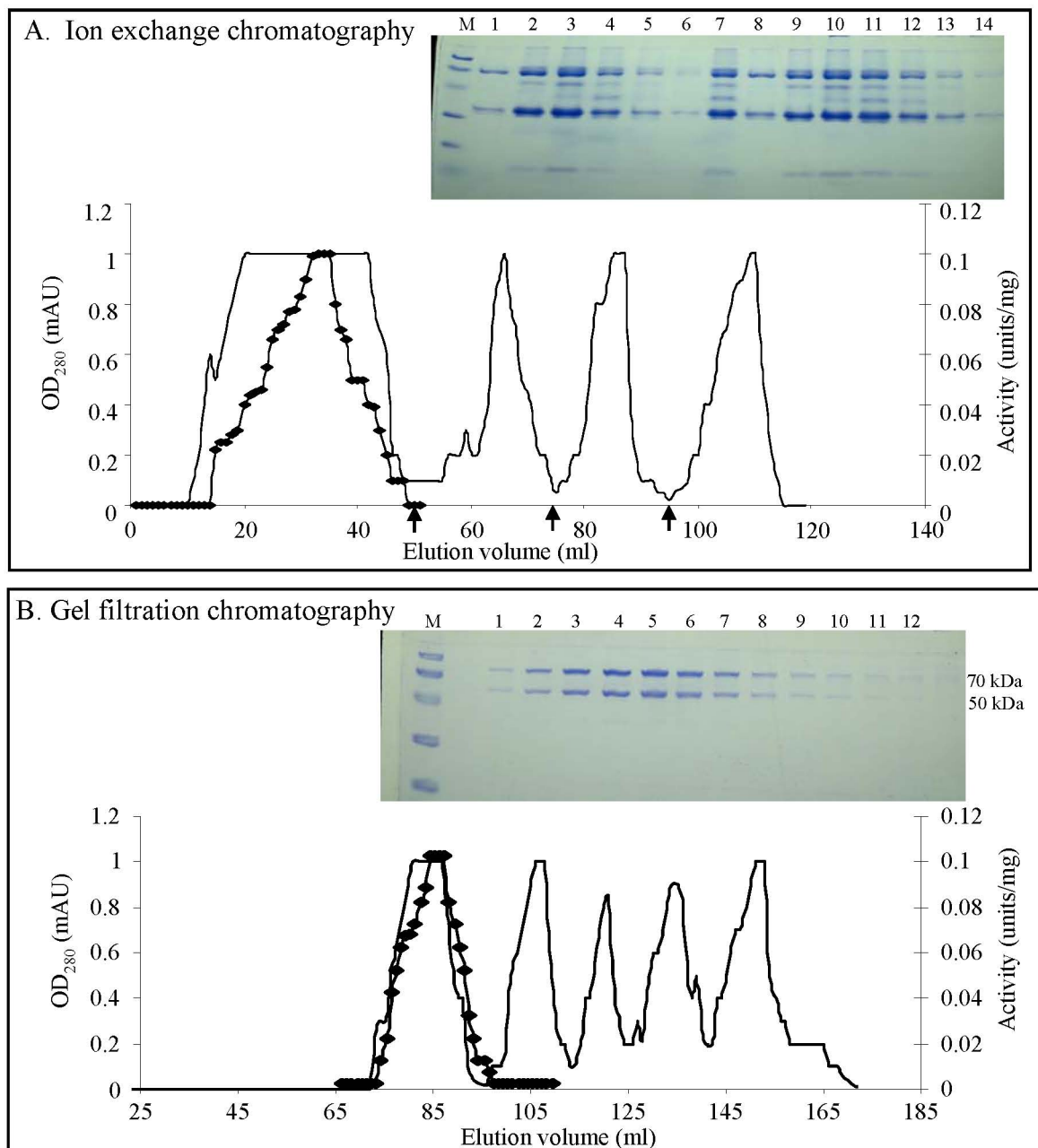
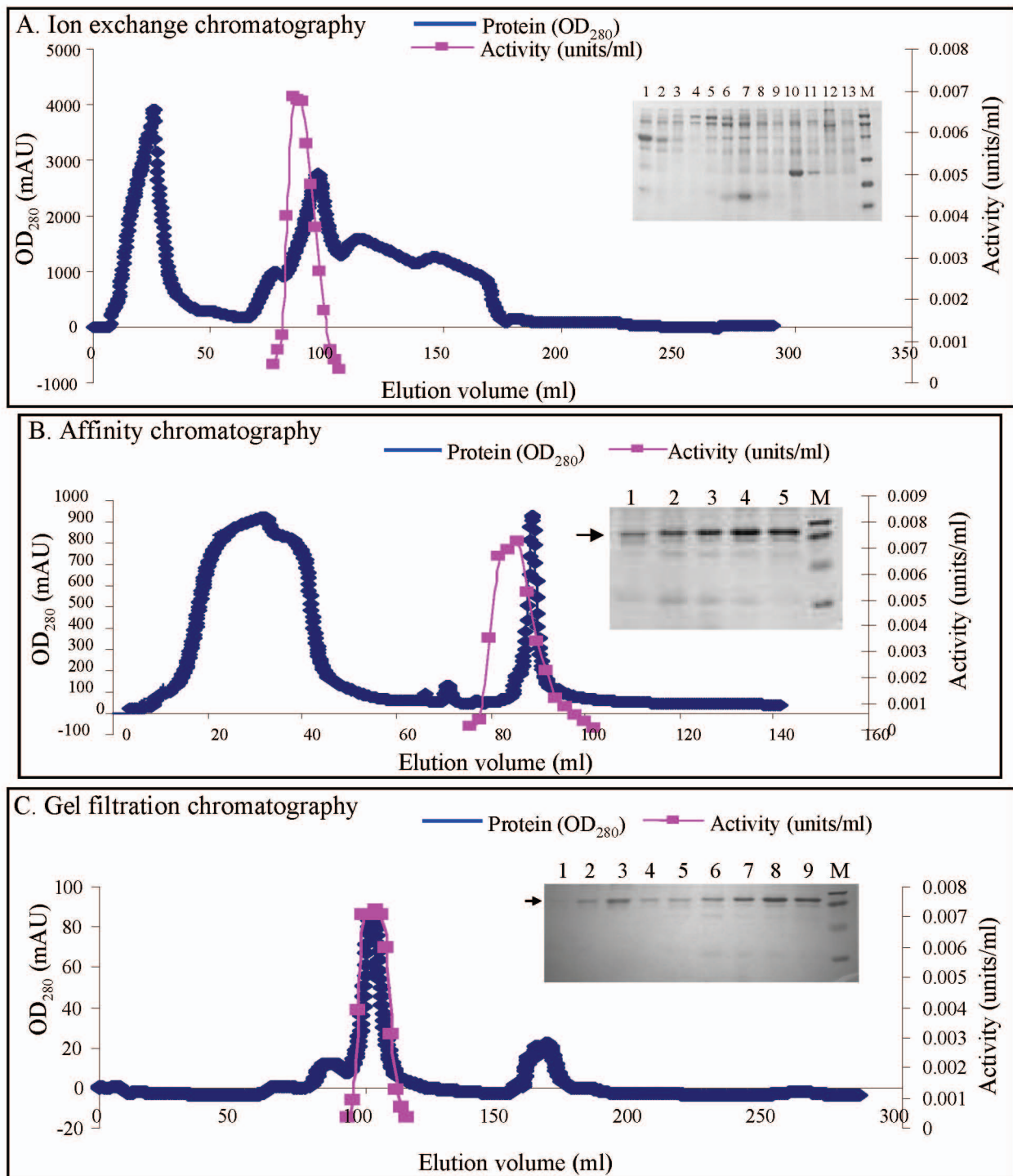


# The *N*-glycan processing enzymes $\alpha$ -mannosidase and $\beta$ -*D*-*N*-acetylhexosaminidase are involved in ripening-associated softening in the non-climacteric fruits of capsicum

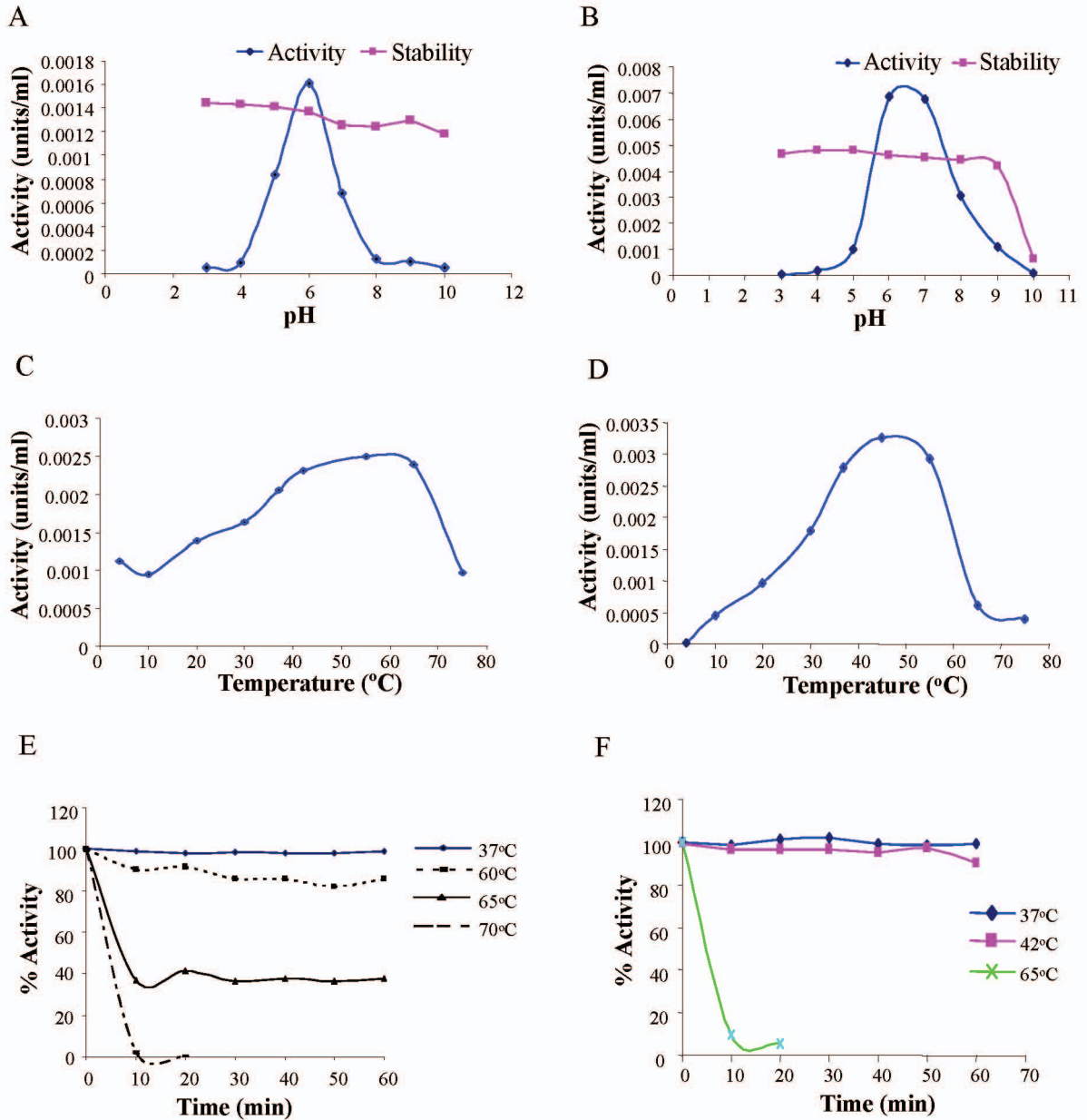
Sumit Ghosh, Vijaykumar S. Meli, Anil Kumar, Archana Thakur, Niranjana Chakraborty, Subhra Chakraborty and Asis Datta



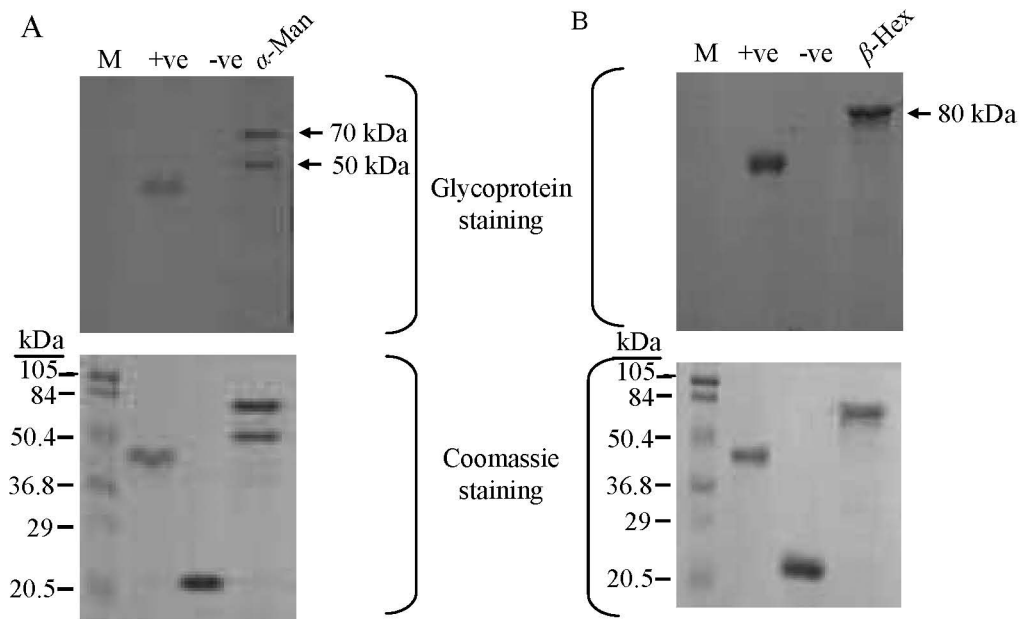
**Supplementary Fig. S1.** Purification of capsicum  $\alpha$ -man. Chromatograms show elution profile during different column chromatography. Fractions with enzyme activity were resolved on 12.5 % SDS-PAGE. M depicts molecular weight marker (kDa- 97.4, 66.2, 45, 31, 21.5, 14.4). (A) Q-sepharose ion-exchange chromatography. Elution was carried out with 0.05, 0.10 and 0.15 M NaCl as depicted by arrows. Enzyme activity was detected in the unbound fractions. (B) Sephadex G100 gel filtration chromatography.



**Supplementary Fig. S2.** Purification of capsicum  $\beta$ -hex. Chromatograms show elution profile during different chromatography. Fractions with enzyme activity were resolved on 12.5 % SDS-PAGE. M depicts molecular weight marker (kDa- 97.4, 66.2, 45, 31, 21.5, 14.4). (A) DEAE sepharose ion exchange chromatography. Bound proteins were eluted with increasing gradient of salt (upto 1 M NaCl).  $\beta$ -Hex was eluted with 120 mM NaCl. (B) ConA sepharose affinity chromatography.  $\beta$ -Hex was eluted with 50 mM  $\alpha$ -D-methylmannopyranoside. (C) Sephadex G100 gel filtration chromatography.



**Supplementary Fig. S3.** Biochemical characterization of capsicum  $\alpha$ -man and  $\beta$ -hex. (A) Optimum pH for the activity and stability of  $\alpha$ -man. (B) Optimum pH for the activity and stability of  $\beta$ -hex. (C) Effect of temperature on  $\alpha$ -man activity. (D) Effect of temperature on  $\beta$ -hex activity. (E) Effect of temperature on  $\alpha$ -man stability. (F) Effect of temperature on  $\beta$ -hex stability.



**Supplementary Fig. S4.** Capsicum  $\alpha$ -man and  $\beta$ -hex are glycoproteins. Purified proteins were resolved on 12.5 % SDS-PAGE and stained for the glycoprotein (PAS staining) as well as Coomassie Brilliant blue. +ve denotes positive control (horseradish peroxidase), -ve denotes negative control (soybean trypsin inhibitors). M is marker (kDa). (A)  $\alpha$ -man. (B)  $\beta$ -hex.

## Multiple sequence alignment

```

gi|22326796|ref|NP_196902.2|-----MDLAKFLCWIVLLLGI 16
gi|79327811|ref|NP_001031878.1|-----MDLAKFLCWIVLLLGI 16
Capsicum (GU356594)-----MKDMAKCEIWFLILMLCGL 19
Tomato (EU244853)-----MKNMGKFEIWFLILMVCGL 19
gi|115485699|ref|NP_001067993.|-----MGASAARLAALLLLLLAAAA 20
gi|108864437|gb|ABG22500.1|-----EXPRESSEDRYZASAT---IVAMGASAARLAALLLLLLAAAA 39
gi|15231611|ref|NP_189306.1|-----MAVKCFSLYLILAAIVI 17
gi|92886171|gb|ABE88173.1|PUTATIVEMEDICAGTRU-NCATULAMRSTGTLAAADSLYVLFLLLCY 49
                                     ::

gi|22326796|ref|NP_196902.2|SLVESRYMVYNTSHTIVPGKLNHVHVPVPHSHDDVGWLKTVDQYYVGSNNSI 66
gi|79327811|ref|NP_001031878.1|SLVESRYMVYNTSHTIVPGKLNHVHVPVPHSHDDVGWLKTVDQYYVGSNNSI 66
Capsicum (GU356594)-VVEAKYMVYNTSQSIVKGLNVHLVPHSHDDVGWLKTVDQYYVGSNNSI 68
Tomato (EU244853)WVVEAKYMVYNTSQSIVKGLNVHLVPHSHDDVGWLKTVDQYYVGSNNSI 69
gi|115485699|ref|NP_001067993.|AVGECVYIPYNTSAGVVGKLNHVHVPVPHSHDDVGWLKTVDQYYVGSNNSI 70
gi|108864437|gb|ABG22500.1|AVGECVYIPYNTSAGVVGKLNHVHVPVPHSHDDVGWLKTVDQYYVGSNNSI 89
gi|15231611|ref|NP_189306.1|GGVTSEYIEYNTKPRIVPEKINVHLVPHSHDDVGWLKTVDQYYVGSNNSI 67
gi|92886171|gb|ABE88173.1|GTIVSAYTKYNTGAGIVKGLNVHLVPHSHDDVGWLKTVDQYYVGSNNSI 99
                                     *  ***  : *  * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
                                     .  *  * *  * : *  * : * : * : * : * : * : * : * : * : * : * : * : *

gi|22326796|ref|NP_196902.2|QVACVQNVLDSIVPALLADKNRKFIYVEQAFFQRWWNEQSEIQRIVKEL 116
gi|79327811|ref|NP_001031878.1|QVACVQNVLDSIVPALLADKNRKFIYVEQAFFQRWWNEQSEIQRIVKEL 116
Capsicum (GU356594)QGACVENVLDSPVALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 118
Tomato (EU244853)QVACVQNVLDLIPALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 119
gi|115485699|ref|NP_001067993.|QGACVQNVLDLIPALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 120
gi|108864437|gb|ABG22500.1|QGACVQNVLDLIPALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 120
gi|15231611|ref|NP_189306.1|QGACVQNVLDLIPALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 120
gi|92886171|gb|ABE88173.1|QGACVENVLDLIPALLADKNRKFIYVEQAFFQRWWRNQSPEIQSTVRQL 120
                                     :  *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
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gi|22326796|ref|NP_196902.2|IHSQGLELINGGCMHDEAAPHYIDMIDQTTLGHFRFIREFNVTPRIGWQ 166
gi|79327811|ref|NP_001031878.1|IHSQGLELINGGCMHDEAAPHYIDMIDQTTLGHFRFIREFNVTPRIGWQ 166
Capsicum (GU356594)INSGQLELINGGCMHDEAATHYIDMIDQTTLGHFRYIKQQFNIAPIRIGWQ 168
Tomato (EU244853)VNSTGLESINGGCMHDEAATHYIDMIDQTTLGHFRYIKQQFNIAPIRIGWQ 169
gi|115485699|ref|NP_001067993.|ISTGRLILINGGCMHDEATVHYIDMIDQTTLGHFRFIKEEFGQIPRIGWQ 170
gi|108864437|gb|ABG22500.1|ISTGRLILINGGCMHDEATVHYIDMIDQTTLGHFRFIKEEFGQIPRIGWQ 189
gi|15231611|ref|NP_189306.1|VDSGQLELINGGCMHDEATPHYIDMIDQTTLGHQFKTEFGQVPRVIGWQ 167
gi|92886171|gb|ABE88173.1|VAAGQLEFVNGGCMHDEATVHYIDMIDQTTLGHFRFIKDQFNTPRAGWQ 199
                                     :  : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
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gi|22326796|ref|NP_196902.2|IDPFGHSAVQAYLLGAIEVGFDSVFFGRIDYQDREKRYKEKTLEVIWRGS 215
gi|79327811|ref|NP_001031878.1|IDPFGHSAVQAYLLGAIEVGFDSVFFGRIDYQDREKRYKEKTLEVIWRGS 215
Capsicum (GU356594)IDPFGHSAVQAYLLGAIEVGFDSLFFGRIDYQDREKRYKEKTLEVIWRGS 217
Tomato (EU244853)IDPFGTFCSSGIPSGSKGWIRLSFLWDALTTKDREKRYKEKTLEVIWRGS 219
gi|115485699|ref|NP_001067993.|IDPFGHSAVQAYLLGTEVGFDSVFFGRIDYQDRDTRKGTKELEVIWRGS 219
gi|108864437|gb|ABG22500.1|IDPFGHSAVQAYLLGTEVGFDSVFFGRIDYQDRDTRKGTKELEVIWRGS 238
gi|15231611|ref|NP_189306.1|IDPFGHSAVQAYLLGAEVGFDSLFFGRIDYQDRAKRLREKTLEVIWRGS 216
gi|92886171|gb|ABE88173.1|IDPFGHSAVQYLLGAEVGFDSVHFARIDYQDRAKRKSDEKTLEVIWRGS 248
                                     *****  .  .  .  * : :  :  :  :  :  :  : * : * : * : * : * : * : * : *
                                     * : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

gi|22326796|ref|NP_196902.2|KSLGSSSQIFAGAFPNTYEPVPPGGFYIEITDD-SPVVQDDPDLFDYNVQE 264
gi|79327811|ref|NP_001031878.1|KSLGSSSQIFAGAFPNTYEPVPPGGFYIEITDD-SPVVQDDPDLFDYNVQE 264
Capsicum (GU356594)KSLSSSTQIFSGAFPQNYEPPSK-FYFEVNDNSLPVQDDVNLFDYNVQE 266
Tomato (EU244853)KSLSSSTQIFSGAFPQNYEPPSK-FYFEVNDNSLPVQDDVNLFDYNVQE 268
gi|115485699|ref|NP_001067993.|KTFGSSADIFAGIFPKNYEPPVGGFYIEVDDT-SPIVQDDPDLFDYNVQE 268
gi|108864437|gb|ABG22500.1|KTFGSSADIFAGIFPKNYEPPVGGFYIEVDDT-SPIVQDDPDLFDYNVQE 287
gi|15231611|ref|NP_189306.1|KSLGSSSQIFTVGFPVHYDPPG-FTFEINDV-SAPIQDDPDLFDYNVQE 264
gi|92886171|gb|ABE88173.1|KTFGSSAQIFANTFPVHYSAPHG-FNFEVSGD-FVPLQDDPDLFDYNVQE 296
                                     * : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
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gi|22326796|ref|NP_196902.2|RVNAFVAAALDQANITRNHIMFTMGTDFRYQYAHTWYRQMDKLIHYVNL 314
gi|79327811|ref|NP_001031878.1|RVNAFVAAALDQANITRNHIMFTMGTDFRYQYAHTWYRQMDKLIHYVNL 314
Capsicum (GU356594)RVNDFVAAALSQANITRNHIMWTMGTDFKYQYAHTWFRNMDKLIHYVNL 316
Tomato (EU244853)RVNDFVAAALSQANITRNHIMWTMGTDFKYQYAHTWFRNMDKLIHYVNL 318
gi|115485699|ref|NP_001067993.|RVDDFVAAAIAQANITRNHVMFTMGTDFKYQYAESWFRQMDKLIHYVNL 318
gi|108864437|gb|ABG22500.1|RVDDFVAAAIAQANITRNHVMFTMGTDFKYQYAESWFRQMDKLIHYVNL 337
gi|15231611|ref|NP_189306.1|RVNDFVAAALQANITRNHIMWLMGTDFRYQYAYSWFRQMDKLIHYVNL 314
gi|92886171|gb|ABE88173.1|RVKDFIDAAITQANVTRNHNIMWTMGDDFQYQYAESWFRQMDKLIHYVNL 346
                                     **  * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
                                     * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

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gi 79327811 ref NP_001031878.1	GTFFPINPEGVPLTVIHGPLVDEVHQQINPWISQITRVYKGEHVEVEFI	708
<b>Capsicum (GU356594)</b>	GSFPIHPEGKVPATILRGPLLDEVHENINSWIYQITRVYKGEHVEVEFT	715
Tomato (EU244853)	GSFPIHPEGKVPATILRGPLLDEVHQNINSWIYQITRVYKGEHVEVEFT	716
gi 115485699 ref NP_001067993.	GTVPVITDGGVPLTVLRGSLDEVHQQINPWIYQINRVYKGDYVETEFI	712
gi 108864437 gb ABG22500.1	GTVPVITDGGVPLTVLRGSLDEVHQQINPWIYQINRVYKGDYVETEFI	729
gi 15231611 ref NP_189306.1	GVLPVSKKEAQLTIVQGPLFDEVHQLNSWISQITRVYKGNHAEIETFT	705
gi 92886171 gb ABE88173.1	ESSPTIVRSVPFKVIRGPLVDEVHQQFNWSWIYQVTRLYKGDHAEIETFT	740
	* . . . . : : * : * : * : * : * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	VGNIPIDDDGIGKEVVTQISSSLKSNKTFYTDSSGRDYIKRIRDYRSDWKL	758
gi 79327811 ref NP_001031878.1	VGNIPIDDDGIGKEVVTQISSSLKSNKTFYTDSSGRDYIKRIRDYRSDWKL	758
<b>Capsicum (GU356594)</b>	VGPIPIDDDGIGKELVTQIQTDIKSNKTFYTDSSGRDFLKRIRDYRADWDL	765
Tomato (EU244853)	VGPIPIDDDGIGKELVTQIQTDIKSNKTFYTDSSGRDFLKRIRDYRADWDL	766
gi 115485699 ref NP_001067993.	VGPIPVDDGNGKELSTEVVNTMATNKTFYTDSSGRDFIKRIRDYRSEWKI	762
gi 108864437 gb ABG22500.1	VGPIPVDDGNGKELSTEVVNTMATNKTFYTDSSGRDFIKRIRDYRSEWKI	779
gi 15231611 ref NP_189306.1	IGPIPADDDGISKEIITKLTMTKNGTFYTDSSGRDFIKRIRDYRSDWDL	755
gi 92886171 gb ABE88173.1	IGPIPADDDGISKEIITKLTMTKNGTFYTDSSGRDFIKRIRDYRSDWDL	790
	: * * * * * : * : * : * : * : * * * * * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	DVNQPIAGNYYPINHGIYLDQSKKEFSVMVDRAFGGSSIVDQVLEMLLHR	808
gi 79327811 ref NP_001031878.1	DVNQPIAGNYYPINHGIYLDQSKKEFSVMVDRAFGGSSIVDQVLEMLLHR	808
<b>Capsicum (GU356594)</b>	QVNVQPAAGNYYPINLGIYLDQSKKEFSVMVDRAFGGSSIVDQVLEMLLHR	815
Tomato (EU244853)	QVNVQPAAGNYYPINLGIYLDQSKKEFSVMVDRAFGGSSIVDQVLEMLLHR	816
gi 115485699 ref NP_001067993.	EVHQPIAGNYYPVNLGIYVEDGSRLESLVDRSVGGASIKDQIEMLLHR	812
gi 108864437 gb ABG22500.1	EVHQPIAGNYYPVNLGIYVEDGSRLESLVDRSVGGASIKDQIEMLLHR	829
gi 15231611 ref NP_189306.1	QVYQPVAGNYYPLNLGIYMQDKTSELSVLDVRAVGGSSLENGQIEMLLHR	805
gi 92886171 gb ABE88173.1	QVYQPVAGNYYPLNLGIYMQDKTSELSVLDVRAVGGSSLENGQIEMLLHR	840
	: * * * * * : * : * : * : * : * * * * * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	RLLLDDSRGVAENLNETVVCVQDK----CTGLTIQGGKYYRIRIDPYGEGAK	853
gi 79327811 ref NP_001031878.1	RLLLDDSRGVAENLNETVVCVQDK----CTGLTIQGGKYYRIRIDPYGEGAK	853
<b>Capsicum (GU356594)</b>	RLLLDDSRGVAENLNETVVCALGK----CMGLTVQGGKYYRIRIDSLGEGAK	860
Tomato (EU244853)	RLLLDDSRGVAENLNETVVCALGK----CMGLTVQGGKYYRIRIDSLGEGAK	861
gi 115485699 ref NP_001067993.	RLLLDDSRGVAENLNETVCFDQ----CEGLVIQGGKYYLIDPQGEAR	857
gi 108864437 gb ABG22500.1	RLLLDDSRGVAENLNETVCFDQ----CEGLVIQGGKYYLIDPQGEAR	874
gi 15231611 ref NP_189306.1	RMQHDDIRGVGEILNETVCLPEG----CKGLTIQGGKYYVQIDKPGDGA	850
gi 92886171 gb ABE88173.1	RLIEDDGRGVGEPDQVCIKADNNSCTDGLTVRGNYYIGIHNVAGSRR	890
	* : * * * * * : * : * : * : * : * * * * * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	WRRTFGQEIYSPDLLLAFQDDGKPMSPFGAASFSGIDPSYSLPDNVALLT	903
gi 79327811 ref NP_001031878.1	WRRTFGQEIYSPDLLLAFQDDGKPMSPFGAASFSGIDPSYSLPDNVALLT	903
<b>Capsicum (GU356594)</b>	WRRSFQGEIYSPDLLLAFTEQDQDKFTKFPVPTFTWIDPSYSLPDNVAIIT	910
Tomato (EU244853)	WRRSFQGEIYSPDLLLAFTEQDQDKFTKFPVPTFTWIDPSYSLPDNVAIIT	911
gi 115485699 ref NP_001067993.	WRRTFGQEIYSPDLLLAFQDDGNNWVNSHVTKFSAMDPAYSLPDNVALLT	907
gi 108864437 gb ABG22500.1	WRRTFGQEIYSPDLLLAFQDDGNNWVNSHVTKFSAMDPAYSLPDNVALLT	924
gi 15231611 ref NP_189306.1	WRRTFGQEIYSPDLLLAFTEQEGDSWINSHKTTFSAFEPSYSLPKNVALLT	900
gi 92886171 gb ABE88173.1	WRRTFGQEIYSPDLLLAFTEKSKNWKSSHLTKGTLMDPNYSLPNVALLT	940
	*** : ***** : * : * : * : * : * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	LQELDDGNVLLRLAHLHYEVEDKELSGVASVELKKLFPKGIKLTMSL	953
gi 79327811 ref NP_001031878.1	LQELDDGNVLLRLAHLHYE-----	921
<b>Capsicum (GU356594)</b>	LQELLEDHTVLLRLAHLHYEVEDKDLSTKAIVELKRLFPKRIKIKEMSL	960
Tomato (EU244853)	LQELLEDHTVLLRLAHLHYEVEDKDLSTKASVELKRLFPKRIKIREMSL	961
gi 115485699 ref NP_001067993.	LQELLEDGTVLLRLAHLHYEAGEHKDLSALASVDLKRVPDPKIVKIVETSLSL	957
gi 108864437 gb ABG22500.1	LQELLEDGTVLLRLAHLHYEAGEHKDLSALASVDLKRVPDPKIVKIVETSLSL	974
gi 15231611 ref NP_189306.1	LQELLEDGTVLLRLAHLHYEAGEHSDSEYSVMAKVELKKLFPKRIKIVETSLSL	950
gi 92886171 gb ABE88173.1	LEELDDGIVLLRLAHLHYEPNEDAQYSALAKVELKKLFPKRIKIVETSLSL	990
	* : * : * : * : * : * : * : * : * : *	
gi 22326796 ref NP_196902.2	SANQERSTMEKKRLVWVKEGEGSYGEEKAKRGREIDPRKLEMELYPMEI	1003
gi 79327811 ref NP_001031878.1	-----	
<b>Capsicum (GU356594)</b>	SANQEREEMKRLKWKAEAPS---DSQDVPRGGPVDPTKLVVELAPMEI	1007
Tomato (EU244853)	SANQERVEMKRLKWKAEAPS---DLRDVARGGPVDPTKLMVELAPMEI	1008
gi 115485699 ref NP_001067993.	SANQERSAMEKRLKWKVEGPP---ADEKIVRGGPVDPSKLVVDLGPMEI	1004
gi 108864437 gb ABG22500.1	SANQERSAMEKRLKWKVEGPP---ADEKIVRGGPVDPSKLVVDLGPMEI	1021
gi 15231611 ref NP_189306.1	SGNQEKAEKRRLLIWKVEGSAG---EEVKRGEAVDAEKLVVELVPMELI	996
gi 92886171 gb ABE88173.1	SANQEKSEMKK---MTWKVEGDKG---QEPQAVRGGPVDSTDFVVELGPMEI	1036

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gi|22326796|ref|NP_196902.2|      RTVLIHLELPSSHSRINRFDA-- 1024
gi|79327811|ref|NP_001031878.1|  -----
Capsicum (GU356594)             RTFVINLQSSSPAPGGWKSHMSL 1030
Tomato (EU244853)                RTFVIDLSQS--VPEGWKSHMSL 1029
gi|115485699|ref|NP_001067993.   RTFLINFAPQSGKQLM----- 1020
gi|108864437|gb|ABG22500.1|      RTFLINFAPQSGKQLM----- 1037
gi|15231611|ref|NP_189306.1|     RTLLIKFDDQIEMVGDKEQQHRL 1019
gi|92886171|gb|ABE88173.1       RTFLLEF----- 1043

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**Supplementary Fig S5.** Multiple sequence alignments of  $\alpha$ -man proteins from different plant species using Clustal W (EBI tool). Following are the plant species used in the alignment with their corresponding accession number. Identical amino acid residues are depicted as \*, while conserved and semiconserved substitutions are marked as : and ., respectively. Dashed lines represent gaps inserted for optimal alignment of the sequences. NP\_1969022: *Arabidopsis thaliana*, NP\_001031878: *Arabidopsis thaliana*, NP\_189306: *Arabidopsis thaliana*, NP\_001067993.1: *Oryza sativa* (Japonica), ABE88173: *Medicago truncatula*.



### Multiple sequence alignment

```

CAO67165_Vitis          -----MAVS-----DKALFSIVFLFTA FVSSISASESQS-- 29
ABE82127_Medicago      -----MLLQLLFLFLFFPFSTTS----- 18
Atlg05590_Arabidopsis  -----MLTLSKF-----HVILIPILFFITLLSPLFSIALP---- 30
EU244854_Solanum       -----MRGE-----KTFSEFFLLFFILISQTTATNYP---- 27
GU356593_Capsicum     -----MRGD-----TTFPFILSLFVIFITQTIATNYP---- 27
Os03g0219400_Oryza     -----MAAL-----PAYLLLLLLLILVILRPAAAPGAASQPP 31
Os07g0575500_Oryza     -----MATK-----NGGFVALLLLLSFLLSSPLPARCDAP-- 30
At3g55260_Arabidopsis  -----
CAO41588_Vitis         -----MAVPYHFSSF-----VFVFTLICALGVSLGFNSTSDLDDS- 35
Os05g0115900_Oryza     -----MPPKLLTY-----LILALLAASAAAARRHSPASSAAAGE- 35
Os01g0891000_Oryza     -----MAQA-----LSLGLLL--AFLAIQSC-IAIELTD- 26
Os05g0415700_Oryza     -----MAPA-----MALRLLVVVAVAAVVSCAADAEGS- 29
Atlg65590_Arabidopsis  -----MRGSG-----AKIAGVLPFLMFLIAGTISAFEDIE- 30

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CAO67165_Vitis          -----QINVWPKPRTFSWPSP-QASLLSPNFSITSP-----N 60
ABE82127_Medicago      -----LNIWPKPRNLWTTPHQTTLLSSTFTITTTTLH--H 52
Atlg05590_Arabidopsis  -----LNIWPKPRFLSWPQH-KAIALSPNFTILAP-----E 60
EU244854_Solanum       -----INVWPKPTTFLWPNP-KSIFLSTNFTISHP-----Y 57
GU356593_Capsicum     -----INVWPKPTTFNWPNP-KIHLPLPNFTISHP-----T 57
Os03g0219400_Oryza     TSEPHLPPPLLAQKVQVWPKPTSISWPSA-VYAPLSPSFSVRAVL----S 76
Os07g0575500_Oryza     -----LPNVWPKPMSMSWAEPMAVRVSSSFHVVAPS----G 64
At3g55260_Arabidopsis  -----
CAO41588_Vitis         -----LVYLWPLPSEFTFGEDVLAVDPDLSLAVGGDGGN--- 69
Os05g0115900_Oryza     -----PVYLWPLPRNFTSGSRTLLVDPDLALDGQGG---A 69
Os01g0891000_Oryza     -----HIDLWPMPTSVSHGTQRLYVSKDITMSMEGSTYPD-G 62
Os05g0415700_Oryza     -----VVEVWMPATASKGGQTLHVSRELRMATAEGSKYAD-G 65
Atlg65590_Arabidopsis  -----RLRIWPLPAQVSHGGRMYLSGDFKLVTEGSKYGD-A 66

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CAO67165_Vitis          HQHLSAVARYLRLLILTEHHHPLVTPVTNITG-----PPLETLT 99
ABE82127_Medicago      NNHLTAAISRYTNLIKTEHNHPLIPKTNLSNNL-----PPLQTLT 93
Atlg05590_Arabidopsis  HQYLSASVTRYHNLRSENYSPLISYPVKLMKR-----YTLRNLV 100
EU244854_Solanum       HRYLTPAVDRYRHLILSEHHRPIITPAINLTSS-----IPLQSLV 97
GU356593_Capsicum     HRYLTPVYRYRLLILSEHYRHIITPSINLTSS-----TPLQHLI 97
Os03g0219400_Oryza     HPSLRQAVAFYTRLIRAERHAPLVPPANYTLR-----VPVRTLT 116
Os07g0575500_Oryza     NAHLLSAARRYAALLLAERYRPLVTPAVNVTAGGAGAGAARGAELGYLT 114
At3g55260_Arabidopsis  -----MVE-----YDITSLK 10
CAO41588_Vitis         SDIVREAFRLRYRGIIFKHSTRFSK---FRGRSM-----YDISKIR 106
Os05g0115900_Oryza     AAVAFAFERYRSLVFSWPAHAAR---NASGG-----YDVGKLT 105
Os01g0891000_Oryza     KGILKDAFQRVVDMKLNHVVDG-----ANPSS-----FVLTVGN 97
Os05g0415700_Oryza     EAILKDAFQRMVTLIELDHVING-----SSQGL-----PLLAGVN 100
Atlg65590_Arabidopsis  SGILKEGFDRMLGVVRLSHVISGDRNNSGTGGS-----ALLQGLH 106

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CAO67165_Vitis          IIVSDLAAPLHHGVDESYTLIVP-RGG-----AANLTAATVWGAMRGL 142
ABE82127_Medicago      ITITNPNTLNHATDESYTLIIT-TP-----TATLTAVTSWGAMHGL 135
Atlg05590_Arabidopsis  VTVTDFSLPLHHGVDESYKLSIP-IGSF-----SAHLLAHSAWGAMRGL 144
EU244854_Solanum       ISVSDVTSPLAHGVNESYSLSTPDSGSA-----SAYISAATVWGAMRGL 142
GU356593_capsicum     ISVSDVTSPLSHGVNESYSLSTP-NGSS-----AAYITAGTVWGAMRGL 141
Os03g0219400_Oryza     LSVSDPDVPLGPVDESYTSLVLPDSG-----SADISAATPWGAIRGL 160
Os07g0575500_Oryza     LAVSDLHAPLQHGVDSESYALEILPAGA-----AATVTAATAWAMRGL 158
At3g55260_Arabidopsis  IIVHSDSEELQLGVDESYTLMVSKKN-EQSIVGAATIEANTVYGALRGL 59
CAO41588_Vitis         IIVHSDSEMLQLGVDESYSLLVAKND-DHSIIGEATIEANTVYGALRGL 155
Os05g0115900_Oryza     VVVASADEKLELGVDESYTIYVAAAGVNSIVGGATIEANTIYGALRGL 155
Os01g0891000_Oryza     VVHSPPEDELKFGVDESYNLSVPTAG---YPLRVQIEAQTVFALHALQ 143
Os05g0415700_Oryza     VVVHLPGDELNFGVDESYNLSVPTAG---SPIYAQIEAQTVFALHALQ 146
Atlg65590_Arabidopsis  VIISSSTDELEYGADSESYKLVVPSPE---KPSYAQLEAKSVYGAHLGLQ 152

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CAO67165_Vitis      TFSQIVWGD-----LRVATGLFVWDSPLFGHRGVMLDTSRNYYGVED 185
ABE82127_Medicago  TFSQLAWGNP-----TRVAVNVRVNDAPLFGHRGIMLDTSRNYYPVKD 178
Atlg05590_Arabidopsis TFSQMIWGTSP-----DLCLPVGIIYQDSPLFGHRGVLLDTSRNYYGVD 189
EU244854_Solanum   TFSQLVYGNP-----TRVSAGVYIHDLPFIFTHRGVMLDTSRNFYGVVDH 185
GU356593_capsicum TFSQLVYGNP-----TRVAAGVYISDLPIFTHRGVMLDTSRNFYGVDD 184
Os03g0219400_Oryza TFSQLAWAGGGAASGGQP IVPSGIEISDRPHFTHRGILLDTA RNFYVPRD 210
Os07g0575500_Oryza TFSQLAWWCGRER---AVLVAAGVRVEDRPLYPHRGLMLDTGRTYFPVAD 205
At3g55260_Arabidopsis TFSQLCAF DYITK--SVQIYKAPWYIQDKPRFGYRGLLIDTSRHYLPIDV 107
CAO41588_Vitis     TFSQLCAF DYGTK--TVQVYNAPWYIQDKPRFVYRGLMLDTSRHYLPIDV 203
Os05g0115900_Oryza TFSQLCVFN YDTK--NVEVRHAPWYIEDEPRFAFRGLLLDTSRHF LFPVDV 203
Os01g0891000_Oryza TFSQLCYFDFTSK---LIELISAPWRI SDTPRFPYRGLLIDTSRHYLPVTV 191
Os05g0415700_Oryza TFSQLCNFDFTSR--LIELQSAPWSITDMRFPYRGLLIDTSRHYLPVPV 194
Atlg65590_Arabidopsis TFSQLCHFNLKKK--VIEILMTPWNIIDQPRFSYRGLLIDTSRHYLPLPV 200
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-----Active site-----
CAO67165_Vitis      ILRTIGAMSANKLNVFHWHTDSHSFPLLLPSEPDLAGKGSYGPQM QYSP 235
ABE82127_Medicago  LLRTIEAMSMNKLNVFHWHTDSHSFPLILPSEPLA EK GAYDVMVYTV 228
Atlg05590_Arabidopsis IMRTIKAMSANKLNVFHWHTDSQSFP LVL PSEPSLAAKGS LGPDMVYTP 239
EU244854_Solanum   LLRLIKAMSMNKLNVFHWHTDSHSFPLVIPSEPELAGK GAYSNEMMYSP 235
GU356593_Capsicum LLRLIKAMSMNKLNVFHWHTDSHSFPLVVPSEPELAGK GAYGNEMMYSP 234
Os03g0219400_Oryza ILHTLRAMAFNKLNVFHWHTDAQSFP I VLP TVPNLANS GSYSP TMRYTE 260
Os07g0575500_Oryza ILRTIDAMAANKMNVFHWHTDSQSFP LELPSEPALAEKGSYGDGMR YTV 255
At3g55260_Arabidopsis IKQIIESMSFAKLNVLHWHIVDEQSFPLETP TYPNLWK-GAYS RWER YTV 156
CAO41588_Vitis     IKHVIESMSYAKLNVLHWHIIDEQSFPLEVP TYPKLWK-GAYTKWERYTV 252
Os05g0115900_Oryza IKQVIDSMSFSKLNVLHWHIIDEQSFPLEVP SYPKLWK-GSYSK LERYTV 252
Os01g0891000_Oryza IKKVIDTMAYSKLNVLHWHIVDAQSFP I EIPSYPKLWN-GSYSF SER YTT 240
Os05g0415700_Oryza IKSVIDSMTYSKLNVLHWHIVDEQSFP I EIPSYPKLWN-GAYSYSERYTM 243
Atlg65590_Arabidopsis IKNVIDSMTYAKLNVLHWHIVDTQSFPLEIPSYPKLWN-GAYSSSQRYTF 249
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CAO67165_Vitis      EDVKKIVEFGLGHRVRLPEIDSPGHTGSWAEAYPEIVTCANMFWWPAAE 285
ABE82127_Medicago  DDVKRVVEFGLDRGVRVIPEIDAPGHTGSWALAYPDIVACANMFWWPAGS 278
Atlg05590_Arabidopsis EDVSKIVQYGFEGHRVRLPEIDTPGHTGSWGEAYPEIVTCANMFWWPAGK 289
EU244854_Solanum   ADVQKIVEYFGMEHGVRVLEIDMPAHTGSWAEAYPEIVTCANMFWWPAGS 285
GU356593_Capsicum ADVEKIVEFEGMEHGVRVLEIDMPAHTGSWAEAYPEIITCANMFWWPAGN 284
Os03g0219400_Oryza NDVRHIVSFAASFGRVVIPEIDMPGHTGSWAGAYPEIVTCANRFWAPHAE 310
Os07g0575500_Oryza DDVKLIVDFAMNRGVRVPEIDTPGHTASWAGAYPELVSCAGEFWLPDAS 305
At3g55260_Arabidopsis EDASEIVRFAMKRGINVMAEVDVPGHAESWGTGYPDLWPSL----- 197
CAO41588_Vitis     EDAYDIVNFAKMRGINVMAEIDIPGHAESWGTGYPDLWPS----- 293
Os05g0115900_Oryza EDARDIVSYARKRGIHVMAEIDVPGHAESWGKGYPKLWPS----- 293
Os01g0891000_Oryza SDAVDIVRYAENRGVNVMAEIDVPGHALSWGVGYP SLWPS----- 281
Os05g0415700_Oryza DDAIDIVQYAERGVNVLAEIDVPGHALSWGVGYP SLWPSA----- 284
Atlg65590_Arabidopsis EDAAEIVNYARRRGIVLAEIDVPGHALSWGKGYPALWPSK----- 290
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CAO67165_Vitis      EWADRLASEPGTGHLNPLNPKTYQVFNVIHDVAALFPEPFYHSGADEII 335
ABE82127_Medicago  DWPDRLAAEPGTGHLNPLNPKTYQVLKNVIRDVTLFPEQFYHSGADEVV 328
Atlg05590_Arabidopsis SWEERLASEPGTGQLNPLSPKTYEVVKNVIQDIVNQFPESFFHGGGDEVI 339
EU244854_Solanum   --SPALAAEPGTGQLNPSIPKTYEVVKNVIQGTIAMFPDSLFGGAD E IN 333
GU356593_Capsicum --SPALAAEPGTGQLNPLIPKTYEVVKNVIHDTIAMFPDSLFGGAD E IN 332
Os03g0219400_Oryza P---ALAAEPGTGQLNPLNPKTYRVAQDVL RDMVALFPDPYLHGGAD E VN 357
Os07g0575500_Oryza DWPSRLAAEPGAGQLNPLEPKTYQVMSNVINDVTS LFPDGFYHAGAD E VT 355
At3g55260_Arabidopsis -----SCREPLDVTKNFTFDVIGSILADMRKIFPFELFHLGGDEVN 238
CAO41588_Vitis     -----SCREPLDVSKFTFDVMSGILDMRKIFPFELFHLGGDEVN 334
Os05g0115900_Oryza -----KCREPLDVTSNFTFEVIGSILSDMRKIFPFGLFHLGGDEVY 334
Os01g0891000_Oryza -----SCKEPLDVSNFTFGVIDGILSDFSKVFKFKFVHLGGDEVN 322
Os05g0415700_Oryza -----TCKEPLDVSSSESTFQVINGILSDFSKVFKFKFVHLGGDEVN 325
Atlg65590_Arabidopsis -----NCQEPDLVSSDFTFKVIDGILSDFSKIFKFKFVHLGGDEVN 331
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-----Active site-----  
CAO67165\_Vitis PGCWKADPTIQTFLSNGGTLSQLLEIFINSTFPYIVS-LNRTVVYEDVL 384  
ABE82127\_Medicago PGCWKTDPTIQKFLSNGGTLSQLLETFINNTLPFILS-LNRTVVYEDVL 377  
At1g05590\_Arabidopsis PGCWKTDPAINSFLSSGGTLSQLEKYINSTLPYIVS-QNRTVVYEDVL 388  
EU244854\_Solanum SDCWNTDLSVQKFVASNGTLSQLEKFINNTLPEILS-LNRTVVYEDVI 382  
GU356593\_capsicum SACWNTDPSIQTFVASNGTQSQLEEMFINNTLPEILS-LNRTVVYEDVI 381  
Os03g0219400\_Oryza TACWEDDPVVRRLAEGGTHDHLLELFINATRPFVAQELNRTVVYEDVL 407  
Os07g0575500\_Oryza PGCWNADPSIQRYLARGGTLSRLLEKFFVGAHPLIVS-RNRTAVYEDVL 404  
At3g55260\_Arabidopsis TDCWKNTHVKEWLQGRNFTTKDAYKYFVLRAQQAISKNWTPVNEETF 288  
CAO41588\_Vitis TDCWNSTPHVQQWLQDHNMTPEAYQYFVLRAQEAISKNWAPVNEETF 384  
Os05g0115900\_Oryza TGCWNATPHVKQWLHERNMTTKDAYKYFVLKAQEAIALNWIPVNEETF 384  
Os01g0891000\_Oryza TSCWTATPHIKKWLDDNQMNVS DAYRYFVLRSQKLAISHGYEVINEETF 372  
Os05g0415700\_Oryza TSCWTSTPRVKAWLAHQMKESDAYRYFVLRAQKIAKSHGYEVINEETF 375  
At1g65590\_Arabidopsis TTCWSATPRIAQWLKHKRMSEKEYQYFVLRAQKIALSHGYEIIINEETF 381  
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CAO67165\_Vitis LDANVKVDPSMLPPEENTILQTNNGPNNTKVVASGYRAIVSSDFYLLD 434  
ABE82127\_Medicago LDDTVHVPSTILPKEHVILQTNNGHNNTKRIVSSGYRAIVSSDFYLLD 427  
At1g05590\_Arabidopsis LDAQIKADPSVLPEKHTILQTNNGPENTKRIVAAGYRVIVSSDFYLLD 438  
EU244854\_Solanum LSGNVKVNPSLLPQNVMQTNNGPNNTKQLVTSGYRVIVSSADYLLD 432  
GU356593\_capsicum L SANVKVDPSLLSPQHVMQTNNGPSNTKQLVTSGYRVIVSSADYLLD 431  
Os03g0219400\_Oryza LGPKVTVGP TILPRETTILQTNNDGPENTKRVAAGYRAIVSSASYLLD 457  
Os07g0575500\_Oryza LDQAVNVTASAIPPETTILQTNNGGNTRLIVRAGYRAIVSSAFYLLD 454  
At3g55260\_Arabidopsis SSFGKDLDP-----RTVIQNHLVS-DICQKAVAKGFRCIFSNQGYWLLD 331  
CAO41588\_Vitis NTFATNLNP-----RTVIHNLGP-GVCPKAVAKGFRCIYSNQGVWLLD 427  
Os05g0115900\_Oryza NSFKENLNP-----LTVVHNLGP-GVCPKVVEKGFRCIMSNQGVWLLD 427  
Os01g0891000\_Oryza NNFQDKLDR-----RTVVHNLGE-DVAPKVVAAGLRCIVSNQDKWLLD 415  
Os05g0415700\_Oryza NNFQDKLDR-----RTVVHNLGG-GVAEKVVAAGLRCIVSNQDKWLLD 418  
At1g65590\_Arabidopsis INFQSKLNR-----KTVVHNLNT-GLVENVTASGLRCIVSNQEFWLLD 424  
. : : : \* \* \* \* \* : \*\*\*

CAO67165\_Vitis CGHGDFLGN-----DKN-----GGSWCGPFKTWQTIYNYD 464  
ABE82127\_Medicago CGHGDFTGNNSIYDNTGSDKND-----GGSWCGPFKTWQNIYNYD 468  
At1g05590\_Arabidopsis CGHGGFLGNDSIYDQ---KESG-----GGSWCAPFKTWQSIYNYD 475  
EU244854\_Solanum CGHGSFVGNDSRYDQPPGTDQGN-----GGSWCGPFKTWETIYNYD 473  
GU356593\_capsicum CGHGSFVGNDSRYDQPPGTDQGN-----GGSWCGPFKTWETIYNYD 472  
Os03g0219400\_Oryza CGHGWVGNDSRYDQEKEREPTPLFNDPGGTGGSWCAPFKTWQRYDYD 507  
Os07g0575500\_Oryza CGHGDFAGNDSAYDDPRSDYGTS-----GGSWCGPYKTWQRYDYD 495  
At3g55260\_Arabidopsis HLD-----VPWEEVYNT 344  
CAO41588\_Vitis HLD-----VPWDGFYNAE 440  
Os05g0115900\_Oryza HLD-----VPWQDFYTSE 440  
Os01g0891000\_Oryza HLD-----ATWEGFYTNE 428  
Os05g0415700\_Oryza HLE-----VTWDGFYMNE 431  
At1g65590\_Arabidopsis HID-----APWQGFYANE 437  
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CAO67165\_Vitis ITYGLS-DEEAKLVLGGEVALSQAADPTVLDARIWPRASAMAEALWSGN 513  
ABE82127\_Medicago ITYGLT-EEEAQLVLGGEVALSQAADPTVLDARSRLWPRTSAMAEALWSGN 517  
At1g05590\_Arabidopsis IADGLLNEEERKLVLGGEVALSQAADSTVLDSRLWPRASALAEALWSGN 525  
EU244854\_Solanum ITYGLT-DEEAPLVIGGEVALSQAADSTVMDSRIWPRASAMAEALWSGN 522  
GU356593\_Capsicum ITYGLT-DKEAQLVIGGEVALSQAADSTVMDSRIWPRASAMAEALWSGN 521  
Os03g0219400\_Oryza ILHGLT-DDEAQLVLGGEVALSQAQSDPTVLDARLWPRAAAAEALWSGN 556  
Os07g0575500\_Oryza VAGGLT-AEEARLVVGGEVAMWTTQVDAAVLDGRVWPRASAMAEALWSGN 544  
At3g55260\_Arabidopsis PLNGIEDPSLQKLVIGGEVCMWGTADTTSVVLQTWPRAAAAAERMWSTR 394  
CAO41588\_Vitis PLEGINASAEQELVLGGEVCMWGTADTSNVLQTWPRAAAAAERLWSKR 490  
Os05g0115900\_Oryza PLAGINNTAQQKLVIGGEVCMWGTADTSDVQQTWPRAAAAAERMWSQL 490  
Os01g0891000\_Oryza PLKGIIDPEQQSLVIGGEVCMWGTADTSDVQQTWPRAAAAAERLWTP 478  
Os05g0415700\_Oryza PLRNIKNPAQQKLVIGGEVCMWGTADTSDVQQTWPRAAAAAERLWTP 481  
At1g65590\_Arabidopsis PFQNTIDKKQQLVLGGEVCMWGTADTSDVQQTWPRAAAAAERLWTPY 487  
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-----Active site-----
CAO67165_Vitis          QDKTGMKRYADAMDRLNEWRYRMVARGIGAEPIQPLWCIRNPGMCNTVHP 563
ABE82127_Medicago      RDEKGLKRYAEATDRLNEWRSRMVSRGIGAEPIQPLWCVRNPGMCNTVHA 567
At1g05590_Arabidopsis  RDEKGVKRCGEAVDRLNLWRYRMVSRGIGAEPIQPLWCLKNPGMCNTVHG 575
EU244854_Solanum       RDETGMKRYAEATDRLNEWRYRMVSRGIGAESIQPLWCLKNPGMCNTVHS 572
GU356593_Capsicum     CDETGMRKRYAEATDRLTEWRYRMVARGIGAEPIQPLWCVKNSGMCNTVHS 571
Os03g0219400_Oryza     KGSNGKKRYANATDRLNDWRHRMVERGIRAEPIQPLWCSLHPGMCNLSQ- 605
Os07g0575500_Oryza     RDATGRKRYAEATDRLTDWRHRMVG RGVRAEPIQPLWCRNRP GMCNLVR- 593
At3g55260_Arabidopsis  EAVSKGNITLTALPRLHYFRCLLN RGVPAAPVDNFYARRPPLGPGSCYA 444
CAO41588_Vitis         EATSGKNITLTALPRLHYRCLLTRRGVEADPVTNKYARQPPNGPGSCYE 540
Os05g0115900_Oryza     EAISAQDLETTTLARLHYFRCLLNHRGIAAAPVTNSYARRPPIGPGSCFI 540
Os01g0891000_Oryza     EKIAED--PRLVTSRLARFRCLLN RGVAAAPVAG-YGRTAPYEPGPCVR 525
Os05g0415700_Oryza     EKLSKEWEIAALSARLARFRCLLNHRGIAAGPVTG-YGRSAPAEPSICK 530
At1g65590_Arabidopsis  AKLAKN--PNNVTTRLAHRFRCLLN RGVAAAPLVG-GRRVVPFEPGSCLA 534
                        * * : * : * * : * . :

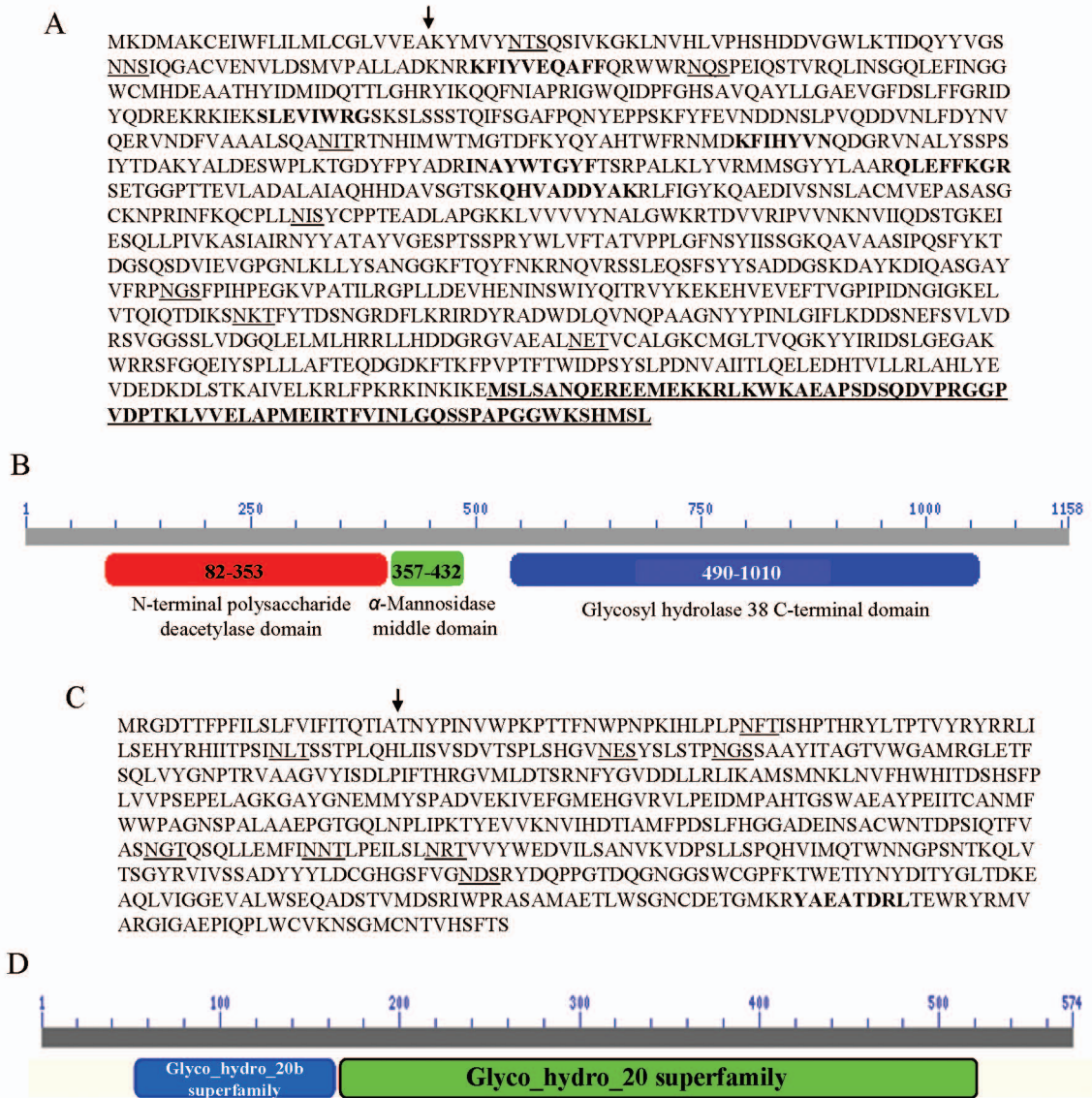
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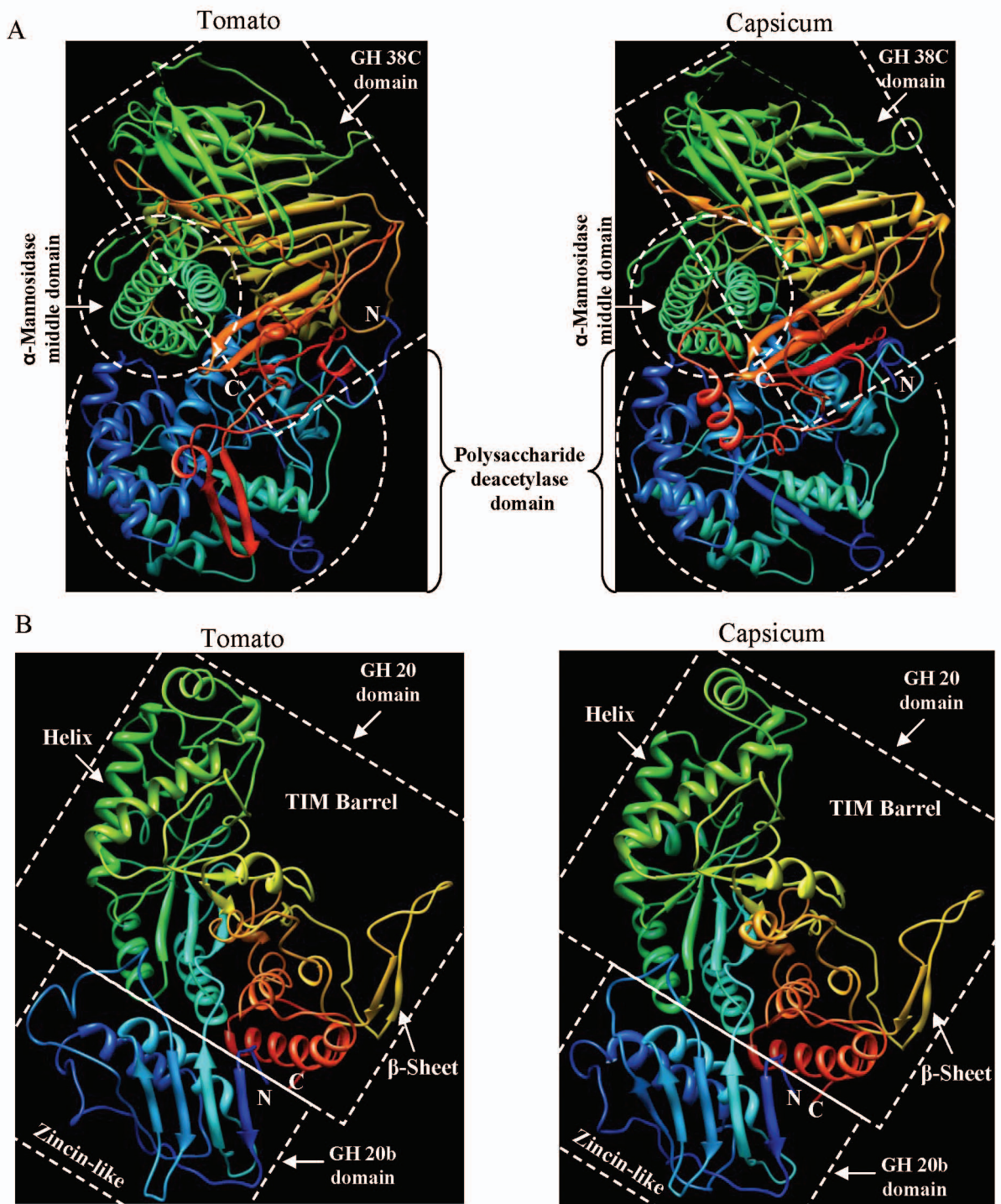
CAO67165_Vitis          FV--- 565
ABE82127_Medicago      I---- 568
At1g05590_Arabidopsis  ALQDQ 580
EU244854_Solanum       FTS-- 575
GU356593_Capsicum     FTS-- 574
Os03g0219400_Oryza     -----
Os07g0575500_Oryza     -----
At3g55260_Arabidopsis  Q---- 445
CAO41588_Vitis         Q---- 541
Os05g0115900_Oryza     Q---- 541
Os01g0891000_Oryza     Q---- 526
Os05g0415700_Oryza     Q---- 531
At1g65590_Arabidopsis  Q---- 535

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**Supplementary Fig S6.** Multiple sequence alignments of  $\beta$ -hex proteins from different plant species. The sequence alignment was performed using ClustalW program. Identical amino acid residues are depicted as \*, while conserved and semiconserved substitutions are marked as : and ., respectively. Dashed lines represent gaps inserted for optimal alignment of the sequences. Eleven residues which compose the conserved feature of the active site of the enzyme are highlighted.

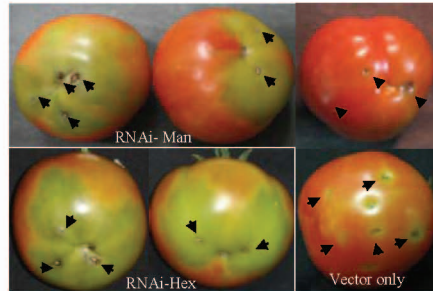


**Supplementary Fig. S7.** The protein sequences of capsicum  $\alpha$ -man and  $\beta$ -hex, deduced from the cloned cDNA sequences. (A)  $\alpha$ -man protein sequence. Peptide sequences obtained by LC-MS/MS analysis are marked in bold letters. Sequences identical to the *McGRF* 15 (geraniol responsible factor 15, AB039375) are marked in bold letters and underlined. Arrow represents the predicted signal peptide cleavage site between 23-24 positions (SignalP). Underlined amino acids are predicted *N*-glycosylation sites. (B) The domain architecture of  $\alpha$ -man protein. Domains were identified in proteins using the conserved domain architecture retrieval tool (CDART, www.ncbi.nlm.nih.gov). (C) The  $\beta$ -hex protein sequence. Peptide sequence identified by the LC-MS/MS analysis is marked in bold letters. Underlined amino acids show the predicted *N*-glycosylation sites and the arrow indicates the site for signal peptide cleavage between amino acid 23-24, as determined by the SignalP program. (D) The domain architecture of  $\beta$ -hex protein.

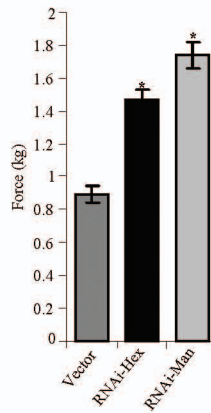


**Supplementary Fig. S8.** Comparative homology modeling of tomato and capsicum  $\alpha$ -man and  $\beta$ -hex using 3D JIGSAW server and Chimera viewer. Different domains are highlighted. N and C indicate N- and C-terminal ends of the protein, respectively. (A)  $\alpha$ -Man proteins. The template used for building these structures was 1HTY (PDB entry) from *Drosophila*. (B)  $\beta$ -Hex proteins. The template used for building these structures was 1now\_B (PDB entry), human  $\beta$ -hexosaminidase  $\beta$ -subunit.

A



B



**Supplementary Fig. S9.** *Agrobacterium*-mediated transient silencing of  $\alpha$ -Man and  $\beta$ -Hex in tomato. (A) Fruits agroinjected with RNAi vector targeting either  $\alpha$ -Man (RNAi-Man) or  $\beta$ -Hex (RNAi-Hex). Blank binary vector (vector only) was used as control. The black arrows show the injection mark on the fruit. (B) Texture of RNAi agroinjected fruits 15 days after agroinjected. Data are mean  $\pm$  s.e.m.,  $P < 0.005$ .  $n = 43$  for RNAi-Man,  $n = 30$  for RNAi-Hex and  $n = 27$  for vector.

**Supplementary Table S1.** Summary of capsicum  $\alpha$ -man and  $\beta$ -hex protein purification

	<b>Purification steps</b>	<b>Total activity (EU)</b>	<b>Specific activity (EU/mg)</b>	<b>Yield (%)</b>	<b>Purification fold</b>
$\alpha$ -Man	<b>Crude extract</b>	77.67	0.21	100	1
	<b>Ammonium sulphate precipitation (40-60%) and dialysis</b>	66.52	0.25	85.64	1.19
	<b>Ion exchange on Q-sepharose, Elution NaCl gradient</b>	21.72	0.96	27.96	4.57
	<b>Gel filtration on Sephadex G-100</b>	5.23	7.69	6.73	36.62
$\beta$ -Hex	<b>Crude extract</b>	146.91	0.57	100	1
	<b>Ammonium sulphate precipitation (40-60%) and dialysis</b>	133.13	0.64	90.62	1.12
	<b>Ion exchange on DEAE-Sepharose, Elution-NaCl gradient</b>	104.89	3.45	71.39	6.05
	<b>Affinity on ConA-Sepharose, Elution- <math>\alpha</math>-D-Methylmannopyranoside</b>	72.75	8.33	49.52	14.61
	<b>Gel filtration on Sephadex G-100</b>	31	100	21.10	175.44

Values are averages of three independent experiments.

One unit (EU) is equivalent to 1  $\mu$ mol *p*NP released/ min.

Specific activity is expressed as  $\mu$ mol *p*NP released/min/mg protein.