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# Differences in the floral anthocyanin content of red petunias and *Petunia exserta*

Toshio Ando[a,*], Fumi Tatsuzawa[b], Norio Saito[c], Motoko Takahashi[a], Yuko Tsunashima[a], Hiroyuki Numajiri[a], Hitoshi Watanabe[a], Hisashi Kokubun[a], Ritsuko Hara[d], Hiroko Seki[d], Goro Hashimoto[e]

[a]*Laboratory of Ornamental Plants Science, Faculty of Horticulture, Chiba University, Matsudo City, 648 Matsudo, Chiba 271-8510, Japan*
[b]*Hokkaido Junior College, Takushoku University, Fukagawa, Hokkaido 074-8585, Japan*
[c]*Meiji-Gakuin University, Totsuka, Yokohama 244-8539, Japan*
[d]*Chemical Analysis Center, Chiba University, Inage, Chiba 263-8522, Japan*
[e]*Centro de Pesquisas de História Natural, Rua Jaime Ribeiro Wright 618, Itaquera, São Paulo 08201-970, Brazil*

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**Abstract**

In order to resolve a conflict between previous papers regarding the floral anthocyanins of red flowers of *Petunia exserta*, a naturally occurring species, the HPLC profile of this species was compared with that of commercial red garden petunias. Both HPLC profiles extremely superficially resemble each other in terms of relative amounts and retention times of the major anthocyanins. However, co-elution on HPLC of the mixed sample resulted in clear separation of the components. Three major anthocyanins in red petunias were determined to be cyanidin 3-sophoroside, cyanidin 3-glucoside and peonidin 3-glucoside, which exhibited similar behaviors on HPLC to delphinidin 3-glucoside, delphinidin-3-rutinoside and petunidin 3-rutinoside, respectively, the major floral anthocyanins of *P. exserta*. 

*Keywords:* Red garden petunias; *Petunia exserta*; Solanaceae; Anthocyanins

## 1. Introduction

*Petunia exserta* Stehmann (Solanaceae) is a natural species unique in the genus; it has red flowers (Stehmann, 1987). Regarding the floral anthocyanin composition, however, two contradictory papers were recently published almost simultaneously (Ando et al., 1999a; Griesbach et al., 1999). Both research groups recognized four major anthocyanins in the flower. The HPLC profiles of floral anthocyanins of *P. exserta* provided by Griesbach et al. (1999) were very similar to ours (Ando et al., 1999b), as both groups performed HPLC using similar solvent systems (Strack and Wray, 1989). Furthermore, the relative amounts of the four anthocyanins also resembled each other. The two research groups seemed to have analyzed the same anthocyanins with different conclusions.

For convenience, herein we would like to call the floral anthocyanins of *P. exserta* pigments **E1**–**E4** according to the order of elution on HPLC, even though previous reports described them otherwise (Ando et al., 1999a; Griesbach et al., 1999). We determined pigment **E1** as delphinidin 3-glucoside (8%), **E2** as delphinidin 3-rutinoside (65%), **E3** as cyanidin 3-rutinoside (3%), and **E4** as petunidin 3-rutinoside (5%) based on FAB mass and $^1$H-NMR analysis of pigments isolated from a sample of ca. 100 g dry weight (Ando et al., 1999a); whereas Griesbach et al. (1999) determined pigment **E1** as cyanidin 3-rutinoside (8%), **E2** as cyanidin 3-glucoside (79%), **E3** as pelar-

* Corresponding author. Fax: +81-47-336-1625.
*E-mail address:* andot@midori.h.chiba-u.ac.jp (T. Ando).

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gonidin 3-rutinoside (6%), and **E4** as pelargonidin 3-glucoside (7%), based on HPLC and spectral analysis of a 1 g fresh weight sample.

Griesbach et al. (1999) stated "*Petunia exserta* flowers were the same color and the same anthocyanin composition as *P.* x *hybrida* 'Red Magic' flowers." Unfortunately, 'Red Magic' is an old cultivar registered in 1966, and is not available to us today. It has been replaced with its new version 'Supermagic Red' registered in 1986 (Price, personal communication). In the present study, therefore, we compared the floral anthocyanins of *P. exserta* with a wide range of commercial red cultivars of petunia including 'Supermagic Red' in order to provide additional evidence for the validity of our conclusion mentioned above. In the following consideration, we will refer to commercial cultivars of *P.* x *hybrida* Vilm. having red flowers as solely red petunias.

## 2. Results and discussion

### 2.1. HPLC profiles of anthocyanins from red petunias and P. exserta

Fig. 1A shows a typical HPLC profile of floral anthocyanins of red petunias, which was obtained from a mixed sample of five cultivars ('Fulcon Red', 'Primetime Red', 'Red Carpet', 'Red Titan' and 'Vacara Red'). Four major pigments were evident as assessed at 530 nm, which will be referred to as pigments **R1**–**R4** according to the order of elution.

HPLC profile of red petunias was rather simple in contrast to that of natural species of reddish-purple flower mostly composed of mono- or di-acylated petunidin and malvidin (see Fig. 1 in Ando et al., 1999b). As shown in Table 1, the anthocyanin composition of various red petunias seems to show general features in the order of relative amount of the four major pigments: pigment **R2** (a mean of 61.0%; 38.9–82.8%), **R1** (22.6%; 7.3–41.9%), **R4** (6.6%; 1.9–10.4%), and **R3** (1.5%; 0.2–5.0%). 'Supermagic Red' seemed to be a standard cultivar (**R2**, 67.4%; **R1**, 19.0%; **R4**, 6.9%; **R3**, 0.6%) in this sense. A red petunia 'Humming Scarlet Star' was the exception, having slightly more of pigment **R1** (41.9%) than **R2** (40.7 %).

Fig. 1C shows an HPLC profile of the floral anthocyanins of *P. exserta*. Interestingly, the profile resembled those of the red petunias (Fig. 1A); the largest peak corresponds to pigment **E2** (70.7%) followed by **E1** (8.0%), **E4** (5.8%), and **E3** (3.7%), which seems to follow the general features of the HPLC profiles of the red petunias mentioned above. Retention times of the four respective pigments of *P. exserta* and those of red petunias were very close as well, and the HPLC profile of *P. exserta* was barely distinguishable from that of red petunias.

When the anthocyanin samples of red petunias and *P. exserta* were mixed together and were subjected to HPLC, however, three pairs of pigment (**R2**/**E2**, **R3**/**E3** and **R4**/**E4**) clearly separated into twin peaks (Fig. 1B) using our gradient elution system. Though pigments **R1** and **E1** did not show any sign of splitting, we conclude that the composition of floral anthocyanin of *P. exserta* is not the same as that of red petunias, at least those listed in Table 1. Since the precise nature of none of the floral anthocyanins of red petunias has been unambiguously determined as yet, we have proceeded to structural analysis as follows.

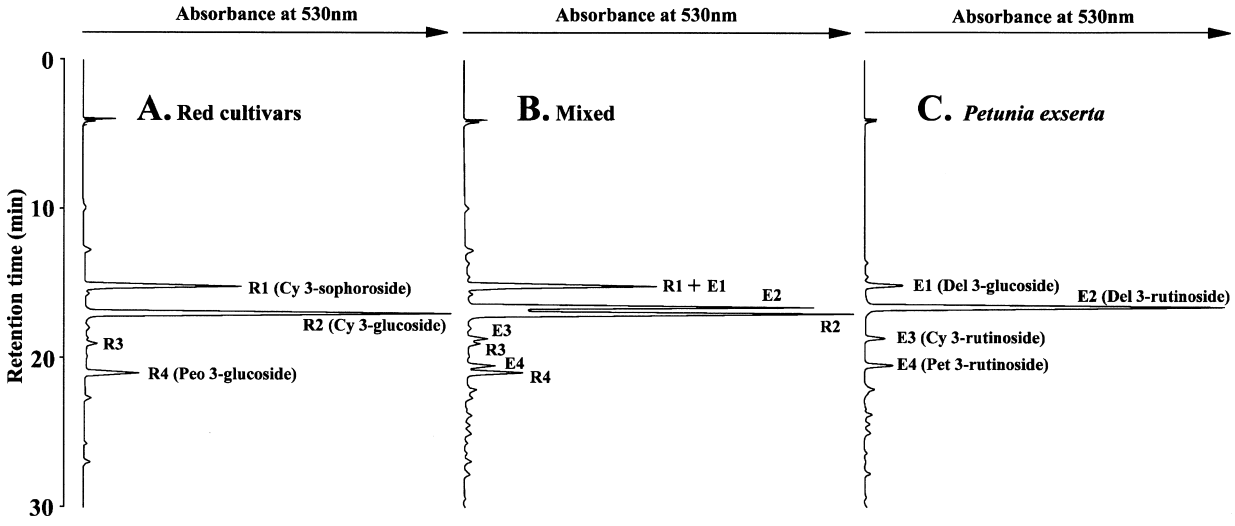


Fig. 1. Comparative HPLC profiles of floral anthocyanins of red petunias (A), *Petunia exserta* (C), and their mixture (B).

### 2.2. Cyanidin 3-glucoside from red petunias

**R2**, the most abundant pigment, gave cyanidin as an aglycone and glucose as the sugar residue on acid hydrolysis. The FAB mass spectrum showed a molecular ion peak at 449 $m/z$. This value is in good agreement with the mass calculated for $C_{21}H_{21}O_{11}$ (449.10833), indicating that **R2** is cyanidin 3-glucoside. By analysis of the chromatographic and spectral data (Table 2), **R2** was determined to be cyanidin 3-glucoside. Further analysis of the $^1$H-NMR spectral data revealed the structure of pigment **R2** to be cyanidin 3-*O*-β-D-glucopyranoside or cyanidin 3-glucoside (see Section 3). Furthermore, **R2** was compared by co-HPLC with the floral anthocyanins of *Cymbidium* Hiroshima Lady 'Sophia' which have been determined to be 3-glucosides of cyanidin and peonidin in a previous paper (Tatsuzawa et al., 1996). The pigment **R2** and cyanidin 3-glucoside of *Cymbidium* co-eluted, yielding a single peak on HPLC.

### 2.3. Cyanidin 3-sophoroside from red petunias

On acid hydrolysis, pigment **R1** gave cyanidin as an aglycone and glucose as the sugar residue. FAB mass spectrometry of **R1** showed a molecular ion peak at 611 $m/z$, in good agreement with the mass calculated for $C_{27}H_{31}O_{16}$ (611.16113) indicating that **R1** is cyanidin diglucoside. By analysis of the chromatographic and spectral data (Table 2), **R1** was proposed to be cyanidin-3-sophoroside.

In order to confirm this structure, the $^1$H-NMR spectrum of **R1** was analyzed as follows (see also Section 3). Six aromatic protons were assigned to be protons of cyanidin as $\delta$ 8.89 (H-4), 8.19 (H-6′), 8.01 (H-2′), 7.11 (H-5′), 7.02 (H-8) and 6.90 (H-6). The proton signals of the sugar moieties appeared in the region of $\delta$ 5.58–2.74. Two anomeric protons were assigned at $\delta$ 5.58 ($d$, $J = 7.3$ Hz, glucose A) and $\delta$ 4.66 ($d$, $J = 7.7$ Hz, glucose B), and all observed coupling constants of these glucose were $J = 7.3$–11.0 Hz, suggesting that

Table 1
Relative amounts of four major floral anthocyanins in commercial red cultivars of petunia

| Cultivar | Source (seed company) | Anthocyanins (%) | | | |
|---|---|---|---|---|---|
| | | **R1** | **R2** | **R3** | **R4** |
| Carnival Scarlet | Takii | 38.2 | 38.9 | 5.0 | 7.6 |
| Cerebrity Red | Bodger | 24.7 | 57.8 | 1.8 | 10.4 |
| Fantasy Red & White | Goldsmith | 38.8 | 44.1 | 2.1 | 4.9 |
| Fulcon Red[a] | Sakata | 14.6 | 69.8 | 1.1 | 7.6 |
| Fulcon Red & White | Sakata | 26.8 | 50.5 | 2.7 | 9.4 |
| Fulcon Red Moon | Sakata | 13.7 | 78.2 | 0.8 | 5.0 |
| Fulcon Red Vein | Sakata | 27.2 | 51.5 | 3.8 | 7.0 |
| Humming Scarlet Star | Takii | 41.9 | 40.7 | 4.2 | 5.6 |
| Polo Red | Novartis | 20.0 | 50.6 | 1.0 | 7.5 |
| Primetime Red[a] | Goldsmith | 20.9 | 64.1 | 1.0 | 8.3 |
| Primetime Red Star | Goldsmith | 16.2 | 75.1 | 0.2 | 2.1 |
| Recoveror Red | Sakata | 7.3 | 82.8 | 0.7 | 5.7 |
| Red Champion (=Ambassador) | Sakata | 25.7 | 59.7 | 1.1 | 5.3 |
| Red Carpet[a] | Pan American | 24.2 | 55.2 | 1.8 | 1.9 |
| Red Cloud | Goldsmith | 20.2 | 65.7 | 1.2 | 5.5 |
| Red Coronet | Sakata | 13.6 | 76.0 | 0.7 | 5.5 |
| Red Dreams | Pan American | 17.0 | 70.1 | 0.9 | 6.4 |
| Red Joy | Goldsmith | 16.4 | 68.8 | 1.5 | 7.7 |
| Red Madness | Pan American | 18.1 | 65.4 | 1.7 | 6.1 |
| Red Picotee | Sakata | 38.0 | 42.7 | 1.7 | 8.5 |
| Red Satin | Bodger | 15.4 | 65.5 | 1.0 | 8.2 |
| Red Titan[a] | Sakata | 32.1 | 52.8 | 1.2 | 6.6 |
| Red & White Titan | Sakata | 17.0 | 70.4 | 0.8 | 6.2 |
| Supercascade Red | Pan American | 15.4 | 65.5 | 1.0 | 8.2 |
| Supermagic Red | Pan American | 19.0 | 67.4 | 0.6 | 6.9 |
| Ultra Red | Goldsmith | 23.7 | 61.0 | 0.7 | 6.5 |
| Ultra Red Star | Goldsmith | 19.8 | 66.8 | 0.8 | 4.9 |
| Vacara Red (=Merlin Red)[a] | Sakata | 27.8 | 52.2 | 1.7 | 8.7 |
| Mean | | 22.6 | 61.0 | 1.5 | 6.6 |
| Standard deviation | | 8.6 | 11.5 | 1.1 | 1.9 |

[a] Cultivars used for the determination of anthocyanins.

these glucose moieties are β-D-glucopyranose forms. By analysis of $^1H$–$^1H$ COSY spectrum of pigment **R1**, a proton signal at $\delta$ 3.95 (H-2 of glucose A) was directly correlated to H-1 proton of glucose A. This proton was assigned to H-2 of glucose A indicating that the OH-2 of glucose A is bound to glucose B through a glycosidic bond. Therefore, **R1** was unambiguously determined to be cyanidin 3-*O*-[2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside] or cyanidin 3-sophoroside.

**R1** was also confirmed to be cyanidin 3-sophoroside by co-elution on HPLC with deacyl anthocyanin (cyanidin 3-sophoroside) of the brownish-red flowers of *Ipomoea purpurea* (L.) Roth., whose major pigment has been determined to be cyanidin 3-diglucosylcaffeoylsophoroside in a previous paper (Saito et al., 1998).

### 2.4. Peonidin 3-glucoside from red petunias

Contrary to our expectation, pigment **R4** gave peonidin as the aglycone and glucose as a sugar residue upon acid hydrolysis. FAB mass spectrometry of **R4** showed a molecular ion peak at 463 *m*/*z* in agreement with the mass calculated for $C_{22}H_{23}O_{11}$ (463.12399). By consideration of the chromatographic and spectral data (Table 2), **R4** was determined to be peonidin 3-glucoside. This structure of **R4** was confirmed to be peonidin 3-glucoside by analysis of HPLC with an authentic sample of peonidin 3-glucoside from *Cymbidium* Hiroshima Lady 'Sophia' as mentioned earlier. However, no NMR spectrum could be obtained, due to the small amount of **R4** obtained.

Since the amount of pigment **R3** obtained was quite limited, further analysis of this pigment was not carried out.

### 2.5. Comparison to the previous papers

Various commercial red petunias are considered to be progeny of 'Fire Chief' (Ando et al., 1999a) or 'Tango' (Griesbach et al., 1999), and are known to be extremely inbred and genetically similar to one another. Similarity in the floral anthocyanin composition of 28 red petunias examined in this study (Table 1) may be an indication of the restricted genetic background.

Because the HPLC profile of floral anthocyanins of red petunias is so characteristic, especially regarding the relative amounts (Fig. 1A and Table 1), it may be useful to compare the HPLC profiles provided by different authors. Since reversed phase chromatography using organic solvent was recommended for isolation of anthocyanins of petunia, Schram et al. (1983) and other researchers including ourselves have been using the system, the order of elution of anthocyanins may also be comparable.

Our HPLC profile of red petunias (Fig. 1A) really resembles that of 'Red Magic' provided by Griesbach et al. (1991, see Fig. 1, whose pigments **3**, **4**, **5**, and **6** seem to correspond to pigments **R1**, **R2**, **R3** and **R4**, respectively, of the present study. Schram et al. (1983) also provided similar HPLC data for a red petunia of their own selection. Their pigments **1** and **2** seem to correspond to our **R1** and **R2**, respectively.

Muszynski (1968) compared the floral anthocyanins of nine red petunias with known anthocyanins using two-dimensional TLC, and concluded that the main pigment is cyanidin glucoside. Schram et al. (1983, their pigment **2**) as well as Griesbach et al. (1991, their pigment **4**) determined that the most prominent anthocyanin of red petunias eluted second on HPLC, and is cyanidin 3-glucoside. We unambiguously confirm it herein (our pigment **R2**).

Table 2
Chromatographic and spectral properties of anthocyanins from red petunias

| Anthocyanin | $R_f$ values (×100) | | | | Spectral data in 0.1% HCl–MeOH | | | $R_t$ (min) | FAB-MS [M+] |
|---|---|---|---|---|---|---|---|---|---|
| | BAW | BuHCl | 1% HCl | AHW | $\lambda_{max}$ (nm) | $E_{440}/E_{max}$ (%) | $AlCl_3$ | | |
| **R1** | 31 | 22 | 36 | 57 | 528,281 | 33 | + | 15.3 | 611 |
| **R2** | 35 | 24 | 7 | 22 | 529,282 | 26 | + | 17.2 | 449 |
| **R4** | 39 | 27 | 8 | 27 | 528,281 | 28 | 0 | 21.3 | 463 |
| Cyanidin 3-sophoroside[a] | 31 | 22 | 36 | 57 | 528,281 | 32 | + | 15.3 | – |
| Cyanidin 3-glucoside[b] | 35 | 24 | 7 | 22 | 529,282 | 27 | + | 17.2 | – |
| Peonidin 3-glucoside[b] | 39 | 27 | 8 | 27 | 528,281 | 28 | 0 | 21.3 | – |
| Cyanidin 3-rutinoside[b] | 36 | 6 | 14 | 34 | 531,282 | 24 | + | 18.6 | – |
| Cyanidin 3,5-diglucoside[c] | 25 | 24 | 16 | 38 | 526,278 | 14 | + | 13.7 | – |

[a] Standard anthocyanin from *Ipomoea purpurea* (Saito et al., 1998).
[b] Standard anthocyanins from *Cymbidium* (Tatsuzawa et al., 1996).
[c] Standard anthocyanin from *Rosa* (Mikanagi et al., 1995).

Muszynski (1968) determined the second most abundant pigment of nine red petunias as cyanidin 3-diglucoside (=cyanidin 3-sophoroside). Schram et al. (1983) determined the second most abundant pigment, which eluted first on HPLC of a red petunia extract (their pigment **1**), to be cyanidin 3-diglucoside. However, Griesbach et al. (1991) suggested it (their pigment **3**) to be cyanidin 3-rutinoside. Our results (our pigment **R1**) supported Muszynski (1968) and Schram et al. (1983) rather than Griesbach et al. (1991). If it is cyanidin 3-rutinoside, there should be characteristic signals (singlet signal at near 4.5 ppm as H-1 of rhamnose, and doublet signal about 1.0 ppm for the protons of $-CH_3$) in the $^1$H-NMR spectrum, but we detected no such signals.

Griesbach et al. (1991) determined that the third most abundant anthocyanin, which eluted fourth (their pigment **6**), was pelargonidin 3-glucoside. However, our results suggest that their pigment **6** is identical to our **R4**, which was determined to be peonidin 3-glucoside in the present study. Schram et al. (1983) did not mention this pigment, nor did Muszynski (1964, 1968) report the existence of pelargonidin in red petunias.

### 2.6. Conclusions

Floral anthocyanins of *P. exserta* and those of red petunias may be nearly indistinguishable when the independently obtained HPLC profiles are compared (Fig. 1C vs. Fig. 1A). Relative amounts and retention times of the respective components are very similar (Fig. 1, Table 1). Thus, *P. exserta* strongly resembles red petunias in terms of the flower color and HPLC profile of the floral anthocyanins. Under such circumstances, we cannot exclude the possibility of erroneous determination of the components. However, their compositions were completely different from each other as shown in the present study. This fact was readily confirmable by high resolution HPLC of the mixed sample. Although pigment **E1** and **R1** did not separate in our system, the remaining pigment pairs, **E2**/**R2**, **E3**/**R3** and **E4**/**R4**, exhibited the respective twin peaks (Fig. 1B).

Pigment **R1** of red petunia was unambiguously determined to be cyanidin 3-sophoroside in the present study, whereas pigment **E1** of *P. exserta* had been determined unambiguously to be delphinidin 3-glucoside in our previous paper (Ando et al., 1999a). The inability of our HPLC system to separate these common and rather simple anthocyanins from each other demonstrates the risk of depending solely on HPLC profiles to identify pigments.

Griesbach et al. (1999) showed the lower pH of corolla tissue of *P. exserta* (pH 5.4) and 'Red Magic' (pH 5.5) as a cause of the red color. Red color in petunia has been considered to be due to the combination of cyanidin glycosides and low pH (de Vlaming et al., 1983; Griesbach, 1996). Actually, red petunias contain a large proportion of cyanidin glycosides (70–92%, pigment **R1** plus **R2** in Table 1) as shown in this study. In contrast, the red color of *P. exserta* is caused by delphinidin (79%, pigment **E1** plus **E2**) and small quantity of petunidin (5%) and cyanidin (3%) (Ando et al., 1999a). The lower pH of corolla tissue should also be responsible for the flower color of this species.

If 'Red Magic' selected by Griesbach et al. (1991) contained pelargonidin (their pigments **5** and **6**) in the flower, it will be a useful parent promising novel colors for garden petunias, since commercial red petunias did not contain detectable amounts of pelargonidin as shown in Table 1 and by Muszynski (1968). If the individual of *P. exserta* used in the work of Griesbach et al. (1999) contained so much cyanidin (87% in total) and pelargonidin (13%) in the flower, it may also be useful as an alternative source of red color in breeding improved red petunias, as they have suggested. However, the segregation of flower color in the second generation of the hybrid between red petunia and our *P. exserta*, having delphinidin as major floral anthocyanidin, is rather complicated as will be reported elsewhere.

## 3. Experimental

### 3.1. Plant materials

Among commercial petunias, 28 cultivars containing the word "Red" or "Scarlet" in the cultivar name were selected, which were donated by respective seed companies in 1991 (Table 1). Source of the seed of *P. exserta* was the same as that used in a previous paper (Ando et al., 1999a). Plants were established following standard floricultural practice. The fresh flowers were collected in June to August in 1991 and 1998, from which corolla-tubes were removed, dried overnight at 37°C, and kept in a refrigerator at about 4°C.

### 3.2. Quantitative analysis of anthocyanins

Fresh corolla-limb (ca. 0.02 g) of each red petunia was extracted with MAW (MeOH–HOAc–$H_2O$, 9:1:10). Quantitative analysis of anthocyanins for these extracts was performed by HPLC (Shimadzu LC-6A) equipped with a diode array detector (Shimadzu SPD-M10A*vp*) on a Waters C18 (4.6$\phi$ × 250 mm) column at 40°C with a flow rate of 1 ml min$^{-1}$, monitoring at 530 nm. The solvent system employed was a linear gradient elution for 40 min from 20 to 85% solvent B (1.5% $H_3PO_4$, 20% HOAc, 25% MeCN in $H_2O$) in solvent A (1.5% $H_3PO_4$ in $H_2O$).

### 3.3. Isolation of anthocyanins

After removing corolla-tube, fresh corolla-limb (ca. 1 kg) of selected red cultivars ('Fulcon Red', 'Primetime Red', 'Red Carpet', 'Red Titan' and 'Vacara Red') was extracted with MAW (2:1:7, 10 l). The extract was concentrated to 500 ml, then purified by Diaion HP-20 CC, PC and HPLC as described previously (Ando et al., 1999a). Solvents used were 15% HOAc, BAW (*n*-BuOH–HOAc–$H_2O$, 4:1:5, upper layer), 10% HOAc–MeOH and MAW for CC and PC. Preparative HPLC was run on a Waters C18 (19ϕ × 150 mm) column at 40°C with a flow rate of 4 ml $min^{-1}$ and monitored at 530 nm, using a linear gradient elution for 40 min from 20 to 85% solvent B in solvent A. The pigment fractions were evaporated in vacuo to dryness. The evaporated residues were dissolved in a small volume of 10% HOAc–MeOH followed by addition of excess $Et_2O$, and then dried to give pigment powder (pigment **R1**, 2.2 mg; **R2**, 5.6 mg; and **R4**, ca. 0.8 mg).

### 3.4. Characterization of anthocyanins

Characterization of pigments was carried out by PC, TLC and UV–Vis spectrometry. Solvents used were BAW, BuH (*n*-BuOH–2 N HCl, 1:1), 1% HCl and AHW (HOAc–HCl–$H_2O$, 15:3:82) for anthocyanins, and *n*-BuOH–HOAc–$H_2O$ (4:1:2), EtOAc–HOAc–$H_2O$ (3:1:1) and EtOAc–HCOOH–$H_2O$ (5:2:1) for sugars. Acid hydrolysis, alkaline deacylation, $H_2O_2$ oxidation and partial acid hydrolysis of anthocyanins were performed according to the standard procedures (Ando et al., 1999a; Harborne, 1984).

FAB mass spectra were recorded on JEOL JMS HX-110A, positive mode, in magic bullet matrix. NMR spectra were recorded at a frequency of 600 MHz for $^1H$ spectra on a JEOL JNM LA 600 in DMSO-$d_6$–DCl (9:1). Chemical shifts are reported relative to a TMS internal standard ($\delta$) and coupling constants ($J$) are reported in Hz.

### 3.5. $^1H$-NMR spectral data (600 MHz, DMSO-$d_6$–DCl)

Pigment **R1** (cyanidin 3-sophoroside): $\delta$ 8.89 (1H, *s*, H-4 of cyanidin), $\delta$ 8.19 (1H, *brd*, $J$ = 8.8 Hz, H-6′ of cyanidin), 8.01 (1H, *brs*, H-2′ of cyanidin), 7.11 (1H, *d*, $J$ = 8.8 Hz, H-5′ of cyanidin), 7.02 (1H, *brs*, H-8 of cyanidin), 6.90 (1H, *brs*, H-6 of cyanidin), 5.58 (1H, *d*, $J$ = 7.3 Hz, H-1 of glucose A), 4.66 (1H, *d*, $J$ = 7.7 Hz, H-1 of glucose B), 3.95 (1H, *t*, $J$ = 8.1 Hz, H-2 of glucose A), 3.68 (1H, *brd*, $J$ = 11.0 Hz, H-6b of glucose A), 3.64 (1H, *t*, $J$ = 8.6 Hz, H-3 of glucose A), 3.57 (1H, *m*, H-5 of glucose A), 3.52 (1H, *m*, H-6a of glucose A), 3.35 (1H, *t*, $J$ = 9.3 Hz, H-4 of glucose A), 3.11 (1H, *t*, $J$ = 8.8 Hz, H-3 of glucose B), 3.06 (1H, *t*, $J$ = 8.6 Hz, H-4 of glucose B), 3.04–3.14 (2H, *m*, H-6a and H-6b of glucose B), 2.96 (1H, *t*, $J$ = 8.3 Hz H-2 of glucose B), 2.74 (1H, *m*, H-5 of glucose B).

Pigment **R2** (cyanidin 3-glucoside): $\delta$ 8.91 (1H, *s*, H-4 of cyanidin), 8.22 (1H, *dd*, $J$ = 2.2, 8.5 Hz, H-6′ of cyanidin), 8.06 (1H, *d*, $J$ = 2.2 Hz, H-2′ of cyanidin), 7.11 (1H, *d*, $J$ = 8.5 Hz, H-5′ of cyanidin), 7.03 (1H, *brs*, H-8 of cyanidin), 6.91 (1H, *brs*, H-6 of cyanidin), 5.37 (1H, *d*, $J$ = 8.0 Hz, H-1 of glucose A), 3.69 (1H, *brd*, $J$ = 10.6 Hz, H-6b of glucose A), 3.52 (1H, *m*, H-6a of glucose A), 3.50 (2H, *m*, H-2 and -5 of glucose A), 3.40 (1H, *t*, $J$ = 9.0 Hz, H-3 of glucose A), 3.26 (1H, *t*, $J$ = 9.0 Hz, H-4 of glucose A).

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