Pergamon

0031-9422(95)00949-3

Phytochemistry, Vol. 42, No. 3, pp. 671–672, 1996

0031-9422/96 $15.00 + 0.00

ACYLATED ANTHOCYANINS IN RED FLOWERS OF *HYACINTHUS ORIENTALIS* REGENERATED *IN VITRO*

KEIZO HOSOKAWA, YUKIO FUKUNAGA,* ERI FUKUSHI† and JUN KAWABATA†

Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024, Japan; *Gakken Institute of Plant Technology, 1-2-14 Honson, Chigasaki, Kanagawa 253, Japan; †Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

(*Received in revised form* 7 *November* 1995)

Key Word Index—*Hyacinthus orientalis*; Liliaceae; regenerated flowers; acylated anthocyanins.

Abstract—Nine acylated anthocyanins were produced by flowers of *Hyacinthus orientalis* cv. Hollyhock that had been regenerated *in vitro*. All anthocyanins were identical to those of field-grown flowers. However, there was a significant difference in composition between regenerated and field-grown flowers. The difference disclosed was an increasing amount of cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) in the regenerated flowers.

INTRODUCTION

We have described the anthocyanins in flowers of *Hyacinthus orientalis* L. cv. Delft Blue [1] and cv. Hollyhock [2, 3]. Recently, we developed the anthocyanin production system of regenerated flowers in cv. Delft Blue, which mainly contained delphinidin derivatives, and found that the regenerated flowers produced the same anthocyanins as field-grown flowers [4]. However, in *Impatiens balsamina* anthocyanidins that were not found in intact flowers were produced in *in vitro* culture and the difference occurred in two genotypes which mainly contained pelargonidin derivatives as floral pigments [5]. It therefore seemed necessary to examine the anthocyanin profile of regenerated flowers of hyacinth, whose field-grown flowers contained mainly pelargonidin derivatives, in order to see whether it was anomalous or not. Hence, the anthocyanin profile of the regenerated flowers of cv. Hollyhock was compared with that in field-grown flowers which mainly contained pelargonidin derivatives.

RESULTS AND DISCUSSION

Nine anthocyanins were detected in red regenerated flowers by HPLC. All anthocyanins (**1**–**9**) were identical with those of field-grown flowers with regard to retention times in HPLC (Table 1). However, the composition was significantly different and the difference was an increase of **5** in the regenerated flowers (Table 1). The concentration of anthocyanins produced in the regenerated flowers was *ca* 6.2 times less than that obtained from field-grown flowers (Table 1).

In order to confirm this finding, the anthocyanins of the regenerated flowers were isolated by column chromatography on Amberlite XAD-7, followed by subsequent preparative HPLC and compared with those of field-grown flowers. From the spectral data, **1**–**9** were identified as pelargonidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**1**), pelargonidin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**2**), pelargonidin 3-*O*-(6-*O*-caffeoyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**3**), cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside (**4**), cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside (**5**), pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside (**6**), pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(4-*O*-malonyl-β-D-glucoside) (**7**), pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**8**) and pelargonidin 3-*O*-(6-*O*-feruloyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**9**), respectively. The results demonstrated that all the anthocyanins (**1**–**9**) were the same as those of field-grown flowers as reported previously [2, 3]. Compound **5**, increasing in regenerated flowers, was a cyanidin derivative.

In two genotypes in the studies of *I. balsamina* flowers in which the anthocyanidin was pelargonidin, the petals cultured *in vitro* produced cyanidin and/or peonidin, in addition to pelargonidin [5].

The main anthocyanidin in field-grown flowers of cv. Hollyhock is pelargonidin. On the other hand, the regenerated flowers contain cyanidin in addition to pelargonidin. Our results indicate that enzymes of anthocyanin biosynthesis in the regenerated flowers of cv. Hollyhock are expressed as found in field-grown flowers. However, the activities of particular enzymes

Table 1. Analytical HPLC data for the anthocyanins in the flowers regenerated *in vitro* and field-grown flowers of *Hyacinthus orientalis* L. cv. Hollyhock

	Regenerated flowers		Field-grown flowers	
Anthocyanin	R_t (min)	Concentration ($\mu g\, g^{-1}$ fr. wt flowers)	R_t (min)	Concentration ($\mu g\, g^{-1}$ fr. wt flowers)
1	4.11	18	4.12	66
2	8.76	1	8.61	13
3	11.76	1	11.61	30
4	13.81	6	13.36	145
5	20.86	106	20.81	49
6	23.86	19	23.66	141
7	26.66	4	26.01	21
8	34.34	187	33.61	1602
9	41.60	8	41.16	103
Total		350		2170

vary. In this case the particular enzyme may be flavonid 3′-hydroxylase, a cytochrome P-450 type.

EXPERIMENTAL

Plant material. Bulbs (*ca* 6 cm in diameter) of *H. orientalis* L. cv Hollyhock, obtained from Kaneko Seed Co. (Utsunomiya, Japan) in September 1990, were kept at 5–10° for 2 months. Some bulbs were also planted in the field.

Tissue culture. Regeneration of flowers was initiated from tepal explants of the bulbs treated as mentioned above. The explants were cultured according to the procedures for cv. Delft Blue and the regeneration and the development of flowers were repeated [4]. Anthocyanins were also produced in the regenerated flowers at 15° for 3 weeks on MS [6] agar medium containing 30 g l^{-1} sucrose, 1 mg l^{-1} GA_3 and 0.5 g l^{-1} casamino acid according to the procedure for cv. Delft Blue [4].

HPLC analysis of anthocyanins. A sample (1 g fr. wt) was collected from field-grown flowers at anthesis and from the regenerated flowers that produced anthocyanins as mentioned above. Extraction and HPLC sepn was performed according to methods reported previously [4] except for monitoring by UV–Vis detection at 510 nm. The concns of anthocyanins were calculated from standard linear curves obtained with each isolated anthocyanin.

Isolation of anthocyanins. From freeze-dried regenerated flowers (27 g), anthocyanins were extracted and isolated according to the methods reported previously [2, 3]. Anthocyanins **1**–**9** were obtained as TFA salts: **1** (3.0 mg), **2** (0.3 mg), **3** (0.3 mg), **4** (1.8 mg), **5** (15.7 mg), **6** (9.5 mg), **7** (0.3 mg), **8** (61.6 mg) and **9** (1.9 mg).

Spectral analysis. UV-Vis spectra were recorded in MeOH containing 0.1% HCl. FAB MS were obtained in positive mode with glycerol as matrix. ^{1}H NMR (500 MHz) were obtained using 10% TFA-*d*-methanol-d_4 as a solvent for **1**–**9**. It was confirmed that spectral data for all isolated anthocyanins were identical with those reported previously [2, 3, 7].

Acknowledgement—The authors are grateful to Prof. Masahiro Mii (Faculty of Horticulture, Chiba University) for helpful discussions and for a critical review of the manuscript.

REFERENCES

1. Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. (1995) *Phytochemistry* **38**, 1293.
2. Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. (1995) *Phytochemistry* **39**, 1437.
3. Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. (1995) *Phytochemistry* **40**, 567.
4. Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. *Phytochemistry*, **41**, 1531.
5. Klein, A. O. and Hagen, C. W. (1961) *Plant Physiol.* **36**, 1.
6. Murashige, T. and Skoog, F. S. (1962) *Physiol. Plant.* **15**, 473.
7. Yoshida, K., Kondo, T., Kameda, K. and Goto, T. (1990) *Agric. Biol. Chem.* **54**, 1745.