

SUPPLEMENTARY DATA

Manuscript title: A *SHATTERPROOF*-like gene controls ripening in the non-climacteric strawberries, and auxin and abscissic acid antagonistically affect its expression.

Authors: Margherita Daminato, Flavia Guzzo, Giorgio Casadoro

Supplementary File 1

Supplementary materials and methods: metabolomics analysis

The liquid nitrogen frozen fruits were powdered and extracted with three volumes of ice cold methanol, sonicated at 40 kHz for 15 min in an ultrasonic bath (Falc Instruments, Bergamo, Italy) at room temperature, centrifuged for 10 min at 16,000g at 4°C and filtered through 0.2 µm pore filters. The samples were diluted with 1:1 (v:v) LC-MS grade water (SIGMA) just before the chromatographic analysis. The extracts were analyzed by reversed phase HPLC-ESI-MS (High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry) in negative ionization mode.

The extracts were analyzed by reversed phase HPLC-ESI-MS in negative ionization mode, using a Beckman Coulter Gold 127 HPLC system (Beckman Coulter, Fullerton, CA) equipped with a C18 guard column (7.5 x 2.1 mm) and an analytical Alltima HP C18 column (150 x 2.1 mm, particle size 3µm) (Alltech Associates Inc, Deerfield, IL). Two solvents were used: 1% (v/v) formic acid, 5% (v/v) acetonitrile in water (solvent A), and 100% acetonitrile (solvent B). A solvent gradient was established from 0 to 10% B in 5 min, from 10 to 20% B in 20 min, from 20 to 25% B in 5 min, and from 25 to 70% B in 15 min, followed by 10 min of 70% acetonitrile isocratic phase. Four untreated fruits, four fruits agro-infiltrated with the pBINplus_intron construct, seven fruits FaSHP up-regulated, eight FaSHP down-regulated,

three pools of either ABA or NAA treated fruits plus three untreated controls were chosen and analyzed in duplicate, with a 20 μ l injection volume and 20 min equilibration between samples. The flow rate was 200 μ l min⁻¹. The HPLC system was coupled on-line with a Bruker ion trap mass spectrometer Esquire 6000, equipped with an electrospray ionization (ESI) source. MS data were collected using the Bruker Daltonics Esquire 5.2- Esquire Control 5.2 software, and processed using the Bruker Daltonics Esquire 5.2-Data Analysis 3.2 software (Bruker Daltonik GmbH, Bremen, Germany). The alternate mass spectra were recorded in the range 50–3000 m/z (full scan mode, 13,000 m/z s⁻¹). For the fragmentation pattern analysis, MS/MS and MS³ spectra were recorded in negative and positive mode in the range 50–3000 m/z, with the fragmentation amplitude set at 1 V. Nitrogen was used as the nebulizing gas (50 psi, 350°C) and drying gas (10 l min⁻¹). Helium was used as the collision gas. The vacuum pressure was 1.4 x10⁻⁵ mbar. Additional parameters were: capillary source, +4000 V; end plate offset -500 V; skimmer: -40 V; cap exit -121 V; Oct 1 DC: -12 V; Oct 2 DC: -1.70 V; lens 1: 5 V; lens 2: 60 V.

Metabolites were identified through the comparison of m/z, retention time and fragmentation pattern with those of authentic standards and, when authentic standards were not available, comparing m/z and fragmentation patterns with those reported in the literature. Chromatogram data extraction and alignment were carried out using MZmine software (<http://mzmine.sourceforge.net>).

Since internal standards were not applied, the relative quantitation (i.e. comparison between samples) was based on the area of each of the signals extracted from the chromatograms and expressed as intensity in arbitrary units.

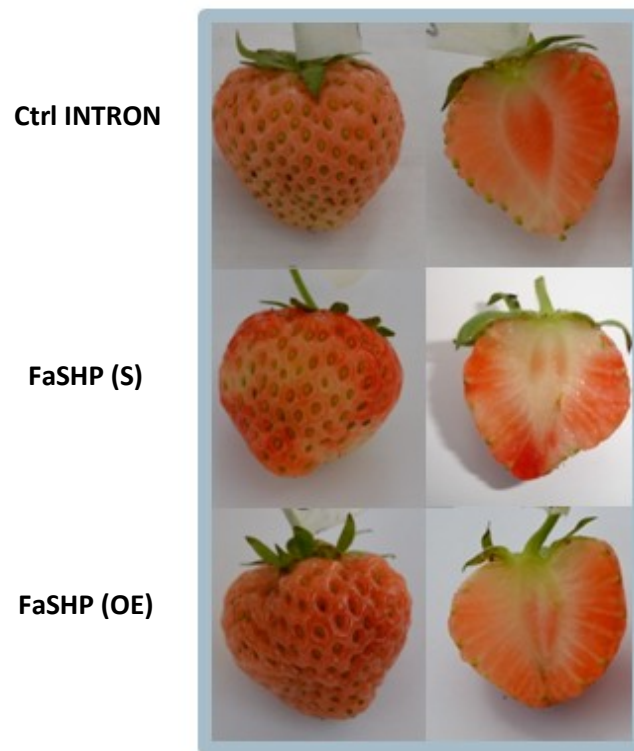


Figure S1. Phenotype of agro-injected fruits harvested at the pink stage. (Ctrl INTRON): fruit injected with the control construct. [FaSHP (S)]: fruit injected with the RNA interference construct (pBINplus_FaSHPi). [FaSHP (OE)]: fruit injected with the over-expression construct (pBinAR_FaSHP).

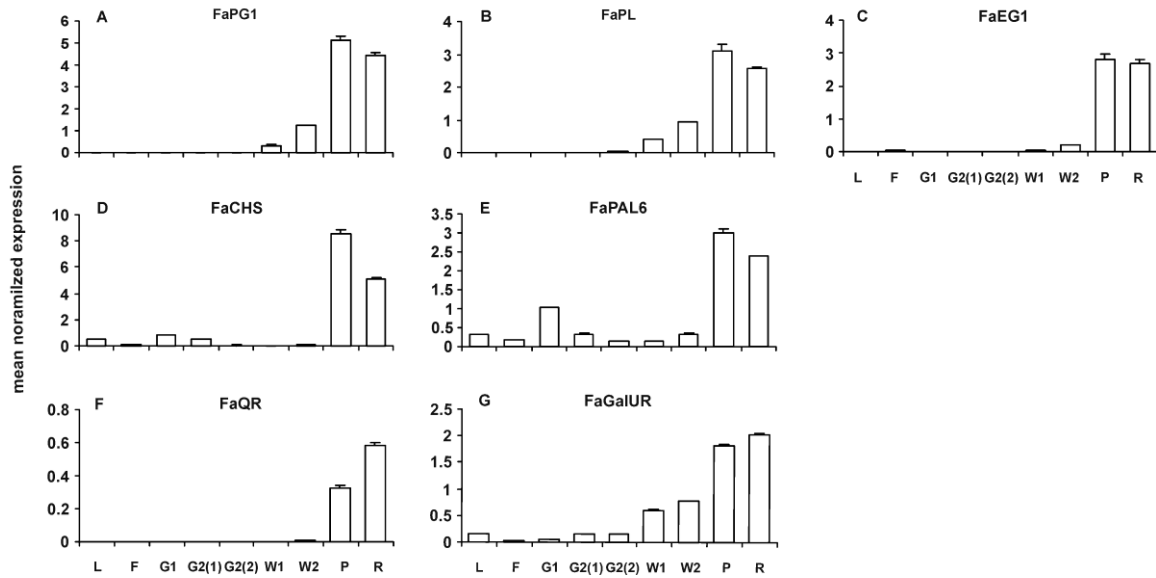


Figure S2. Relative expression profiles of ripening-related genes in leaf (L), flower (F), and fruits at different developmental stages: small green (G1), large green [G2(1)], large green with enlarged achenes [G2(2)], white with green achenes (W1), white with brown achenes (W2), pink (P) and red (R). [*FaPG1*, polygalacturonase; *FaPL*, pectate lyase; *FaEG1*, endo-beta-1,4-glucanase; *FaCHS*, chalcone synthase; *FaPAL6*, phenylalanine ammonia lyase, *FaQR*, quinone oxidoreductase; *FaGalUR*, D-galacturonate reductase].

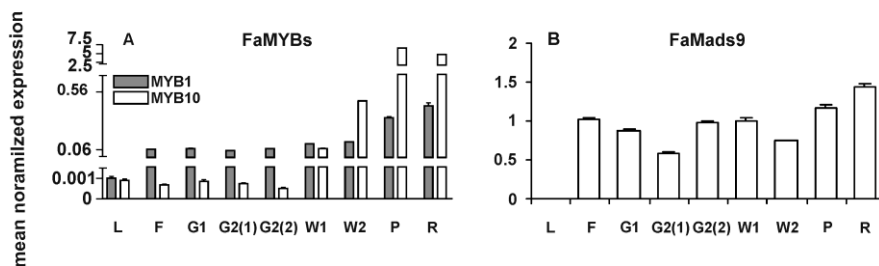


Figure S3. Relative expression profiles of transcription factor encoding genes in leaf (L), flower (F), and fruits at different developmental stages: small green (G1), large green [G2(1)], large green with enlarged achenes [G2(2)], white with green achenes (W1), white with brown achenes (W2), pink (P) and red (R).

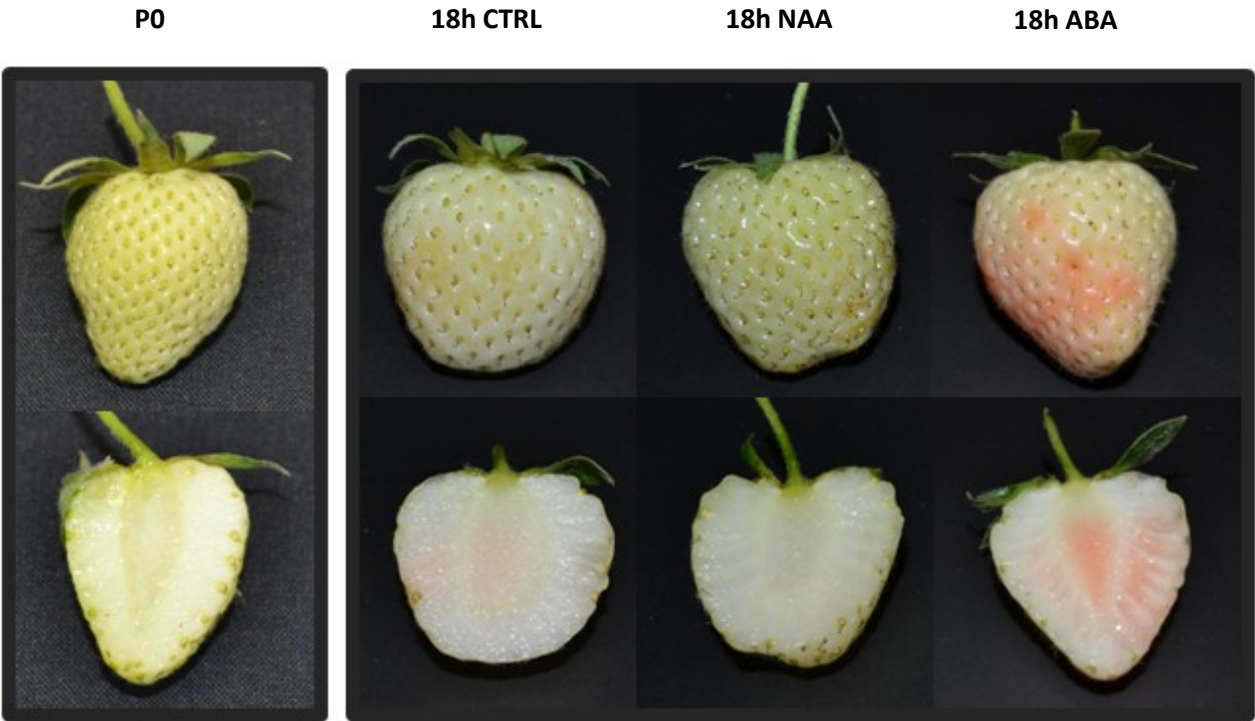


Figure S4. Phenotype of hormone treated white fruits. (P0) Fruit at the beginning of the treatment. (18h CTRL) Fruit sprayed with the control solution: a faint pink color can be seen in the central part of the fruit. (18h NAA) Fruit sprayed with the NAA solution: the fruit is completely white. (18h ABA) Fruits sprayed with the ABA solution have acquired a distinct pinkish color.

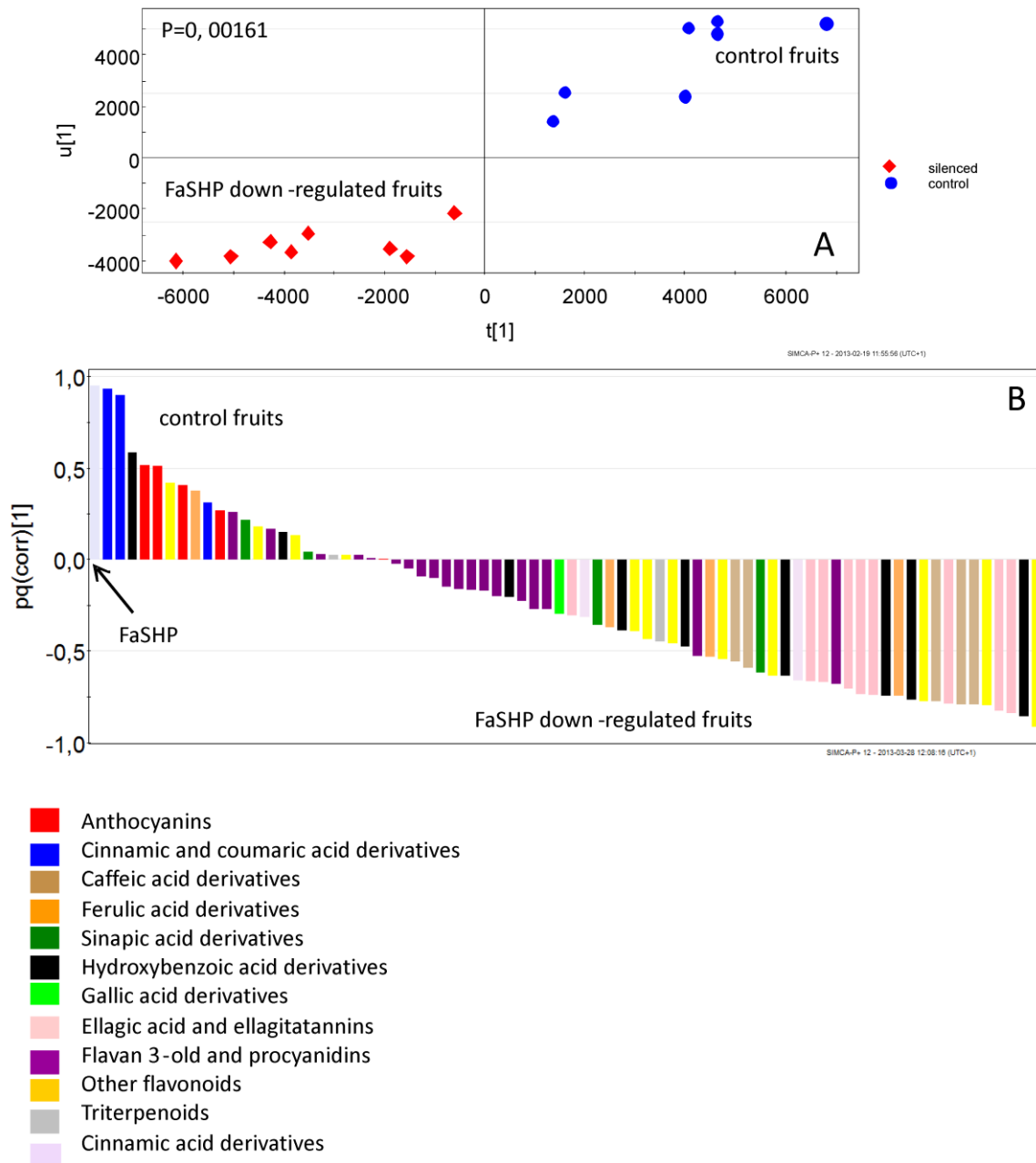


Figure S5. OPLS analysis of *FaSHP* down regulated and control fruits (either untreated fruits or fruits agroinfiltrated with the pBINplus_intron construct; one of the control agroinfiltrated fruit has been removed as outlier); variable Y: mean normalized *FaSHP* expression; variables X: HPLC-ESI-MS quantified metabolites. A: t_1 - u_1 score plot; B: $pq(corr)_1$ (correlation between t , the metabolite content, and u , the gene expression) loading plot, showing the contribution of the various metabolites on the linear gene expression-metabolite function shown in the score plot.

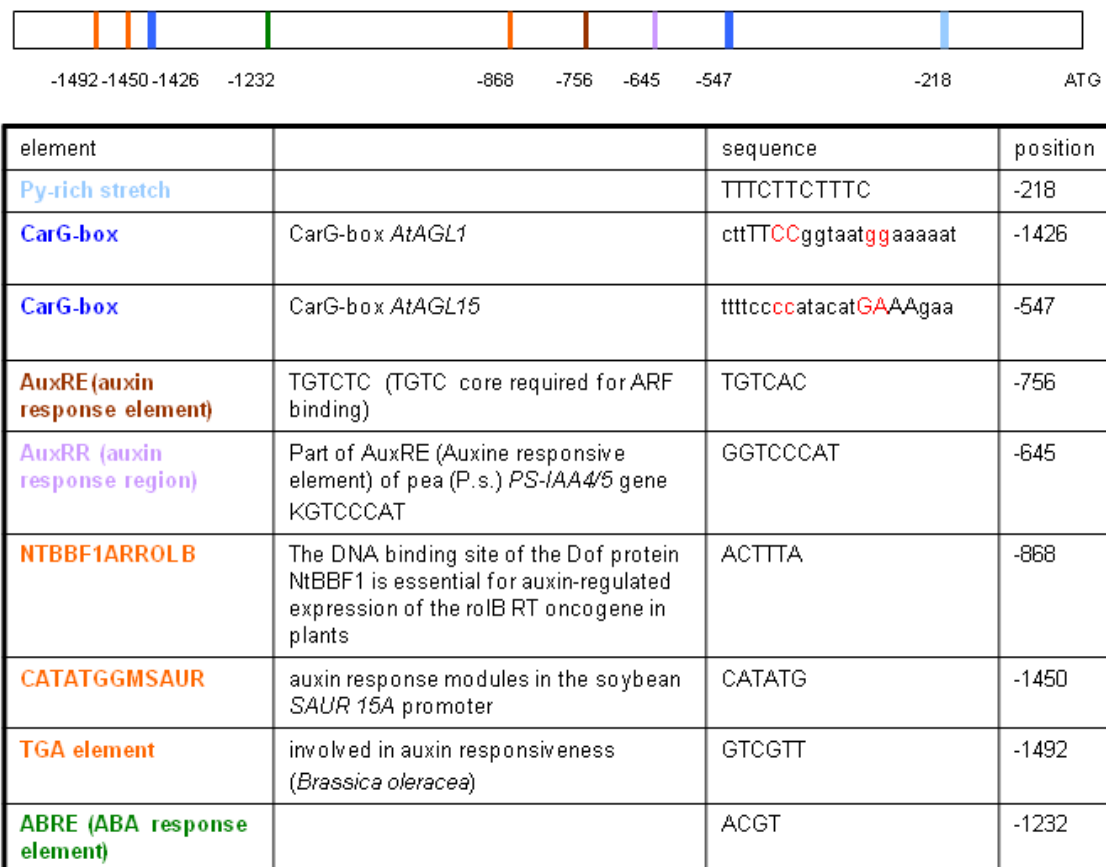


Figure S6. Bioinformatic analysis of the *FaSHP* gene promoter. The position of the indicated cis-acting element is expressed relative to ATG codon.

m/z (-)	m/z(+)	rt (min)	ms/ms		ms3		putative identification
			m/z	I (%)	m/z	I (%)	
430,94		17,3	268.8	100.0	146.7	100.0	pelargonidin 3-O-glucoside
			269.5	15.1	240.8	21.0	
			146.7	4.0	224.8	14.2	
					147.8	12.6	
472,87		22,5	268.8	100.0	146.7	100.0	pelargonidin-3-O-acetylglucoside
			269.7	19.0			
446,92		15,9	284.7	100.0	256.7	100.0	cyanidin 3-O-glucoside
			285.5	13.0	240.8	95.3	
					198.7	89.8	
					216.8	83.9	
					162.7	69.9	
	534,90	30,3	287.0	100.0			cyanidin-3-O-malonylglucoside
			288.0	9.6			
	518,99	22,5	271.0	100.0	121.0	100.0	
			271.8	13.7	197.0	55.7	pelargonidin-3-O-malonylglucoside
					145.1	46.8	
					224.9	40.5	
					169.1	36.4	
					173.0	36.2	
					215.0	31.2	
					186.8	29.1	
486,90		20,8	440.9	100.0	292.8	100,0	cinnamoyl desoxyhexose d, fa
			292.8	15.3	146.7	57.4	
			146.7	11.8			

308,95		21,7	146.8	100.0			cinnamoyl O-hexose
324,86		12,1	144.8	100.0	118.8	100.0	coumaric acid hexose
			162.8	87.8			
			288.8	33.5			
			186.7	31.4			
			118.9	10.7			
			204.7	7.8			
			116.9	7.7			
			264.8	7.1			
			234.8	6.2			
			163.7	5.7			
			145.8	4.8			
			187.8	3.7			
			265.8	2.8			
			158.8	2.6			
			160.8	2.5			
			216.8	2.2			
			172.7	1.8			
			289.8	1.6			
			119.8	1.5			
336,96		16,0	190.7	100.0			coumaroyl quinic acid
			191.7	6.8			
			162.8	4.4			
324,95		18,0	162.7	100.0			coumaric acid hexose
			118.8	83.1			
			160.7	49.1			
340,93		7,7					caffeic acid hexose

			178.7	100.0	134.8	100.0	
			160.8	42.1			
			134.8	19.4			
			179.7	9.8			
			202.7	5.9			
			132.8	3.6			
340,87		11,9	178.7	100.0	134.8	100.0	caffeic acid hexose
			134.8	25.7			
			179.7	9.3			
352,86		12,4	190.7	100.0	84.9	100.0	caffeoyl quinic acid
			191.7	6.7			
			178.9	3.1			
520,99		24,6	326.9	100.0	326.9	100.0	caffeic acid hexose d
			472.9	90.2	340.9	16.5	
			178.7	66.7			
			340.9	50.0			
566,88		25,9	520.7	100.0	178.8	100,0	caffeic acid d fa
			178.7	49.9	160.6	27,0	
354,96		14,1	192.8	100.0	133.8	100.0	ferulic acid hexose
			216.7	72.3	148.8	34.7	
			174.8	53.2			
			133.8	11.9			
			159.9	11.3			
			234.8	7.5			
			193.8	7.2			
			201.7	4.8			
			294.8	4.8			

516,92		16,8	192.7	100.0	133.7	100,0	ferulic acid hexose d
			174.8	67.0	148.8	81.3	
			234.8	41.3			
			294.8	27.4			
			336.8	22.1			
			264.8	21.6			
			159.7	12.5			
536,96		17,9	178.7	100.0	130.7	100.0	dihydroferulic acid d
			356.8	80.3	96.9	93.1	
			354.8	52.0	88.9	69.0	
			194.9	26.6	148.8	69.0	
			324.9	24.6	142.7	37.9	
			160.8	20.3			
448,93		18,3	354.8	100.0	192.7	100.0	ferulic acid hexose d
			192.8	66.4			
			268.8	59.4			
			286.8	40.5			
			355.7	18.3			
			328.8	16.5			
			148.7	15.8			
			287.7	11.5			
			164.8	8.3			
431,01		14,0	384.9	100.0	152.8	100.0	sinapic acid hexose fa
			222.9	21.0	222.8	77.9	
			152.9	16.2			

			160.9	13.3			
			204.9	10.6			
431,01		14,7	384.9	100.0	152.8	100.0	sinapic acid hexose fa
			222.8	19.6	204.8	96.9	
			160.8	14.0	222.8	53.2	
			204.9	9.3			
			152.9	9.1			
			381.0	5.4			
			382.1	5.0			
384,96		15,1	222.8	100.0	207.7	1,0	sinapic acid hexose
			204.8	83.1			
			246.7	50.5			
			163.8	16.7			
402,97		21,8	222.8	100.0	178.8	100,0	sinapic acid d
			178.8	58.5			
314,91		4,9	152.7	100.0	108.8	100.0	hdihydroxybenzoic acid hexose
			151.8	23.8	122.8	32.0	
			107.9	11.1			
			164.7	9.3			
			108.8	9.1			
			153.7	6.7			
			162.7	5.9			
			224.8	2.3			
			176.6	1.9			
			194.8	1.6			
			206.7	1.5			
			122.8	1.3			

			150.8	1.3			
314,91		6,7	152.7	100.0	134.7	100.0	dihydroxybenzoic acid hexose
			108.8	9.5	108.8	53.5	
			153.7	6.3	122.9	23.3	
298,95		7,1	112.8	100.0			hydroxybenzoic acid hexose
			118.8	94.7			
			178.8	76.6			
			100.8	70.4			
			142.8	66.7			
			88.9	61.4			
			160.8	57.6			
			136.6	3.8			
344,93		7,1	298.8	100.0	118.8	100.0	hydroxybenzoic acid hexose fa
					178.8	97.6	
					112.8	76.8	
					142.7	76.6	
					160.7	68.5	
284,88		9,0	152.7	100.0	108.9	100.0	dihydroxybenzoic acid pentose
			151.9	21.7			
			107.9	9.7			
			108.9	8.5			
			153.7	4.8			
			164.7	4.1			
			162.6	3.2			
			178.7	2.3			
298,90		9,2	136.8	100.0	92.9	100.0	hydroxybenzoyl hexose
			137.7	3.7			

330,90	20,5	136.8	100.0	108.7	100.0	hydroxybenzoic acid d
		286.8	50.9			
		270.8	31.4			
		312.8	27.3			
		288.8	13.8			
		268.8	9.5			
		244.9	9.0			
		313.8	6.2			
		124.8	6.2			
		287.8	6.1			
		228.8	5.5			
		108.9	4.4			
		262,89	31,1	152.8	100.0	
		218.8	44.8			
543,02	42,5	335.0	100.0	98.9	100.0	vanillic acid d, fa
				180.6	71.5	
				136.8	70.7	
				166.8	45.5	
				202.9	43.9	
				167.7	42.3	
				162.9	41.5	
				96.8	27.6	
				317.8	19.5	
				150.7	17.1	
				264.9	13.0	
300,89	5,9	167.7	100.0	149.7	100.0	gallic acid pentose

			168.7	81.3	150.7	25.1	
			282.7	39.4			
			124.8	26.3			
			283.7	15.3			
			149.8	13.3			
			166.9	8.6			
			150.7	8.3			
			169.7	7.1			
864,84		3,8	694.9	100.0	242.6	100,0	procyanidin P3 type
			576.8	28.0	288.9	16.9	
			286.8	27.7			
			695.7	24.4			
			574.9	20.2			
			404.8	15.7			
			542.9	14.9			
576,94		9,7	424.9	100.0	406.8	100.0	procyanidin P2 type
			406.9	89.6	407.4	20.6	
			288.9	35.3	272.9	8.2	
			450.8	29.6	380.9	7.0	
			407.8	22.9	228.8	2.7	
			425.6	12.3	338.9	2.6	
			286.9	9.1	150.7	2.2	
			244.8	8.5	273.8	2.2	
			272.8	7.8	280.8	2.0	
864,86		9,8	694.9	100.0	542.8	100.0	procyanidin P3 type
			286.8	89.1	406.8	50.9	
			576.8	54.5	524.8	50.0	

			406.8	52.4	242.9	41.7	
			448.7	35.0	462.8	35.2	
			424.9	32.9	677.1	26.9	
			542.9	32.5	254.8	16.7	
			712.8	29.2	450.9	15.7	
			739.0	26.9	288.7	14.8	
			450.7	26.0	404.7	13.9	
			577.8	21.8	362.8	13.0	
			695.9	21.1	346.8	10.2	
			413.0	18.3	293.7	9.3	
			574.8	18.1	584.4	9.3	
864,88		10,9	694.9	100.0	242.7	100.0	procyanidin P3 type
			406.9	75.6	542.8	50.6	
			576.8	58.6	524.8	25.9	
			286.9	51.6	677.9	22.2	
			738.8	51.3	404.8	19.8	
			448.9	48.7	499.0	12.3	
			450.9	42.2	450.8	11.7	
			574.8	42.2	544.3	11.7	
			288.9	38.8	204.7	8.6	
			404.9	34.6	677.0	8.6	
1152,82		12,0	1027.1	100.0			Procyanidin tetramer
			406.7	55.6			
			694.9	54.3			
			1027.9	43.2			
			737.0	39.5			
			982.7	37.0			

			983.1	32.1			
			448.8	30.9			
			576.8	25.9			
			865.0	23.5			
288,88		12,0	244.8	100.0	202.8	100.0	catechin
			204.8	35.9	187.8	26.6	
			178.8	16.2	186.8	24.6	
			245.7	10.8	226.7	21.8	
			202.9	10.0	160.8	19.0	
			164.9	6.9	174.8	11.8	
			136.9	6.6	161.7	8.4	
			160.9	5.6	203.7	6.5	
			230.8	5.5	230.8	5.5	
			124.9	5.5			
864,87		12,7	694.9	100.0	288.9	100.0	procaynidin P3 type
			576.9	76.1	242.3	88.7	
			286.9	68.5			
			406.9	68.3			
			424.9	45.1			
			574.9	43.4			
			738.9	34.7			
1152,84		12,7	864.8	100.0	407.0	100.0	Procyanidin tetramer
			983.8	97.3			
			846.9	75.7			
			982.8	71.2			
			695.0	60.4			

			1026.9	55.9			
			1134.8	55.9			
			862.7	55.0			
			448.8	50.5			
			1000.8	47.7			
576,78		13,3	424.8	100.0	406.8	100.0	procyanidin P2 type
			406.9	75.5	407.7	19.5	
			288.9	31.7	272.7	11.3	
			286.9	21.5			
			450.8	21.4			
			407.9	16.1			
			425.6	13.9			
			298.8	8.7			
			289.9	5.0			
560,87		13,7	288.8	100.0	244.8	100.0	propelargonidin dimer (afzelechin-catechin)
			434.9	37.8	204.8	63.0	
			270.9	25.8			
			542.8	19.5			
			406.9	19.1			
			289.9	16.4			
			328.9	12.8			
			244.9	11.6			
			424.9	10.2			
719,98		14,2	1152.8	100.0			B-type procyanidin pentamer (M-2H)--/2
			1151.9	83.4			
			862.9	70.7			
			864.9	69.2			

			1152.8	55.6			
848,87		14,7	406.8	100.0			B type procyanidin trimer (epi)afzelechin-(epi)catechin-(epi)catechin
			570.9	100.0			
			722.9	89.1			
			571.9	78.2			
			558.8	76.2			
			288.8	55.8			
			432.8	52.4			
			286.8	51.7			
			450.8	51.7			
			577.8	49.0			
576,83		15,2	424.8	100.0	406.8	100.0	procyanidin P2 type
			406.9	75.5	407.7	19.5	
			288.9	31.7	272.7	11.3	
			286.9	21.5			
			450.8	21.4			
			407.9	16.1			
			425.6	13.9			
			298.8	8.7			
			289.9	5.0			
575,98		16,6	406.9	100.0	242.8	100.0	procyanidin B type tetramer (M-2H)--/2 adduct
			288.9	98.0	280.7	52.2	
			424.8	70.1			
			286.8	46.2			
			490.9	42.1			
			499.8	39.7			

			1026.8	34.7			
			405.9	34.5			
			863.0	29.1			
			864.8	27.1			
			738.8	26.9			
864,87		17,2	406.8	100.0			procyanidin P3 type
			694.9	93.9			
			286.8	51.9			
			576.9	45.0			
			424.9	41.6			
			574.8	36.2			
			542.9	32.8			
			696.0	27.9			
			404.9	27.6			
			719.7	27.1			
			448.8	24.9			
576,86		18,6	424.9	100.0	406.8	100.0	procyanidin P2 type
			406.8	78.3	272.7	20.2	
			288.8	36.2			
			286.9	33.8			
			425.7	18.9			
			450.8	18.6			
			407.9	8.4			
			289.9	7.4			
			451.7	7.0			
			244.9	6.7			
			272.9	6.5			

864,86		19,6	694.8 406.8 738.8 560.7 576.7	100.0 95.5 72.8 40.9 38.3			procyanidin P3 type
434,96		25,4	314.9 315.5 344.8	100.0 11.7 10.3	166.7 208.7 124.8 209.8	100.0 45.9 6.0 5.2	(Epi)afzelechin (or phloretin) hexose
632,75		13,9	300.7 301.6 462.7	100.0 13.2 4.0	300.7 256.6 229.6 201.5	100.0 89.3 49.1 41.1	HHDP galloyl hexose
462,91		19,0	300.7 300.0 301.4	100.0 30.5 8.1	256.7 299.7 228.7 184.8 257.7 300.5	100.0 58.0 27.9 16.5 14.0 11.9	ellagic acid hexose
934,74		19,2	300.7 632.9 633.7 301.7 299.9 274.8	100.0 67.4 20.9 15.7 6.4 5.3	256.8 283.8	100.0 76.5	ellagic acid d
466,95		19,2	390.9 300.8	100.0 24.8	300.7 274.8	100.0 64.1	casuructin/potentillin like ellagitannin

			274.7	7.8	305.7	56.7	
			301.9	6.8	168.8	43.3	
			632.7	3.5	376.8	42.3	
					292.8	28.9	
					262.7	21.5	
					381.8	20.8	
					340.6	20.1	
446,89		22,3	300.7	100.0	300.8	100.0	ellagic acid desoxyhexoside (rhamnoside)
			300.1	16.4	299.7	93.2	
			301.6	14.5	256.7	55.5	
					257.8	29.0	
432,89		22,5	300.7	100.0	299.7	100.0	ellagic acid pentose
			299.8	61.2	228.6	14.9	
			301.7	17.5	184.8	11.2	
					256.5	7.8	
466,90		23,4	300.8	100.0	256.7	100.0	casuructin/potentillin like ellagitannin
			457.8	30.5	144.9	77.2	
			381.9	28.1	184.6	53.2	
			301.6	17.2	300.6	49.4	
			632.7	12.4	298.5	46.8	
			390.8	9.2	270.5	32.9	
300,83		23,4	300.7	100.0	184.6	100.0	ellagic acid
			256.7	34.8			
			228.8	27.8			
			200.7	9.5			
			257.7	8.3			
			184.7	8.0			

			283.7	7.5			
			258.7	6.6			
			299.8	6.6			
			254.7	5.8			
933,94		23,8	896.8	100.0	744.7	100.0	ellagic acid d
			1566.8	89.4	300.8	50.0	
			300.7	76.1			
			1567.5	44.6			
			1084.7	41.6			
			1264.7	41.1			
			1566.4	38.2			
			897.7	30.0			
			1568.6	26.4			
			898.7	21.7			
446,89		12,5	400.8	100.0	268.8	100.0	trihydroxyflavone O-pentose, fa
			268.9	35.7	160.8	24.9	
			130.8	6.8	130.8	11.7	
416,88		12,7	151.8	100.0			kaempferol O -pentose
			240.8	88.1			
			162.8	33.4			
			150.7	32.7			
			284.8	25.2			
			152.7	19.2			
			178.8	17.8			
446,97		13,7	400.9	100.0	268.8	100.0	trihydroxyflavone O-pentose, fa
			313.1	6.0	160.7	62.9	
460,99		15,4	414.9	100.0	268.8	100.0	trihydroxyflavone-desoxyhexoside fa

			268.9	6.4	160.7	47.5	
					269.8	19.3	
					142.8	8.7	
448,91		17,3	286.8	100.0	258.8	100.0	tetrahydroxyflavanone O-hexoside
			258.9	69.7			
			268.8	50.0			
			259.8	11.0			
			287.8	10.7			
			269.8	9.9			
			242.9	5.4			
432,94		19,3	270.7	100.0			naringenin C-hexose
			252.8	75.2			
462,95		23,9	300.7	100.0	150.7	100.0	quercetin -O-hexose
			301.4	11.0	178.7	82.9	
					270.7	35.5	
					272.7	35.4	
476,89		24,1	300.8	100.0	178.7	100.0	quercetin O-glucuronide
			301.7	14.4	150.7	86.4	
446,90		27,2	284.6	100.0	254.7	100.0	kaempferol O-hexose
			283.9	66.4	255.8	51.6	
			254.8	21.7	226.7	43.2	
			326.8	12.3	228.8	40.1	
			285.6	10.7			
	463,00	27,7	287.0	100.0			kaempferol-O-glucuronide
			288.0	21.3			
488,89		30,3	284.8	100.0			kaempferol-O-acetyl hexose

			285.5	21.1			
592,91		38,5	284.8	100.0			kaempferol coumaroyl hexose
			285.7	17.2			
			446.8	7.6			
463,01		31,8	417.0	100.0	160.8	100.0	sesquiterpenoid hexose, fa
			417.9	21.3			
			415.0	15.7			
695,15		41,5	487.3	100.0	469.1	100.0	triterpenoid hexose fa
			649.0	16.1	470.0	34.0	
					425.2	13.4	
					407.0	7.1	
					426.2	6.2	
					442.9	5.1	

Table S1. Mass to charge ratio (m/z) in negative (-) and positive (+) ionization mode, retention time (rt, in minutes), fragmentation pattern (ms/ms and ms3 of the ms/ms fragment underlined in bold), relative intensity (I %) of the fragments, putative identification, based on comparison with authentic standards, comparison with public databases and comparison with literature (especially: Del Bubba et al.,2012, J. Mass Spectrom. 47, 1207–1220, Fait et al., 2008, Plant Physiol. 148, 730-750, Hanhineva et al., 2008, Phytochemistr 69, 2463–2481).

	OE	C	S	pq(corr)1	ABA	C	NAA
FaSHP expression	++	+	-	0,946209			
cinnamoyl O-hexose	+/-	+	-	0,930756	++	+	-
coumaric acid hexose	+/-	+	-	0,901673	++	+	-
dihydroxybenzoic acid derivative	+	+	-	0,597937	++	+	-
pelargonidin-3-O-acetylglucoside	+/-	+	-	0,526407	++	+	-
pelargonidin-3-O-malonylglucoside	+/-	+	-	0,521076	++	+	-
sinapic acid hexose	+	+	++	-0,61096	-	+	++
dihydroxybenzoic acid hexose	++	+	++	-0,63283	-	+	+/-
trihydroxyflavone-desoxyhexoside	+	+	+	-0,633278	+	+	+
coumaroyl quinic acid	++	+	++	-0,660334	++	+	++
ellagic acid desoxyhexose	+	+	++	-0,673076	-	+	+
casuructin/potentillin like ellagitannin	++	+	++	-0,679878	-	+	+
Procyanidin tetramer	+/-	+	++	-0,681829	-	+	-
casuructin/potentillin like ellagitannin	++	+	++	-0,716418	-	+	++
ellagic acid pentose	+	+	+	-0,731163	-	+	++
hydroxybenzoyl hexose	++	+	++	-0,740757	-	+	-
ellagic acid derivative	+	+	++	-0,751597	-	+	++
hdihydroxybenzoic acid hexose	+/-	+	++	-0,756686	-	+	+/-
ferulic acid hexose derivative	++	+	++	-0,756927	++	+	++
caffeoyl quinic acid	++	+	++	-0,776746	-	+	++
kaempferol coumaroyl hexose	++	+	++	-0,778017	-	+	++
trihydroxyflavone O-pentose	+	+	++	-0,790763	-	+	+/-
HHDP galloyl hexose	+	+	++	-0,798734	-	+	+
caffeic acid derivative	++	+	++	-0,80117	-	+	++
caffeic acid hexose	++	+	++	-0,802114	-	+	++
ellagic acid hexose	-	+	++	-0,829421	-	+	++
ellagic acid	+/-	+	++	-0,839532	-	+	++
dihydroxybenzoic acid pentose	-	+	++	-0,845747	-	+	+/-
trihydroxyflavone O-pentose	+	+	++	-0,912101	-	+	+/-

Table S2. pq(corr) loadings of OPLS analysis and comparison with metabolite levels in *FaSHP* up- and down- regulated fruits, and in fruits treated with hormones. + = level of metabolite in control fruits; +/- = slight variation (increase or decrease) compared to the control; ++ = increase of metabolite level compared with level in control fruits; - = decrease of metabolite level compared with level in control fruits. OE = *FaSHP* over-expressing fruits; S = *FaSHP* silenced fruits; C = control fruits; ABA, NAA = fruits treated with either ABA or NAA, 18 h after the beginning of the treatment.

Gene	primer FW name	primer FW sequence (5'-3')	primer RV name	primer FW sequence (5'-3')	Genbank Ac. number
FaITS - Fragaria x ananassa internal transcribed spacer	FaSHP_ITS_rtfor	CGTCACGCGGTTGGCATAAATAC	FaSHP_ITS_rtrev	GACGCGCTCACGGCTAAGGAA	FJ356167
FaSHP - Fragaria x ananassa SHATTERPROOF	FaSHP_rtfor2	GGCACAGCAGCAGCAAGCAAATA	FaSHP_rtrev2	CTTGGCGATTGTAATTGTGATTGG	KC676787
FaSHP - Fragaria x ananassa SHATTERPROOF	FaSHP_rtiRNAfor	TGGAGAAAGGGATCAGCAGAATAA	FaSHP_rtiRNArev	TATGCTGCCAGGAACATCGTCCG	KC676787
FaPG1- Fragaria x ananassa polygalacturonase 1	FaPG1_rtfor	TCGGAAGTCTCGGAAGATACGACA	FaPG1_rtrev	CGAGGCGGTGGTAGCTTTAGGA	AY282613
FaPL - Fragaria x ananassa pectate lyase	FaPL_rtfor	CCATATTCGGATCTAGCCACATTT	FaPL_rtrev	AGGAGTCACTATGCCCAACAGC	U63550
FaEG1 - Fragaria x ananassa endo-beta-1,4-glucanase 1	FaEG1_rtfor1	GCGTACAGCTCCAGCCTCAAAAA	FaEG1_rtrev1	ATTCTCTGTACTGCCCTTCTCG	AJ00634
FaCHS - Fragaria x ananassa chalcone synthase	FaCHS_rtfor1	GCCCGTCCGTCGTAAGCGTCTC	FaCHS_rtrev1	CTAGGCCACGGAAGGTCACG	A1795154
FaPAL6 - Fragaria x ananassa phenylalanine ammonia lyase 6	FaPAL6_rtfor	GACAAGGTGTTCAACGCAATCTGC	FaPAL6_rtrev	AATCAAACCAATTCAGCCAAAAG	HM641823
FaGalUR – Fragaria x ananassa galacturonate reductase	FaGalUR_rtfor1	TGCCCAAGCCAAAGGAAAATCAAC	FaGalUR_rtrev1	TCCTCCTCGGTCAAGCAGAAAGTCG	AF139082
FaQR – Fragaria x ananassa quinone oxidoreductase	FaQR_rtfor1	CCTTGGGTGCTGCTGATTTG	FaQR_rtrev1	GTGGCGTTGCTGGACCTACTATT	AY048861
FaMYB10 – Fragaria x ananassa MYB 10	FaMYB10_rtfor2	TCCAAATAAGCCCATGAGAATAA	FaMYB10_rtrev2	CACCAAACAGCCAGCAGCAGAAT	EU155162
FaMYB1 – Fragaria x ananassa MYB 1	FaMYB1_rtfor1	GCTGTTTGGTGCCTGAGTTGAATC	FaMYB1_rtrev1	TAAAAGAAGCCCCGAGAGGAA	AF401220
FaMads9 – Fragaria x ananassa MADS 9	FaMads9_rtfor1a	AGCCAACAGAGATTTGAAAACGAA	FaMads9_rtrev1a	AGGCTGGAACATAAGTCCCTGTGT	AF484683
STAG1 – strawberry AGAMOUS 1	STAG1_rtfor	CTGCCAAGCTGCATAACCAGATA	STAG1_rtrev	ATTCGCGCAAACAAGAGTTCATTC	AF168468

Table S3. sequences of the forward (FW) and reverse (RV) oligonucleotides used in the real-time PCR experiments performed in this work. In the last column the GenBank accession numbers are reported.

Sequence name	Genbank Ac. number
Rosa rugosa MASAKOD1	BAA90743.1
Malus x domestica MADS14	ADL36737.1
Prunus persica PpPLE	ACL31234.1
Arabidopsis thaliana SHP1	NP_191437.1
Arabidopsis thaliana SHP2	AAU82079
Antirrhinium majus AmPLE	AAB25101
Petunia hybrida FBP6	AAQ72516.1
Solanum lycopersicum TAGL1	NP001234187
Rosa rugosa MASAKOC1	BAA90744.1
Malus x domestica MADS15	CAC80858
Fragaria x ananassa STAG1	AAD45814
Arabidopsis thaliana AG	NP_567569
Antirrhinium majus AmFAR	CAB42988
Petunia hybrida PMADS3	CAA51417.1
Solanum lycopersicum TAG1	AAM33099
Arabidopsis thaliana SEP1	AAA32732
Arabidopsis thaliana SEP2	AAA32734
Arabidopsis thaliana SEP3	AAB67832
Arabidopsis thaliana SEP4	NP_973411.1
Fragaria x ananassa FaMads9	AAO49380.1
Solanum lycopersicum RIN	AAM15775.1
Arabidopsis thaliana AP3	NP_191002.1

Table S4. Genbank accession numbers of the MADS-box protein sequences used to construct the phylogenetic tree.