

FSL 7: HPLC analysis of anthocyanins and flavonols and expressions of different copies of *F3'5'H* in grapevine transgenic lines



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Abstract

Transgenic lines of grapevine containing silencing constructs of *F3'5'H* based on ihpRNA mechanism were studied for their flavonoids and for the expression of *F3'5'H* gene copies. 83-89 % of transgenic lines showed various degrees of silencing, and increase of di-hydroxylated anthocyanins and kaempferol were observed in the leaves of transgenic lines.

Keywords: *Vitis vinifera*, anthocyanin, flavonol, *F3'5'H*, silencing

Introduction

Grape berries are valuable nutraceuticals due to the presence of different types of flavonoids such as anthocyanins and flavonols. Beside, flavor is an important popularity character of Grape berries and their byproducts. *F3'5'H* and *F3'H* are the major genes for flavonoid biosynthesis. In order to study *F3'5'H* and *F3'H* functions and their interaction in the grapevine flavonoids biosynthetic pathway, transgenic lines containing silencing constructs of *F3'H* and *F3'5'H/F3'H* were created using ihpRNA mechanism. In this paper the results of the flavonoids analysis and gene expression assays of *F3'5'H* regenerated transgenic lines are presented.

Materials and Methods

Among several grapevine regenerated lines, independent single copy lines were identified through southern blot analysis. The anthocyanin and flavonol compositions (HPLC analysis was performed according to Downey and Rochfort, 2008) as well as expressions of different copies of *F3'5'H* were studied in the leaves of transgenic lines in three replicates.

Results

Among studied *F3'5'H* copies in the leaves, the highest suppression was observed in *F3'5'H-f*, *F3'5'H-g*, *F3'5'H-j*, *F3'5'H-1* and *F3'5'H-p*, respectively. In all of *F3'5'H* copies, except *F3'5'H-p*, 83-89 % of transgenic lines showed various degrees of silencing (Fig 1). Increase of di- and decrease of tri-hydroxylated anthocyanins and increase of kaempferol and decrease of myricetin were observed in the leaves of transgenic lines in comparison to control plants (Tables 1 and 2). It seems that the biosynthetic pathway has shifted toward the increase of cyanidin, peonidin and caempferol due to inactivation of *F3'5'H*.

Table 1 Anthocyanin compositions (%) of *F3'5'H* transgenic lines and cv. Shiraz

Sample code	Delphinidin		Cyanidin		Petunidin		Peonidin		Malvidin	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
F2	1.68	0.04	0.00	0.00	0.00	0.00	94.65	0.15	3.67	0.19
F4	1.52	0.27	0.00	0.00	0.00	0.00	95.89	0.18	2.60	0.11
F6	0.35	0.02	0.16	0.00	0.00	0.00	92.89	0.11	6.61	0.12
F10	2.52	0.19	0.90	0.10	0.00	0.00	95.02	0.18	1.56	0.30
F13	4.17	0.33	0.85	0.07	0.00	0.00	94.29	0.31	0.69	0.10
F16	7.64	0.10	6.21	0.37	0.00	0.00	86.15	0.47	0.00	0.00
F17	0.76	0.18	0.38	0.04	0.00	0.00	91.41	0.24	7.46	0.02
F19	0.74	0.04	0.39	0.03	0.00	0.00	95.37	0.08	3.50	0.04
F24	4.64	0.62	7.52	0.07	0.00	0.00	87.84	0.68	0.00	0.00
F28	2.72	1.12	3.66	0.19	0.00	0.00	93.36	0.83	0.26	0.14
F29	7.44	0.92	1.17	0.09	0.00	0.00	91.39	0.96	0.00	0.00
F30	0.00	0.00	2.07	0.12	0.00	0.00	96.88	0.19	1.05	0.10
F35	9.14	0.32	0.93	0.10	0.00	0.00	89.92	0.42	0.00	0.00
F38	0.81	0.03	0.56	0.04	0.00	0.00	95.87	0.18	2.76	0.11
F41	0.61	0.01	0.30	0.02	0.00	0.00	95.53	0.03	3.56	0.02
F43	7.43	0.37	3.29	0.05	0.00	0.00	89.27	0.42	0.00	0.00
F44	6.15	0.17	4.12	0.14	0.00	0.00	89.74	0.22	0.00	0.00
F47	7.88	0.87	5.11	0.47	0.00	0.00	87.02	1.34	0.00	0.00
F21 (control)	6.75	0.16	0.59	0.06	0.87	0.15	55.67	1.15	36.13	1.17
Shiraz (control)	7.75	0.05	0.67	0.05	1.09	0.07	55.25	1.09	35.25	1.24

Table 1 Anthocyanin compositions (%) of *F3'5'H* transgenic lines and cv. Shiraz

Sample code	Dihydroxylated		Trihydroxylated		Glucoside		Acetylglucoside		Coumaroyl		Petunidin Caffeoylglucoside	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
F2	94.65	0.15	5.35	0.15	18.09	0.85	14.60	0.41	67.31	1.23	0.00	0.00
F4	95.89	0.18	4.11	0.18	23.57	1.35	13.02	0.39	63.41	1.74	0.00	0.00
F6	93.05	0.11	6.95	0.11	20.11	0.35	10.40	0.13	69.49	0.47	0.00	0.00
F10	95.92	0.12	4.08	0.12	25.65	0.71	12.47	0.45	61.88	1.03	0.00	0.00
F13	95.14	0.37	4.86	0.37	23.00	0.99	13.58	0.24	63.41	0.86	0.00	0.00
F16	92.36	0.10	7.64	0.10	43.92	1.88	13.91	0.41	42.17	2.26	0.00	0.00
F17	91.79	0.20	8.21	0.20	17.83	0.49	9.31	0.32	72.86	0.78	0.00	0.00
F19	95.76	0.06	4.24	0.06	23.33	0.39	10.52	0.08	66.15	0.43	0.00	0.00
F24	95.36	0.62	4.64	0.62	43.16	1.10	11.23	0.90	45.61	1.41	0.00	0.00
F28	97.02	0.99	2.98	0.99	37.61	0.79	11.32	1.09	51.07	1.67	0.00	0.00
F29	92.56	0.92	7.44	0.92	33.63	1.54	16.12	1.49	50.25	2.85	0.00	0.00
F30	98.95	0.10	1.05	0.10	35.98	0.72	7.33	0.25	56.69	0.57	0.00	0.00
F35	90.86	0.32	9.14	0.32	29.66	1.11	17.91	0.82	52.43	1.40	0.00	0.00
F38	96.43	0.15	3.57	0.15	18.82	0.32	12.02	0.19	69.16	0.29	0.00	0.00
F41	95.83	0.01	4.17	0.01	18.01	0.16	13.63	0.11	68.36	0.27	0.00	0.00
F43	92.57	0.37	7.43	0.37	26.74	0.64	16.93	0.82	56.33	1.44	0.00	0.00
F44	93.85	0.17	6.15	0.17	33.74	1.35	13.41	0.15	52.85	1.32	0.00	0.00
F47	92.12	0.87	7.88	0.87	33.16	0.73	18.21	1.63	48.63	1.20	0.00	0.00
F21 (control)	56.25	1.09	43.75	1.09	37.19	0.51	16.35	0.60	46.47	1.10	0.00	0.00
Shiraz (control)	55.92	1.14	44.08	1.14	40.28	0.48	17.48	0.03	42.24	0.45	0.00	0.00

Table 2 Flavonol compositions (%) of *F3'5'H* transgenic lines and cv. Shiraz

Sample code	Myricetin -3-O-glucoside		Quercetin -3-O-glucuronide		Quercetin -3-O-glucoside		Laricitrin -3-O-galactoside		Kaempferol -3-O-glucoside	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
F2	0.00	0.00	75.46	0.57	20.67	0.47	0.15	0.07	0.37	0.00
F4	0.00	0.00	70.68	0.56	25.10	0.38	0.33	0.03	0.30	0.05
F6	0.14	0.02	77.32	0.30	18.23	0.26	0.00	0.00	0.19	0.02
F10	0.00	0.00	66.15	0.51	29.56	0.46	0.00	0.00	0.65	0.05
F13	0.00	0.00	64.66	1.31	30.36	0.45	0.00	0.00	0.60	0.25
F16	0.09	0.01	62.43	0.55	34.54	0.53	0.00	0.00	0.28	0.00
F17	0.12	0.01	75.56	0.49	19.56	0.34	0.00	0.00	0.26	0.02
F19	0.00	0.00	74.21	0.47	22.28	0.41	0.00	0.00	0.20	0.00
F24	0.09	0.01	63.03	0.93	33.66	0.88	0.00	0.00	0.30	0.01
F28	0.07	0.01	55.68	0.61	41.03	0.47	0.00	0.00	0.37	0.02
F29	0.00	0.00	58.37	0.67	37.34	0.47	0.00	0.00	0.52	0.01
F30	0.10	0.01	54.17	0.76	41.31	0.44	0.00	0.00	0.54	0.00
F35	0.00	0.00	50.93	0.64	45.33	0.90	0.00	0.00	0.61	0.07
F38	0.11	0.01	64.44	0.46	31.43	0.26	0.61	0.38	0.32	0.02
F41	0.00	0.00	66.95	0.15	28.32	0.12	0.00	0.00	0.39	0.06
F43	0.00	0.00	55.34	0.25	42.50	0.24	0.00	0.00	0.23	0.01
F44	0.05	0.01	54.95	0.42	41.58	0.41	0.00	0.00	0.36	0.00
F47	0.15	0.02	56.49	0.70	39.38	0.34	0.00	0.00	0.49	0.01
F21										
(control)	0.21	0.01	83.78	0.47	13.00	0.35	0.00	0.00	0.00	0.00
Shiraz										
(control)	0.68	0.05	67.86	0.37	29.73	0.41	0.00	0.00	0.17	0.00

Table 2 Flavonol compositions (%) of *F3'5'H* transgenic lines and cv. Shiraz

Sample code	Laricitrin -3-O-rhamnose -7-O-trihydroxycinnamicacid		Kaempferol-3-O- caffeoylate		Isorhamnetin -3-O- glucoside		Syringetin -3-O-galactoside	
	mean	SE	mean	SE	mean	SE	mean	SE
F2	0.00	0.00	1.49	0.03	1.87	0.02	0.00	0.00
F4	0.00	0.00	1.50	0.11	2.09	0.07	0.00	0.00
F6	0.00	0.00	1.31	0.03	2.81	0.02	0.00	0.00
F10	0.00	0.00	2.41	0.02	1.23	0.08	0.00	0.00
F13	0.00	0.00	3.21	0.56	1.16	0.26	0.00	0.00
F16	0.00	0.00	1.52	0.00	1.13	0.00	0.00	0.00
F17	0.00	0.00	1.66	0.17	2.85	0.02	0.00	0.00
F19	0.00	0.00	1.10	0.04	2.21	0.02	0.00	0.00
F24	0.00	0.00	1.71	0.03	1.21	0.00	0.00	0.00
F28	0.00	0.00	1.93	0.02	0.93	0.16	0.00	0.00
F29	0.00	0.00	2.75	0.02	1.02	0.19	0.00	0.00
F30	0.00	0.00	2.89	0.03	1.00	0.32	0.00	0.00
F35	0.00	0.00	2.40	1.01	0.73	0.04	0.00	0.00
F38	0.00	0.00	1.68	0.01	1.41	0.02	0.00	0.00
F41	0.00	0.00	2.51	0.03	1.83	0.01	0.00	0.00
F43	0.00	0.00	1.56	0.01	0.37	0.01	0.00	0.00
F44	0.00	0.00	1.95	0.01	1.12	0.00	0.00	0.00
F47	0.00	0.00	2.44	0.02	1.05	0.37	0.00	0.00
F21 (control)	0.00	0.00	0.39	0.09	2.63	0.04	0.00	0.00
Shiraz (control)	0.00	0.00	1.04	0.00	0.52	0.01	0.00	0.00

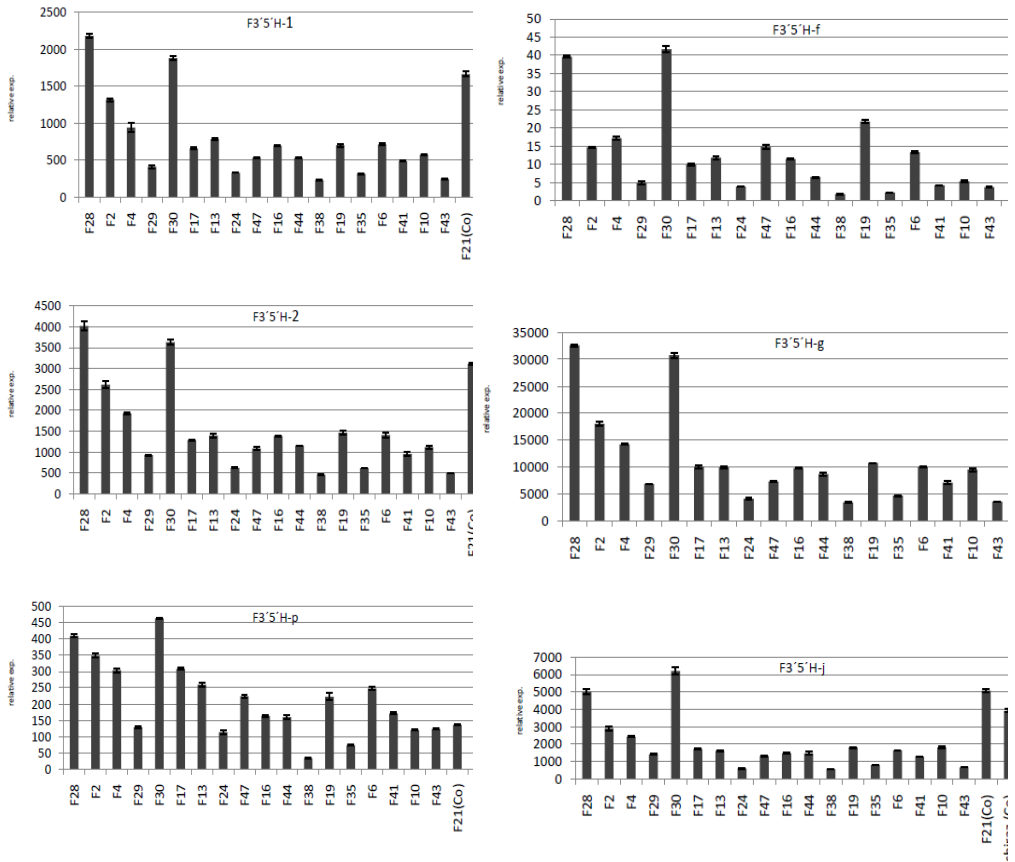


Fig1 Relative expression of different copies of *F3'5'H* studied in transgenic lines and cv. Shiraz

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