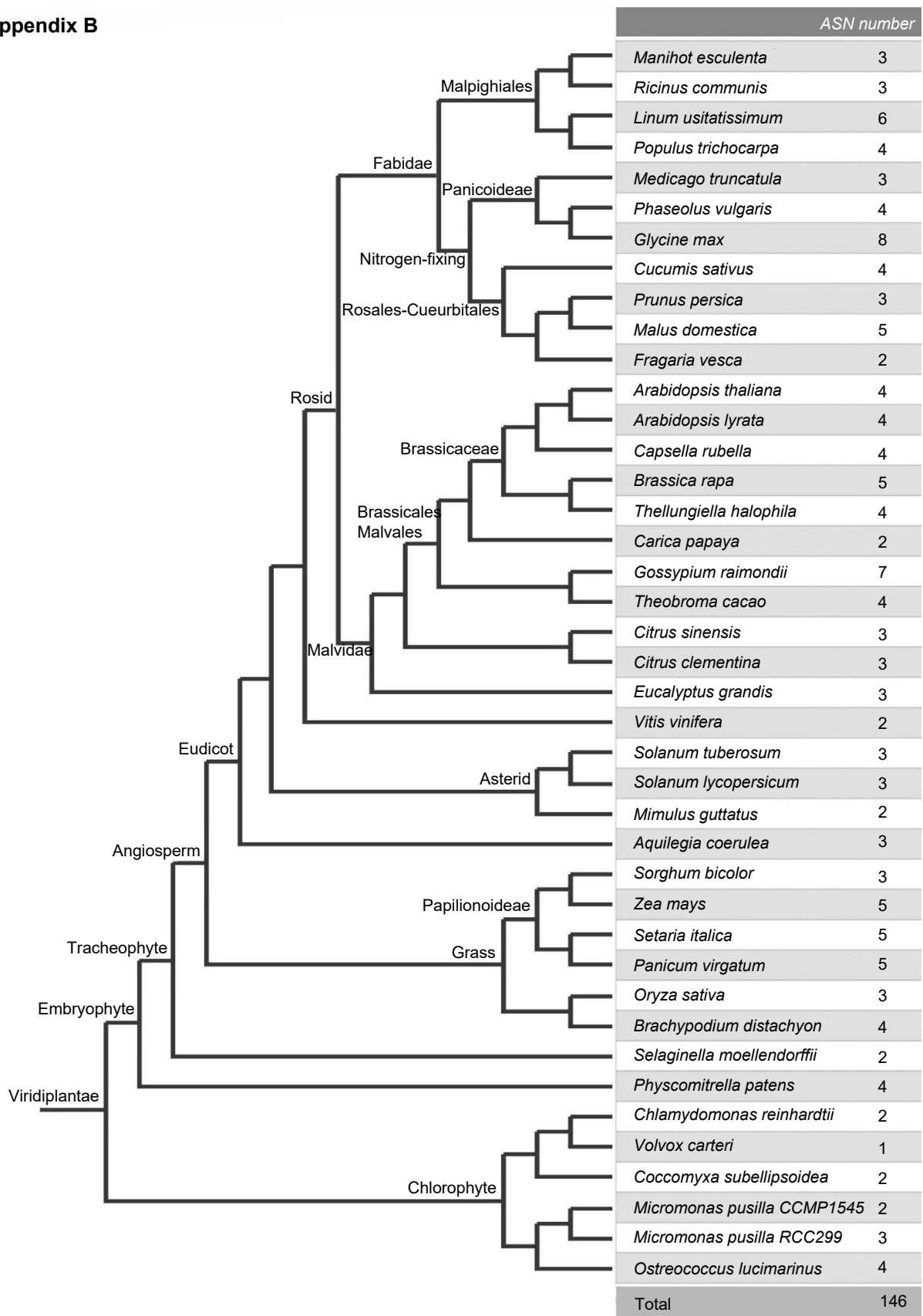


## Appendix A. Primers used in this study

Purpose	Name	Accession number of gene	Sequence (5'-3')
qRT-PCR	MdASN1 F	MDP0000292272	CCATGAACATTGATCCAGA
	MdASN1 R	MDP0000292272	AACACCATCACTGAACTG
	MdASN2 F	MDP0000096208	AAGCACATTCTGTATAGGC
	MdASN2 R	MDP0000096208	TTCAGCATTTCATCATCATCT
	MdASN3 F	MDP0000119630	TGGTAGGGCTATACTTGAA
	MdASN3 R	MDP0000119630	TTCATCTGAGGTACATTGTC
	MdASN4 F	MDP0000302187	TATAATAAGGAATAGACCAGAGTG
	MdASN4 R	MDP0000302187	CATGGCATAGAATTGAGGA
	MdASN5 F	MDP0000319314	CTTCTTGTTGGTTCAGGT
	MdASN5 R	MDP0000319314	CAATTCATTCATCGTTTAGTG

## Appendix B



### Appendix B. The numbers and phylogenetic relationships of ASNs in 41 species via MEGA5

Phylogenetic tree indicating the presence or absence of ASN family members in the indicated species. ASN members showed higher similarity between apple and *Prunus persica*.

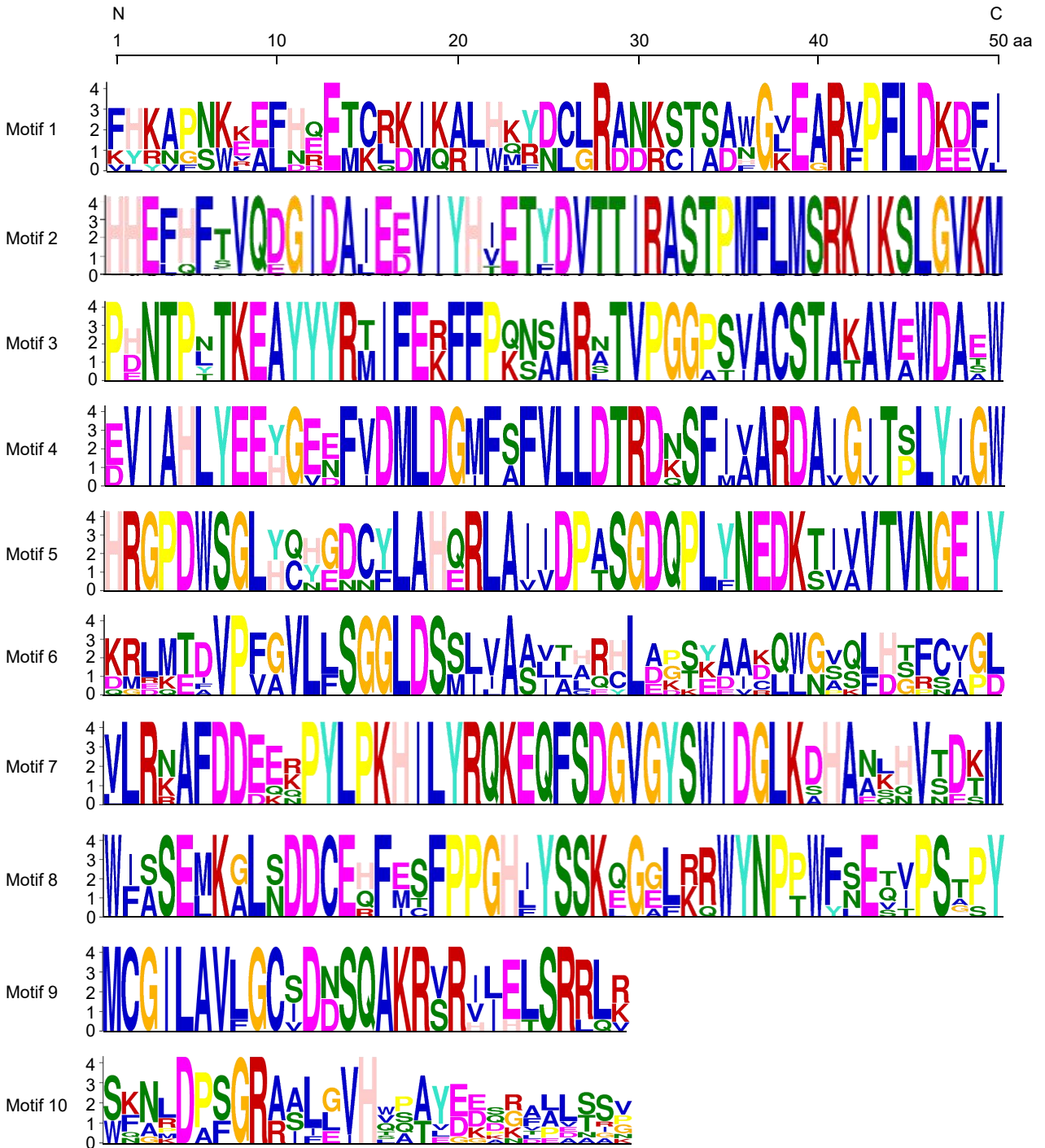
## Appendix C

AtASN1	MCGLAVLGCSDDSQAKRVRVLELSR.....	RRHRGPDNSGLYQHGDNYLAHQRLAIVDPASGDQPLFNEDKTIIVTVNG	76				
AtASN3	MCGLAVLGCVDNSQAKRSRIIELSR.....	RRHRGPDNSGLCHCYDCYLALHERLAIVDPTSGDQPLYNEDKTIIVTVNG	76				
AtASN2	MCGLAVLGCIDNSQAKRSRIIELSR.....	RRHRGPDNSGLCHCYDCYLALHERLAIIDPTSGDQPLYNEDKTIIVTVNG	76				
MdASN1	MCGLAVFGCIDNSQAKRSRIIELSR.....	RLKHRGPDNSGLCHGCHNYLAHQRLAIVDPASGDQPLYNEDKTIIVTVNG	76				
MdASN2	MCGLAVLGCSDDSQAKRVRHLSR.....	LKHRGPDNSGLYQHGDNYLAHQRLAIVDPASGDQPLYNEDKTIIVTVNG	76				
MdASN3	MCGLAVLGCSDDSQAKRVRVLELSR.....	RLKHRGPDNSGLYQHGDNYLAHQRLAIVDPASGDQPLYNEDKTIIVTVNG	120				
MdASN4	MCGLALICGIRFHLSSFLINSLTLPVLSNDQDLTFVVG.....	TEQLAFDIDDLREARRRGPDLSLSTKLLLSPIINRNLPLRGVSPVQPLDLSAKN.....	93				
MdASN5	MCGLALTIWGRFDLSSLLNPTPLPNSD.....	QLAFADIDDLRKLARRRGPDLSLSTKLLLSHSAVSNRNLVREIVSSIEGVKKEBAGXEGDEGK	91				
AtASN4	MCGLAVVCGVRIELSTLSSSLSETETPFER.....	LQVSVEDVKAVLSQRGPDSSVEKTIHLRPNCSQESATLSVVETSEGTVYKLEETSLGELH	91				
	MCGL	I	GFD				
AtASN1	EIYNHEELR.....	KRLKNHKFRTEGSDCEVIAHLYEEYGV....DFVDMLDLDFSVLLDTRDNSFMVARDAIQVTSLYIGWG.	150				
AtASN3	EIYNHKALR.....	ENLKSQFRTEGSDCEVIAHLYEEHGE....EFVDMLDLDFMFAVLLDTRDKSFIAARDAIGTFLYIGWG.	150				
AtASN2	EIYNHKILR.....	EKLKSQFRTEGSDCEVIAHLYEEHGE....EFIDMLDLMFMAVLLDTRDKSFIAARDAIGTFLYIGWG.	150				
MdASN1	EIYNHKQLR.....	ENLKSQFRTEGSDCEVIAHLYEEHGE....EFVDMLDLDFMFAVLLDTRDKSFIAARDAIGTFLYMGWG.	150				
MdASN2	EIYNHEELR.....	SXLPNHKFRTEGSDCEVIAHLYEEYGE....NFVDMLDLDFMFAVLLDTRDNSFIVARDVAGTSLYIGWS.	150				
MdASN3	EIYNHEELR.....	SRLPNHKFRTEGSDCEVIAHLYEEYGE....NFVDMLDLDFMFAVLLDTRDNSFIVARDAIQVTSLYIGWS.	194				
MdASN4	..ILVYN.....	GEIFGGIDIGSDENGEVLLQLLGECCSGSIPGVLRSRIKPEWAIYQWESAKTLWFARDAFGRRSLVHWP	170				
MdASN5	CFSLNKRTPHLHTFSTVPSSSAELHFLGATQLRGMVDIEFGFFVKGEIFGGIDIGSDENGEVLLQLLQCCSGSVPDVL	SRRIKPEWAIYQWESSKTLWFARDAFGRRSLVHWP	211				
AtASN4	FIGSTLQLRGTSPRIQPLVDSSGNILAYN.....	GEVFGGIELNSYDNTTEVLLKSLKAKS.LVPDVLMLIKPEWAIYQWESSRTLWFQDPPFRRSLVHWP	191				
		D	V	G	D	G	L
AtASN1	..LDGSVMISSEMKGLNDCCHEFFFPFPHIYSSKLGKQWYNPWFNESEVSPSTPYEPLAIRAFENAVIKRIMT.DV	PFGVLLSGGSDSLVASITARHLA..GTAKAQWQGLHSTFC	266				
AtASN3	..LDGSVMFASMKALSDCCQMFPPFPHIYSSKQGGRLRWYNPWFSEVSPSTPYDPLVVRNTEFAKAVIKRIMT.DV	PFGVLLSGGSDSLVASVALRHL..KSEAAQWQSKLHTFC	266				
AtASN2	..LDGSVMFASMKALSDCCQMFPPFPHIYSSKQGGRLRWYNPWFSEVSPSTPYDPLVLRNTEFAKAVIKRIMT.DV	PFGVLLSGGSDSLVAVALRHL..KSEAAQWQGLHSTFC	266				
MdASN1	..LDGSVMFASMKALSDCCERFISFPFPHIYSSKQGGRLRWYNPWFLEQTPSAYDPIVLRKAFKAVIKRIMT.DV	PFGVLLSGGSDSLVAAVACRYLA..DSEAAQWQGLHSTFC	266				
MdASN2	..LDGSVMISSELKGLNDCCHEFFFPFPHIYSSKLGKQWYNPWFSEVSPSTPYDPLVLRNTEFAKAVIKRIMT.DV	PFGVLLSGGSDSLVASITARHLA..GTAKAQWQGLHSTFC	266				
MdASN3	..LDGSVMISSELKGLNDCCHEFFFPFPHIYSSKLGKQWYNPWFSEVSPSTPYDPLVLRNTEFAKAVIKRIMT.DV	PFGVLLSGGSDSLVASITARHLA..GTAKAQWQGLHSTFC	310				
MdASN4	EEDSRLLSSVSPVSSNEHSSDIEAENGTKLNFWEELRCGIYSISMADPDVGLVGEVKKHDTNPNVLEELIKWERTYVEK	PPEDLHSHSK.....SLTGKHDTRLVNSDIV	280				
MdASN5	LEDNRFLSSVSPVSSNERSFDFAEANGTKLNFWEELRCGIYSISMADPDGLVGEVQKHEWTVNGLVEELIKWERTYVEK	PPEDLHSHSK.....TLTGKRDHMSVN...	317				
AtASN4	VEDPRFLSSVSPASS..VQGLDTEGNSLHRYWEEELPCGVYSISLFGVSEL..IHGEVTKHEWRNTIKPELIEWERKLV	PRPELSSLSGVQEDKSVSTSLGPAQTVLVVLIKESV	307				
	D	S	G	L	S		
AtASN1	VGLEGSPLDKAG..KEVADYLGTVHHEFHFTVQDGDIAEDVIYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHK	377				
AtASN3	IGLKGSPDLKAG..REVADYLGTRHHEHFTVQDGDIAEEVYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHQ	377				
AtASN2	IGLKGSPDLKAG..REVADYLGTRHHEHFTVQDGDIAEEVYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHQ	377				
MdASN1	IGLKGSPDLKAA..REVADYLGTRHHEHFTVQDGDIAEEVYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHL	377				
MdASN2	VGLEGSPLDKAA..KEVADYLGTVHHEFHFTVQDGDIAEDVIYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHM	377				
MdASN3	VGLEGSPLDKAG..KEVADYLGTVHHEFHFTVQDGDIAEEVYHETVYDVTTRASTPEFLMSRKIKSLGKVMVLSG.....	EGSDEIFGGYLYFHKAPNKEFHETCRKIKALHM	421				
MdASN4	PIKSGRLDMRK..EIVFVAVLFGSGLDSMIIAALLHQCLDPSYDIDLLNVSFDGRSADRI SARAG..VNELRRIAP.....	SRKWLVEINABLSTLIFETKHMVSLINP	382				
MdASN5	..SGRHDEQ..EFVFAVLFGSGLDSMIIAALLHCELDPSYDIDLLNVSFDGRSADRI SARAGLWIKVLDGLTPFLMLMVF	TLCKXWCWKLVEIDABLSTLIFETKHMVSLINP	431				
AtASN4	RRRTSLHSTYQGDKEAIVAVLFGSGLDSMIIAALLHQCLDPSYDIDLLNVSFDGRSADRI SARAG..IKELKKIAP.....	FRRKWLVEIDABLSTLIFETKHMVSLINP	412				
	V	V	V	P	ET		
AtASN1	YD..CLRANKSTSAFGLLEARVFLDKDFINTAMSLDPEKMKIPEEGRIEKWVLRRAFDEERPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKHAAQVNDKXMSNAGHIFPHN	377				
AtASN3	YD..CLRANKSTSAWGEARVFLDKDFINAVMSIDPEWKMIKEDLGRIEKWVLRNRAFDEKPNPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKHANKHVSDEMTLMSNAGHIFPHN	377				
AtASN2	FD..CLRANKSTSAWGEARVFLDKDFINAVMSIDPEWKMIKEDLGRIEKWVLRNRAFDEERPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKHANKHVSDEMTLMSNAGHIFPHN	377				
MdASN1	YD..CLRANKSTSAWGEARVFLDKDFINAMNIDPEWKMIKEDLGRIEKWVLRNRAFDEEQPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKHANSQVDSMTLMSNAGHIFPHN	377				
MdASN2	YD..CLRANKSTSAWLEARVFLDKDFIKVAMDIDPEFKMIKDGRIEKWVLRKRAFDEEQPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKSHALHVTDKMMNAERHIFPHN	377				
MdASN3	YD..CLRANKSTSAWLEARVFLDKDFINAVMIDIDPEFKMIKDGRIEKWVLRKRAFDEEKPYLPKHILYRQKE.....	QFSDGVGYSWIDGLKHAHALHVTDKMMLNAERHIFPHN	421				
MdASN4	ANTYMDLNIETALWLAAGDGVWYEENTN..YNDEDRCIKYKS..KARILLVSGGAEQACAGYGRHRTKYRNG.....	SWLALNEEMKLDQRIWKRNLGRDDRDIADN	382				
MdASN5	ANTYMDLNIETALWLAAGDGVWYEENTN..YNEDCQCVKYKS..KARILLVSGGAEQACAGYGRHRTKYRSGGYEIKLQ	GCVRVYFSWRALNDEMKLDQRIWKRNLGRDDRDIADN	431				
AtASN4	ADTYMDLNIETALWLAARGDGIHEDNGNPSVEENNRVYKYS..DARILLVAGAEQACAGYGRHRTKYRNG.....	SWVALDQEMKLDQRIWKRNLGRDDRDIADN	412				
	N	D	Y	YR			
AtASN1	TFNTEKAYYYRMIFFERFFPQ..NSARNTVPGGSAVACSTAKAVEDAWSNNDPSGRAAIGVLSAYDGNKVALTIPPLKAI	DNMPMMMG..QGVVIQS.....	584				
AtASN3	TFNTEKAYYYRMIFFERFFPK..SAARATVPGGSAVACSTAKAVEDAWSONLDPSGRAALGVVLSAYGDKTED....	SRPEKLQKLAETPAIV.....	578				
AtASN2	TFNTEKAYYYRMIFFERFFPK..SAARATVPGGSAVACSTAKAVEDAWSONLDPSGRAALGVVLSAYGDKTKED....	AAKAGSDLVPLPKNGT.....	578				
MdASN1	TFNTEKAYYYRMIFFERFFPK..NAARSTVPGGSAVACSTAKAVEDAWSONNDPSGRAALGHTAAAYEAGDAENG	NVLSSGSKVGEIVKKAUV.....	582				
MdASN2	TFNTEKAYYYRMIFFERFFPQ..NSARNTVPGGSAVACSTATAVADAEWSKNLDPSGRAIFEVHQYQAYDKQ	GAPLTSIGPEIIDNVPMKASTVGVAIRS.....	586				
MdASN3	TFNTEKAYYYRMIFFERFFPQ..NSARNTVPGGSAVACSTATAVADAEWSKNLDPSGRAILEVHQYQAYDKQ	GAPLTSIGPEIIDNVPMKASTVGLAHS.....	630				
MdASN4	GKEARFPFLDEDVIRILLGFPLWEVNTLDQPSGIGDKKILREVAELGLHEAASLKRAIQMSEHRSSLSMNXNGNXXKE	QWRABDAIAGNAEDLMSQTHRLPSPLLSSSPKARSQ	603				
MdASN5	GKEARFPFLDEDVIRILLGFPLWEVANLDQPSGIGDKKILRGVAELGLHEAASLKRAIQMSEHRSNKRNYGNSRAN	QASAGSVAIHKRLD.....	643				
AtASN4	GKEGRFPFLDEDVIKTLIDPLWEIADLEQPSGIGDKKILRQVALLGVHEVAKMKRAIQMSEIARESNNKFN	SGNSRANQASAGSVRFLAPSH.....	610				
	P	G	P				

### Appendix C. Alignment of multiple MdASN and AtASN sequences

MdASNs and AtASNs were performed with multiple sequence alignment by DNAMAN. Conserved sequence characteristics of ASNs are indicated with various colors. Black represents conserved amino acid residues and red indicates the relatively conserved amino acid residues.

## Appendix D



**Appendix D.** Sequences of the 10 conserved motifs in MdASN and AtASN proteins identified via MEME A WebLogo representation of the conservative property of the amino acids in various motifs.

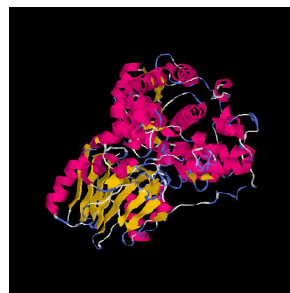
## Appendix E



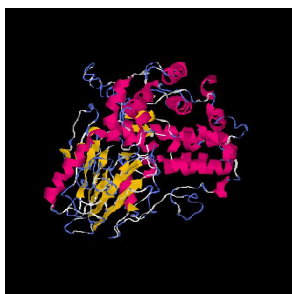
AtASN1  
C-score = -0.60  
Estimated TM-score =  $0.64 \pm 0.13$   
Estimated RMSD =  $9.0 \pm 4.6$  Å



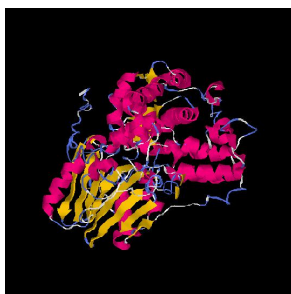
AtASN2  
C-score = -0.47  
Estimated TM-score =  $0.65 \pm 0.13$   
Estimated RMSD =  $8.7 \pm 4.6$  Å



AtASN3  
C-score = -0.47  
Estimated TM-score =  $0.65 \pm 0.13$   
Estimated RMSD =  $8.7 \pm 4.6$  Å



AtASN4  
C-score = -0.48  
Estimated TM-score =  $0.65 \pm 0.13$   
Estimated RMSD =  $8.9 \pm 4.6$  Å



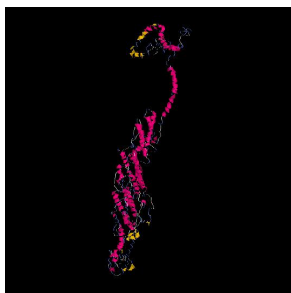
MdASN1  
C-score = -0.82  
Estimated TM-score =  $0.61 \pm 0.14$   
Estimated RMSD =  $9.6 \pm 4.6$  Å



MdASN2  
C-score = -0.73  
Estimated TM-score =  $0.62 \pm 0.14$   
Estimated RMSD =  $9.4 \pm 4.6$  Å



MdASN3  
C-score = -0.74  
Estimated TM-score =  $0.62 \pm 0.14$   
Estimated RMSD =  $9.6 \pm 4.6$  Å



MdASN4  
C-score = -1.11  
Estimated TM-score =  $0.58 \pm 0.14$   
Estimated RMSD =  $11.5 \pm 4.5$  Å



MdASN5  
C-score = -1.05  
Estimated TM-score =  $0.58 \pm 0.14$   
Estimated RMSD =  $10.4 \pm 4.6$  Å

### Appendix E. Three-dimensional structures of MdASNs and AtASNs predicted by I-TASSER website

## Appendix F. Gene ontology annotation of the *At*ASNs

Classification	Gos	Number	Annotation
Molecular function	GO:0005488	3	binding
	GO:0097159	3	organic cyclic compound binding
	GO:0008144	3	drug binding
	GO:0036094	3	small molecule binding
	GO:0097367	3	carbohydrate derivative binding
	GO:1901363	3	heterocyclic compound binding
	GO:0043167	3	ion binding
	GO:0005515	3	protein binding
	GO:0003824	4	catalytic activity
	GO:0016874	4	ligase activity
Cellular component	GO:0055044	1	symplast
	GO:0009506	1	plasmodesma
	GO:0030054	1	cell junction
	GO:0005911	1	cell-cell junction
	GO:0044464	3	cell part
	GO:0044424	3	intracellular part
	GO:0005622	3	intracellular
	GO:0005623	3	cell
	GO:0044464	3	cell part
Biological process	GO:0008152	4	metabolic process
	GO:0044237	4	cellular metabolic process
	GO:0071704	4	organic substance metabolic process
	GO:0044281	4	small molecule metabolic process
	GO:0006807	4	nitrogen compound metabolic process
	GO:0009056	1	catabolic process
	GO:0044238	4	primary metabolic process
	GO:0009058	4	biosynthetic process
	GO:0009987	4	cellular process
	GO:0044237	4	cellular metabolic process
	GO:0051716	1	cellular response to stimulus
	GO:0007154	1	cell communication
	GO:0050896	2	response to stimulus
	GO:0051716	1	cellular response to stimulus
	GO:0009605	1	response to external stimulus
	GO:0006950	2	response to stress
	GO:0009628	2	response to abiotic stimulus
	GO:0042221	1	response to chemical

## Appendix G. Gene ontology (GO) annotation of the *MdASNs*

Classification	Gos	Gens number	Annotation
Molecular function	GO:0005488	1	binding
	GO:0097159	1	organic cyclic compound binding
	GO:1901363	1	heterocyclic compound binding
	GO:0036094	1	small molecule binding
	GO:0008144	1	drug binding
	GO:0097367	1	carbohydrate derivative binding
	GO:0043167	1	ion binding
	GO:0003824	5	catalytic activity
	GO:0016787	1	hydrolase activity
	GO:0016874	5	ligase activity
Biological process	GO:0009987	5	cellular process
	GO:0044237	5	cellular metabolic process
	GO:0008152	5	metabolic process
	GO:0044237	5	cellular metabolic process
	GO:0006807	5	nitrogen compound metabolic process
	GO:0044281	5	small molecule metabolic process
	GO:0071704	5	organic substance metabolic process
	GO:0009058	5	biosynthetic process
	GO:0044238	5	primary metabolic process