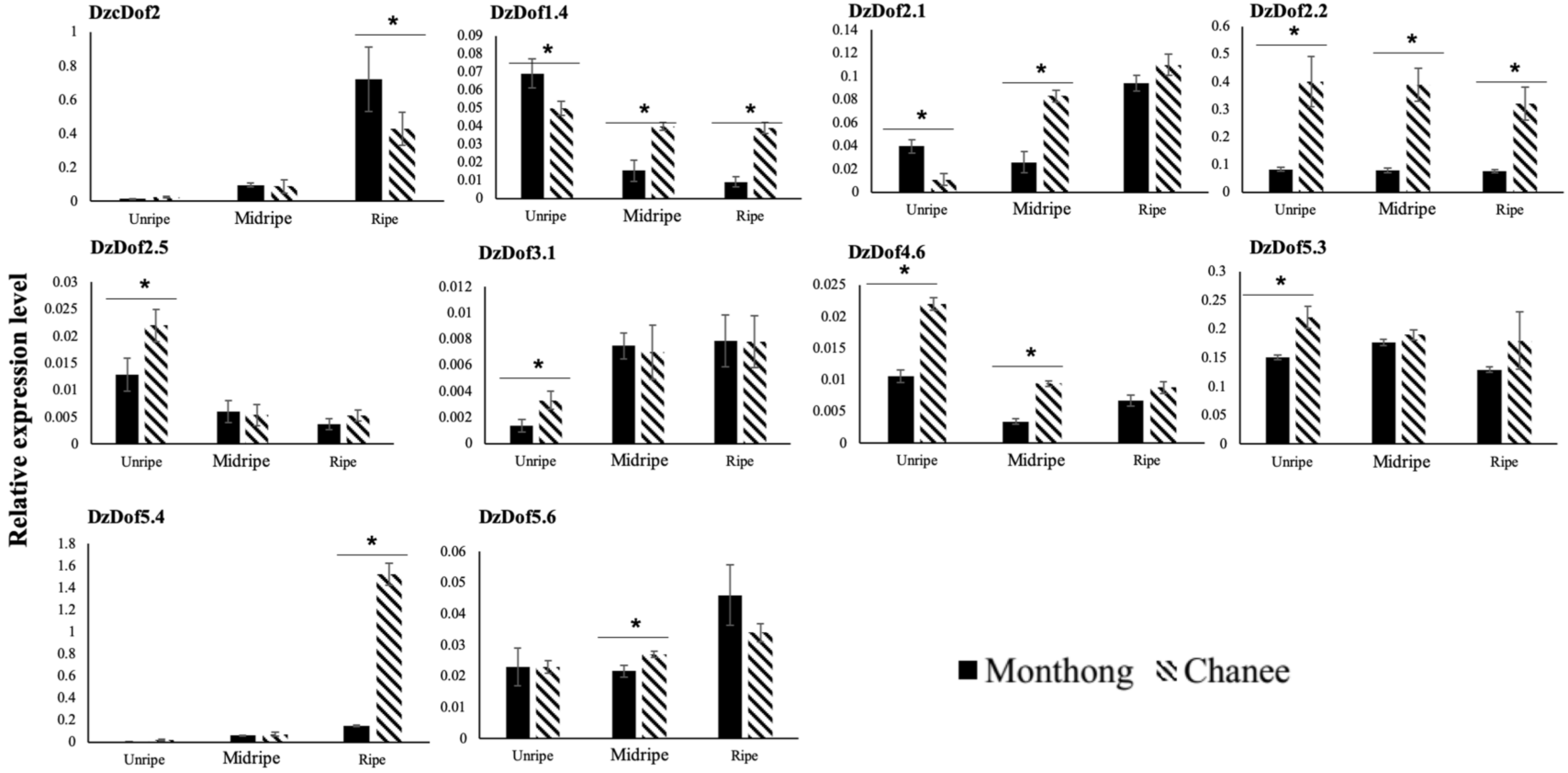
Supplementary Fig. S3



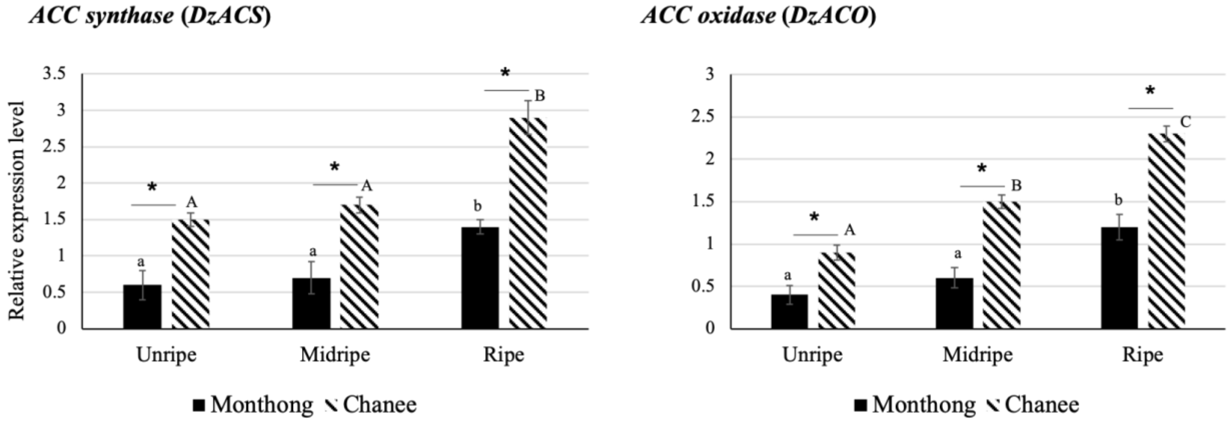
Supplementary Fig. S4



Supplementary Fig. S5



Supplementary Fig. S6



Supplementary Fig. S7

Promoter region of *DzTAA1*

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CCCCCCTATAATACCCATCTCCACGTTCCACTATCCTTCCCTGAGAGAGGTCCCTTTATTATATATACAG
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Promoter region of *DzYUCCA4*

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TTATATTTTAAATTCACTATTAATTAATAATAAATAAAATTATTTATTTAGGTTCAAAATTTTTCACAAA
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Supplementary Fig. S8

Promoter region of *NbTAA1*

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TTAGTAATAGAGCTTAGGAGTTACATTTCGGGAGCCTTAGGATTTAGTTTTAGTGTCAATGCAAATGAGT
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Promoter region of *NbYUCCA3*

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