

# SUPPLEMENTAL MATERIAL

## Figures

### Higher expression of the strawberry xyloglucan endotransglucosylase/hydrolase genes *FvXTH9* and *FvXTH6* accelerates fruit ripening

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Figure S1. Expression levels of putative XTHs in *Fragaria vesca* varieties.

Figure S2. Phylogenetic tree of XTH candidate genes from *F. vesca*.

Figure S3. qPCR analysis of *FvXTH9* and *FvXTH6*.

Figure S4. Amino acid sequence alignment of group I/II XTHs.

Figure S5. Purification of *FvXTH9*-His and *FvXTH6*-His.

Figure S6. pH optimum of *FvXTH9* and *FvXTH6*.

Figure S7. Metabolite analysis of *F. × ananassa* fruit after agroinfiltration.

Figure S8. Amino acid sequence alignment.

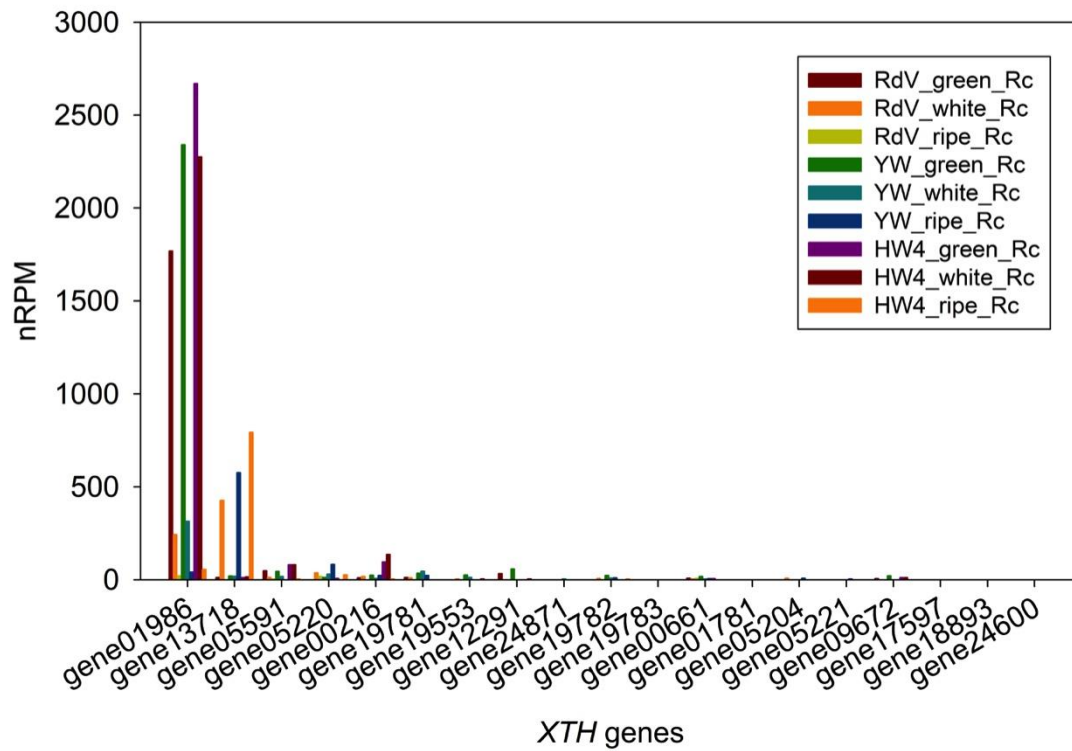


Figure S1. Expression levels of putative XTHs in *Fragaria vesca* varieties Reine de Vallées, Hawaii 4 and Yellow Wonder.

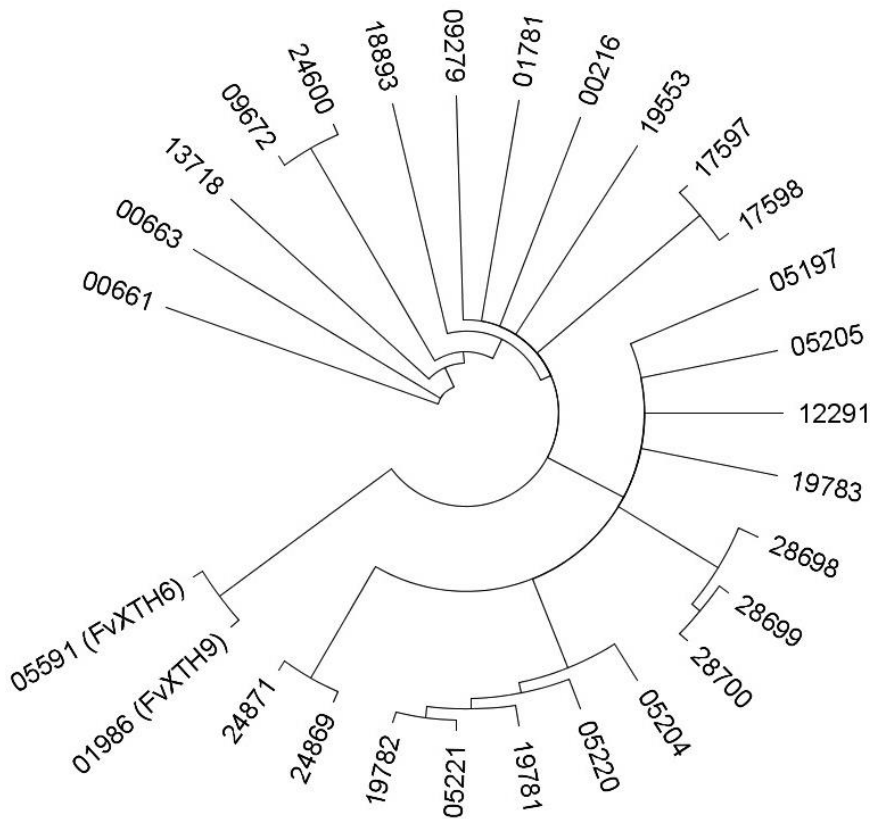
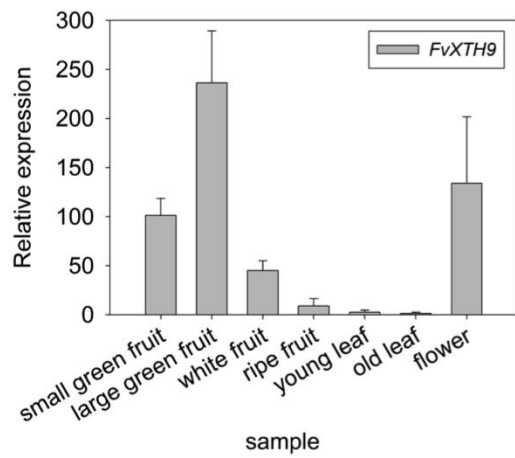


Figure S2. Phylogenetic tree of *XTH* candidate genes from *F. vesca*. The tree was constructed using Genious v5.6 software with the Neighbor-Joining method with 5000 bootstrap replications. Amino acid sequences were retrieved from the *F. vesca* Genome Browser database and their accession numbers are as follows: gene00216, gene00661, gene00663, gene01781, gene01986, gene05197, gene05204, gene05205, gene05220, gene05221, gene05591, gene09279, gene09672, gene12291, gene13718, gene17597, gene17598, gene18893, gene19553, gene19781, gene19782, gene19783, gene24600, gene24869, gene24871, gene28698, gene28699, and gene28700.

a.



b.

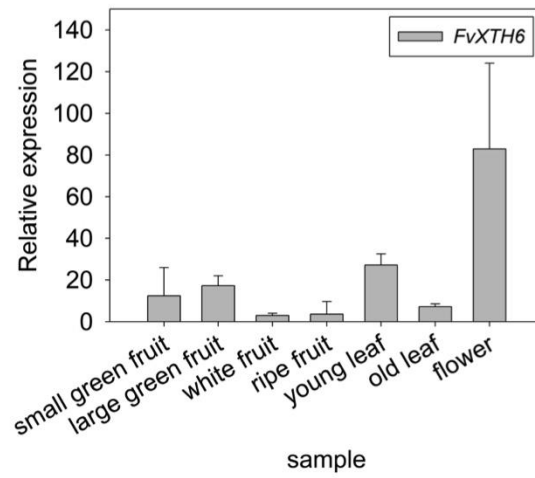


Figure S3. qPCR analysis of *FvXTH9* (a) and *FvXTH6* (b) at different fruit maturation stages (small green, large green, white and ripe fruit) as well as other tissues (young leaf, old leaf and flower) of *F. vesca*. Hawaii 4. Relative expression levels are normalized to the reference gene (interspacer gene).

Section 1

	(1)	1	10	20	30	40	55
At-XTH10	(1)	---	MTLINRSKPFVLLVGF	SIISLLLVWSQASV	VSSGDF	NKDF	FVTVSPTHVN
At-XTH6	(1)	----	MAKIYSP-SFP	GTLCCLCIPTLL	TLMFIRVSAR	PATF	VEDFKAAWSESHIR
At-XTH7	(1)	----	MVVSLSFSSRN	VFYTL	SLCLFAAL---	YQPVMSRPAK	FEDDFRIAWS
FvXTH6	(1)	----	MYP	SLRSG-SVIASISL	CFLSLFS---	LSAFARPATF	LQDFQVTVSDSHIR
Sl-XTH7	(1)	MATL	TCCSLKNS-AFV	LILVYAL	TFSFS---	LVS-ARPATF	LQDFKIAWSDSHIK
At-XTH9	(1)	-----	MVGMDL	FKCVM	MIMV---	LVVSCGEAVS	GAKFDELYRSSWAMDHCV
FvXTH9	(1)	-----	MASASL	FLSVIL	GLS---	LFLG---	PVSSAKFDELFPYWASDHFT
At-XTH8	(1)	--	METERRIIT	SCS	SAMTALFL	FMTALMASS	SIATPTQS-FEDNFNIMWSENHFT
Sl-XTH1	(1)	-----	MGI	IKGVL	F	SIVL	INLSLVVFCGYPRRPVDVFPWKNYEP
Sl-XTH4	(1)	-----	MKG	VLVAF	V	LINLSILAS	CGAPRKVIDVFPWNNYEP
At-XTH4	(1)	-----	MTVSS	SPWAL	MALFL	MVSST	MVMAIPPRKAI
At-XTH5	(1)	-----	MG-RLS	STLCL	TFLIL	LAT--	VAFGVPPKKSINVPGRNYFP
Fc-XTH1	(1)	-----	MASS	Q	CTLLLS	SIM	L
Md-XTH1	(1)	-----	MAS	CKQ	WTV	F	LSLLCLV--ATVAAPP
PttXET16A	(1)	-----	MAA	AY	P	W	T
Consensus	(1)		S	V	L	L	L

Section 2

	(56)	56	70	80	90	100	110
At-XTH10	(52)	TSND	G	R	S	R	T
At-XTH6	(50)	QMED	G	K	A	I	Q
At-XTH7	(49)	QIDG	G	R	A	I	Q
FvXTH6	(48)	QIDG	G	R	A	I	Q
Sl-XTH7	(51)	QLDG	G	R	G	I	Q
At-XTH9	(44)	N--	E	G	E	V	T
FvXTH9	(41)	Y--	E	G	E	L	L
At-XTH8	(53)	TSD	D	G	E	I	W
Sl-XTH1	(48)	FLNG	G	T	T	T	D
Sl-XTH4	(45)	YLNG	G	T	A	E	L
At-XTH4	(50)	QFNG	G	S	E	L	Q
At-XTH5	(47)	YLNG	G	S	E	V	H
Fc-XTH1	(48)	YFNG	G	N	E	I	Q
Md-XTH1	(48)	YFNG	G	K	E	I	Q
PttXET16A	(48)	YFNG	G	N	E	I	Q
Consensus	(56)	NGG	I	Q	L	LD	SG

Section 3

	(111)	111	120	130	140	150	165
At-XTH10	(107)	---	PNR	DE	I	D	F
At-XTH6	(105)	--	ATV	R	E	L	D
At-XTH7	(104)	--	DSV	R	E	L	D
FvXTH6	(103)	--	DAV	R	E	L	D
Sl-XTH7	(106)	--	DNV	R	E	L	D
At-XTH9	(97)	---	PNH	N	E	F	D
FvXTH9	(94)	---	PLH	N	E	F	D
At-XTH8	(108)	GAG	P	E	R	D	E
Sl-XTH1	(103)	---	A	E	H	D	E
Sl-XTH4	(100)	---	A	E	H	D	E
At-XTH4	(105)	---	N	E	H	D	E
At-XTH5	(102)	---	S	E	H	D	E
Fc-XTH1	(103)	---	A	E	H	D	E
Md-XTH1	(103)	---	N	E	H	D	E
PttXET16A	(103)	---	S	E	H	D	E
Consensus	(111)		E	H	D	E	I

Section 4										
	166	180	190	200	210	220				
At-XTH10 (159)	LWNIHQIVFVMDQIP	IRLYRNHGEK	GVAA	YPRLQPM	SVQASL	WNGES	WATR	GGH		
At-XTH6 (158)	LWSHKHIVFYVDDVPI	REYKNN	EAKNIA	YPTSQPM	GVYSTL	WEADD	WATR	GGGL		
At-XTH7 (157)	SWNHLRIVFYVDNVPI	RVYKNN	EARKVP	YPRFQPM	GVYSTL	WEADD	WATR	GGGI		
FvXTH6 (156)	LWNHHHIVFYVDDVPI	RLYKNN	EAKGIP	YPKLQPM	GVFSTL	WEADD	WATR	GGGL		
SI-XTH7 (159)	FWNHHQAVFVSDGIPI	RVYKNN	EAKGIP	FPKFQPM	GVYSTL	WEADD	WATR	GGGL		
At-XTH9 (149)	LWSKRSVVFVMDVETPI	RVQKN	LEEKGIP	FAKDQAM	GVYSSI	WNADD	WATR	QGGL		
FvXTH9 (146)	FWNQRQVVFLVDETP	RVHTN	MESKGLP	FPKDQAM	GVYSSI	WNADD	WATR	QGGR		
At-XTH8 (163)	LWNNHQLVFFVDRVPI	RVYKNS	DKVPN	NDFPNQK	MYLFSS	IW	NADD	WATR	GGGL	
SI-XTH1 (155)	LWNTYLIVIFVDDVPI	RAFKN	SKDLGVK	FPFNQPM	KIYSSL	WDADD	WATR	GGGL		
SI-XTH4 (152)	LWNTYQIAIFVDDVPI	RVFKNS	KDIGVK	FPFNQPM	KIYSSL	LW	NADD	WATR	GGGL	
At-XTH4 (157)	LWNMYQIVFFVDNIP	RTFKN	AKDLGVR	FPFNQPM	KLYSSL	LW	NADD	WATR	GGGL	
At-XTH5 (154)	LWNMYQIVFFVDDVPI	RVFKNS	KDVGVK	FPFNQPM	KIYSSL	LW	NADD	WATR	GGGL	
Fc-XTH1 (155)	LWNLYQIVFFVDDIPI	RVFKNS	KDLGVK	FPFNQPM	KLYSSL	LW	NADD	WATR	GGGL	
Md-XTH1 (155)	LWNLYQIVFLVDDIPI	RVFKNS	KDLGVK	FPFNQPM	KLYSSL	LW	NADD	WATR	GGGL	
PttXET16A (155)	LWNMYMIVFLVDDVPI	RVFKN	CKDLGVK	FPFNQPM	KIYSSL	LW	NADD	WATR	GGGL	
Consensus (166)	LWN YQIVFFVDDVPI	RVFKN	DLGV	FP NQPM	VYSSLW	NADD	WATR	GGGL		

Section 5										
	221	230	240	250	260	275				
At-XTH10 (212)	DKIDWSKGPFFVAS	FGDYKIDACI	WIGNTSFCN	-----	GESTEN	WWW	KN	EFSS		
At-XTH6 (211)	EKIDWSKAPFFYAY	KDFDIEGCP	VPGPTF	-----	CPSNPH	NW	WE	YAYQS		
At-XTH7 (210)	EKINWSRAPFFYAY	KDFDIEGCP	VPGPAD	-----	CPANSK	NW	WE	SAYHQ		
FvXTH6 (209)	EKINWSKAPFFYAY	KDFDIEGCS	VGPAN	-----	CASSAQ	NW	WE	TAYQA		
SI-XTH7 (212)	EKINWSKSPFFAY	KDFDIEGC	AMPAN	-----	CASNPS	NW	WE	PAYQQ		
At-XTH9 (202)	VKTDWSHAPFFVAS	YKEFQIDACE	IPTTDD	-----	LSKCN	GDQK	FW	DEPTVSE		
FvXTH9 (199)	VKTDWSHGPFVAS	YKGFIDINAC	CECPAS	VAGAENAKK	CSSS	NGDK	KY	WWD	DEPVLSE	
At-XTH8 (218)	EKTDWKKAPFFVSS	YKDFAVEGCR	WKDP	PACV	-----	STTTEN	WW	DQYDAWH		
SI-XTH1 (208)	EKTNWANAPFTAS	YTSFHDVGC	EAA	TPQEVQV	-----	CNTKGM	KW	WDQKAFQD		
SI-XTH4 (205)	EKTNWSGAPFIAS	YTSFHIDGCE	AVTP	QEVQV	-----	CNTNGM	KW	WDQKAFQD		
At-XTH4 (210)	EKTNWANAPFFVAS	YKGFHDGCE	AS	VEAKY	-----	CATQGR	MW	DQKEFRD		
At-XTH5 (207)	EKTNWEKAPFFVAS	YRGFHDGCE	AS	VNAKF	-----	CETQGR	WW	DQKEFQD		
Fc-XTH1 (208)	EKTDWSKAPFFVAT	YRGFHDGCE	AS	VQARF	-----	CATQGR	WW	DQKEFQD		
Md-XTH1 (208)	EKTDWSKAPFIAS	YRGFHDGCE	AS	VEAKY	-----	CATQGR	WW	DQKEFQD		
PttXET16A (208)	EKTDWSKAPFIAS	YRSFHIDGCE	AS	VEAKF	-----	CATQGR	WW	DQKEFQD		
Consensus (221)	EKTDWSKAPFFVAS	YK F IDGCE				C TQG	WWDQ	FQD		

Section 6										
	276	290	300	317						
At-XTH10 (259)	LTRVQKRWFKWR	RYHLIYDYCQ	DYGRFNNKLP	KKECSLPKY						
At-XTH6 (256)	LNAVEARRYRWR	RVNHMVYDYCT	DRSRFP	VPPPECRA						
At-XTH7 (255)	LSPVEARSYRWR	RVNHMVYDYCT	DKSRFP	VPPPEC	SAGI					
FvXTH6 (254)	LNALEYRRYKWR	VMNHMIYDYCS	DRSRYP	KPPPEC	VAGL					
SI-XTH7 (257)	LSPVQARQYRWR	VMNHMIYDYCT	DKSRNP	VPPPEC	RAGI					
At-XTH9 (250)	LSLHQNHQLIWR	ANHMIYDYCF	DATRFP	VTPL	ECQHRRHL					
FvXTH9 (254)	LNVHQNHQLVWV	KNHMHVYDYCT	SARFP	VT	PVECVHRRH					
At-XTH8 (265)	LSKTQKMDYAWV	QRNLVVYDYCK	DSESRFP	TL	PWEC	SISPWA				
SI-XTH1 (256)	LDALQYRRLRWR	RQKYTVYNYCT	DKARYP	VPPPEC	TKDRDI					
SI-XTH4 (253)	LDGPEYRKLHR	VRQNFXYNYCT	DRKRYP	TL	PLECTR	DRDL				
At-XTH4 (256)	LDAEQWRRRLKWR	VMKWTIYNYCT	DRTRFP	VM	PAECKRDRDA					
At-XTH5 (253)	LDANQYKRLKWR	VRKRYTYNYCT	DRVRFP	VPPPEC	RDRDI					
Fc-XTH1 (254)	LDAYQWRRRLRWR	RQFTIYNYCT	DRTRYP	TL	PAEVQRDRDI					
Md-XTH1 (254)	LDAQQWRRRLRWR	RKFTIYNYCT	DRVRYP	SMP	PECKRDRDI					
PttXET16A (254)	LDAFYQYRRLSWR	RQKYTYNYCT	DRSRYP	SMP	PECKRDRDI					
Consensus (276)	L A QYRRLRWR	HMIYDYCTDRSRFP	V PPEC	R						

Figure S4. Amino acid sequence alignment of group I/II XTHs using AlignX Vector NTI Advance V.11.5. Sequences correspond to the following GenBank accession numbers: *A. thaliana* (At-XTH4 (NP 178708), At-XTH5 (NP 196891), At-XTH6 (NP 569019), At-XTH7 (NP 195494), At-XTH8 (NP 563892), At-XTH9 (NP 192230), At-XTH10 (NP 179069), *S. lycopersicum* (SI-XTH1, BAA03923), SI-XTH4 (AAG43444), SI-XTH7 (AAS46243), *M. x domestica* (Md-XTH1, AAN07897), *F. chiloensis* (Fc-XTH1, ADE42488) and *Populus tremula x Populus tremuloides* (PttXET16A, AAN87142). Identical amino acids in the sequences are shaded in black. The red box indicates the conserved motif and asterisks (\*\*\*) mark the N-linked glycosylation motif.

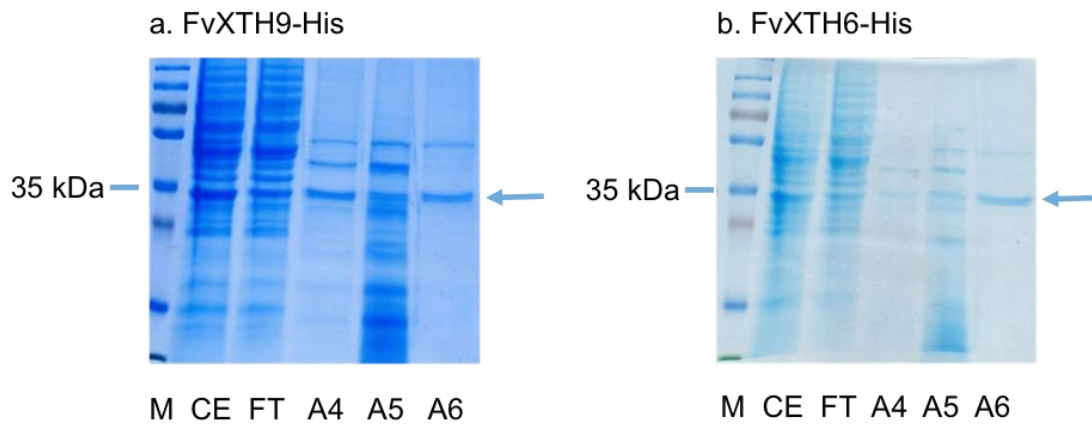


Figure S5. Purification of FvXTH9-His and FvXTH6-His. M, protein marker; CE, crude extract; FT, flow through; A4-, A5-, and A6-fractions were eluted with 250 mM imidazole. Target protein (33 kDa) is shown by arrow.

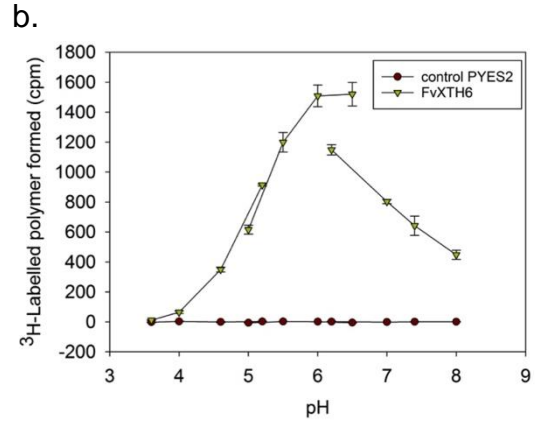
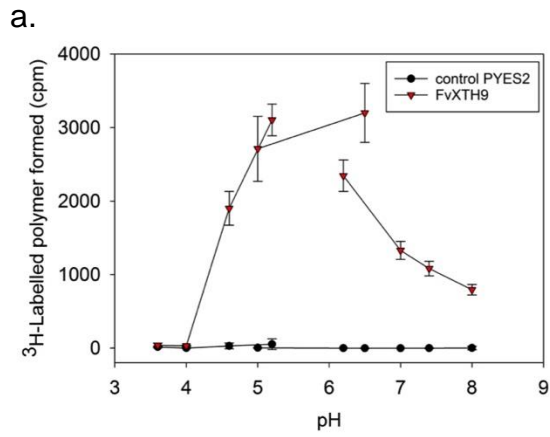
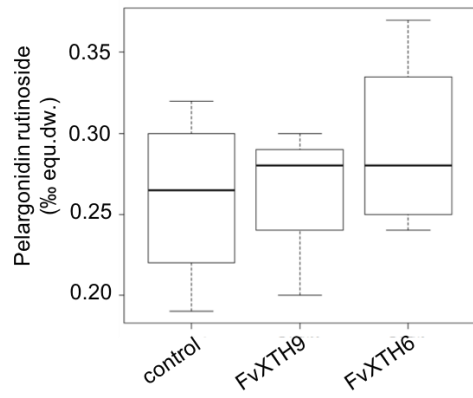


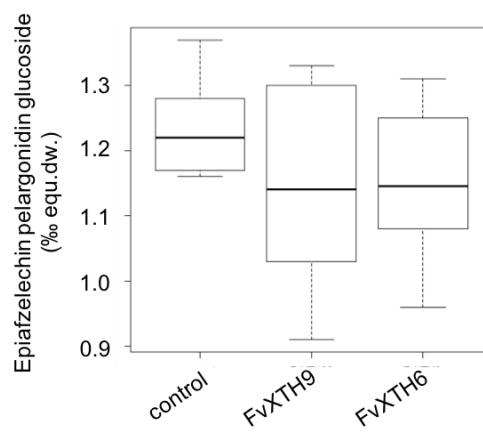
Figure S6. pH optimum of FvXTH9 (a) and FvXTH6 (b). No XET activity was present in control PYES2. Different buffers were used as follows: sodium acetate buffer (pH 3.6, pH 4.0, pH 4.6, and pH 5.2), sodium succinate buffer (pH 5.0, pH 5.5, pH 6.0, and pH 6.5) and sodium phosphate buffer (pH 6.2, pH 7.0, pH 7.4, and pH 8.0). Each assay was performed as 4 replicates; error bars are standard deviation



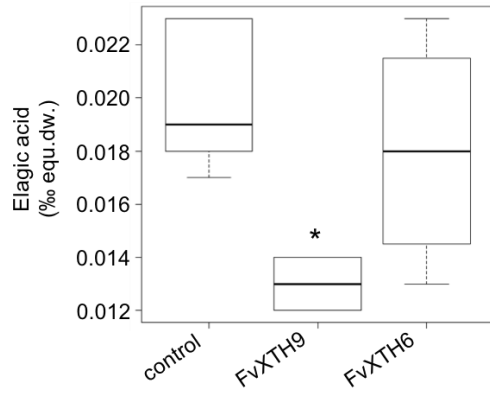
**a.**



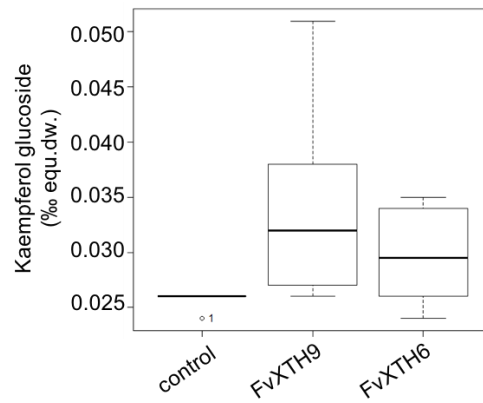
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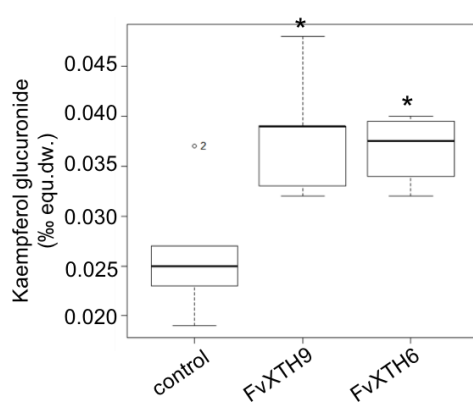
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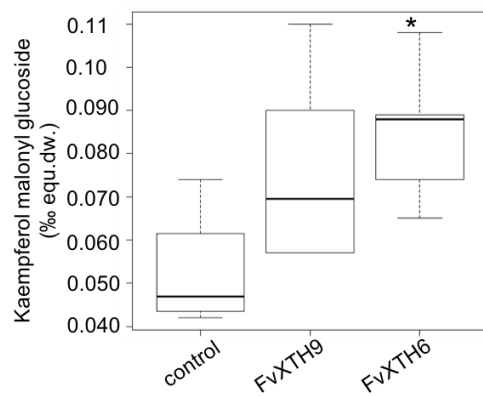
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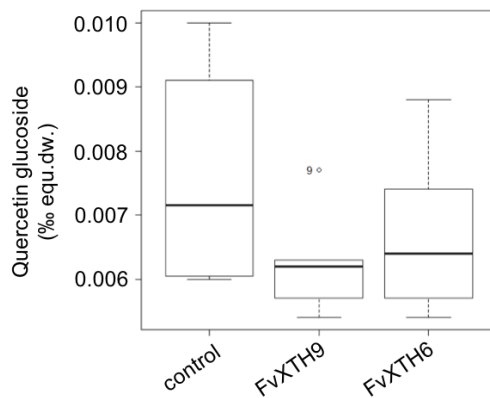
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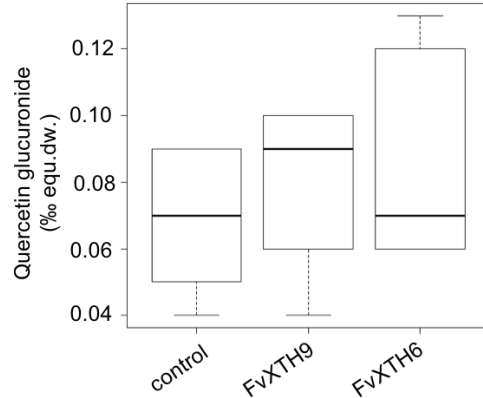
**f.**



**g.**

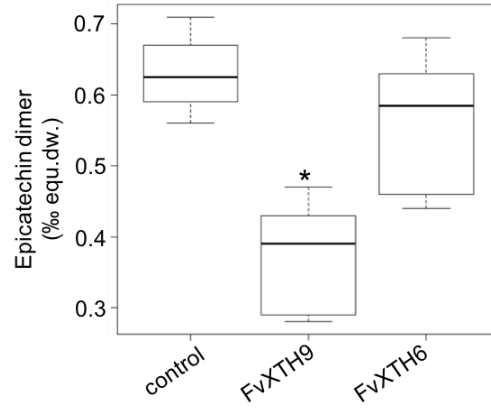
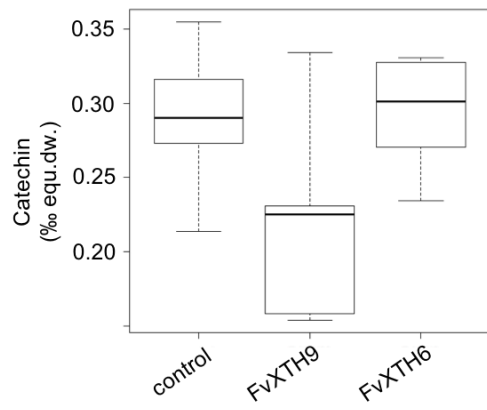


**h.**

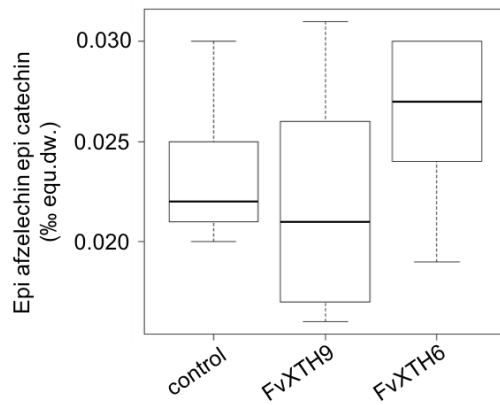


**i.**

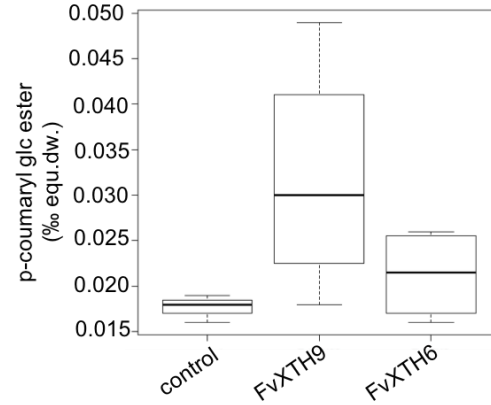
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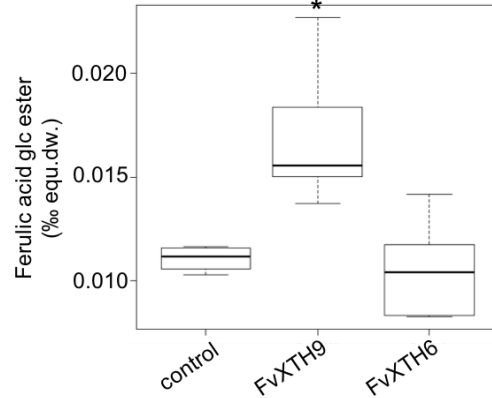
**k.**



**l.**



**m.**



**n.**

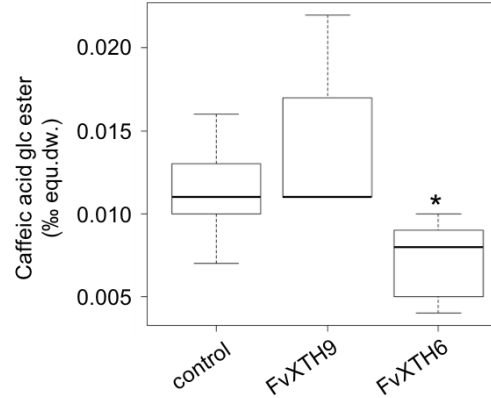
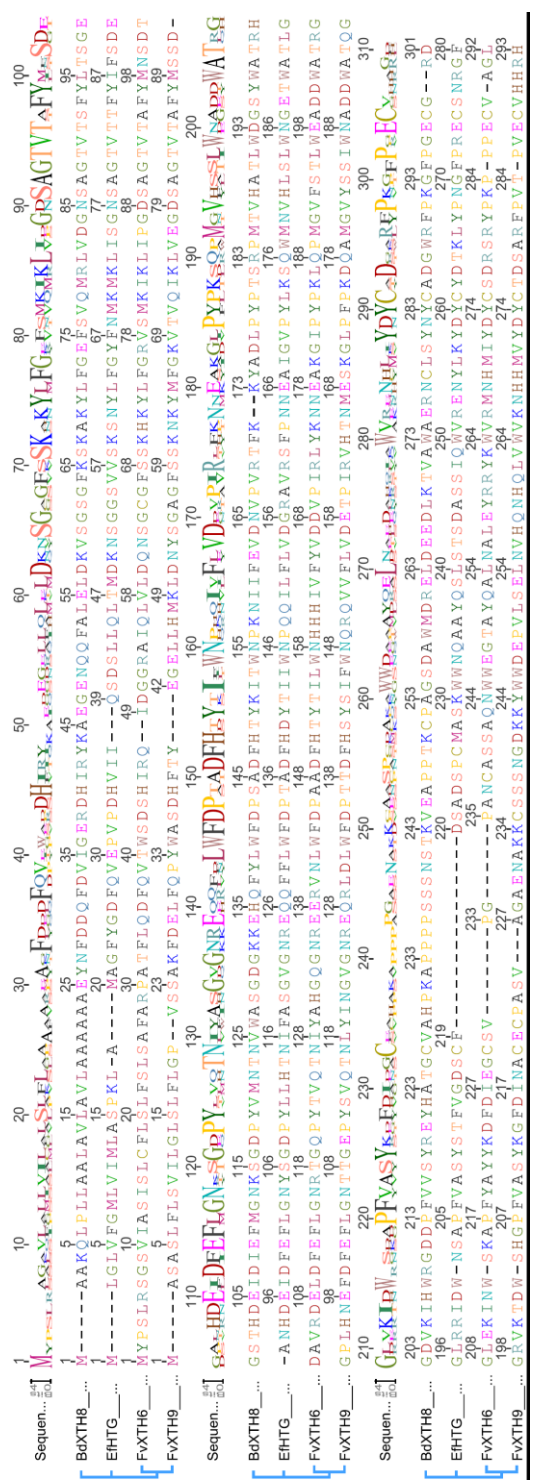


Figure S7. Metabolite analysis of *F. x ananassa* fruit after agroinfiltration. Relative concentration (‰ equivalent of internal standard of the dry weight) of (a) pelargonidin rutinoside, (b) epiafzelechin pelargonidin glucoside, (c) ellagic acid, (d) kaempferol glucoside, (e) kaempferol glucuronide, (f) kaempferol malonyl glucoside, (g) quercetin glucoside, (h) quercetin glucuronide, (i) catechin, (j) epicatechin dimer, (k) epiafzelechin-epicatechin, (l) p-coumaryl glc ester, (m) ferulic acid glc ester, and (n) caffeic acid glc ester. The data were obtained by analysing 4-6 fruits (10 DPI) for each sample. Control fruit was infiltrated with *A. tumefaciens* AgL0 contained pBI121 empty plasmid. The asterisk indicates statistically significant differences ( $p < 0.05$ ) between agroinfiltrated fruits with *XTHs* and the empty plasmid.



	BdXTH8	EfHTG	FvXTH6	FvXTH9
BdXTH8	100	38.2	35.5	35.6
EfHTG	38.2	100	44.6	39.1
FvXTH6	35.5	44.6	100	45.7
FvXTH9	35.6	39.1	45.7	100

Figure S8. Amino acid sequence alignment of BdXTH8 (XP\_003573584.1), EfHTG (CEH24720.1), FvXTH6 (gene05591), and FvXTH9 (gene01986) as well as pairwise sequence identities using Geneious software with default values.