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Anthocyanin Pigments of Red Tetraploid Grape Cultivars (*Vitis vinifera* L. \times *V. labrusca* L.)

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The anthocyanin pigments of red-fruited tetraploid grapes, 'Red Queen', 'Olympia', 'Beni Zuihō', 'Beni Fuji', 'Ryuhō', and 'Honey Red' were examined by thin-layer chromatography and reflection densitometry. Eight spots were separated, and they were identified as pigments by their *R_f* values, molybdate shift and color reactions in the visible and ultra-violet regions. 'Ryuhō' contained five anthocyanins; decreasing order of concentration, cyanidin-3-monoglucoside acylated with p-coumaric acid, cyanidin-3-monoglucoside, delphinidin-3-monoglucoside, acylated with p-coumaric acid and delphinidin-3,5-diglucoside, 'Beni Zuihō' contained six, the same as 'Ryuhō' plus cyanidin-3, 5-diglucoside, 'Beni Fuji' and 'Honey Red', the same as 'Beni Zuihō' plus petunidin-3-monoglucoside, 'Red Queen', the same as 'Beni Fuji' plus peonidin-3, 5-diglucoside but lacked delphinidin-3, 5-diglucoside, and 'Olympia', the same as 'Red Queen', lacking delphinidin-3, 5-diglucoside acylated with p-coumaric acid.

The aglycone level pigments were cyanidin and delphinidin in 'Beni Zuihō' and 'Ryuhō', and cyanidin, delphinidin and petunidin in 'Beni Fuji' and 'Honey Red'. Peonidin was also present in 'Red Queen' and 'Olympia'. All cultivars lacked malvidin. The red tetraploid grape cultivars which lack malvidin may be useful as breeding materials for fresh red-skinned large berries.

INTRODUCTION

A fresh red skin color is visually very appealing in grape berries. The Ikawa new hybrid grape cultivars (*Vitis vinifera* L. \times *V. labrusca* L.) show a promise for contributing varicolored fruits for marketing. Because of their high pulp quality and large berry size, the red skinned cultivars are well suited for viticulture under structures in Japan.

Anthocyanins are the principal phenolic substances of grape berries (Rankine et al. 1958). In a recent studies, a rapid visual estimation of anthocyanin contents of muscadine grapes on thin-layer chromatography plates indicated a potential for screening seedlings in a breeding program for quality of wine colors (Nesbitt et al. 1974).

Our objectives were to identify the anthocyanin constitutions and to determine accurately the quantities of the anthocyanins in a number of tetraploid cultivars of red large-size berries. These investigations should provide a technique to study the inheritance of anthocyanins responsible for beautiful color, and also supply additional background for the development of methods to identify fruits of red tetraploid seedlings capable of producing red color grapes.

MATERIALS AND METHODS

Ripe grapes of six cultivars, 'Olympia', 'Red Queen', 'Beni Zuihō', 'Beni Fuji', 'Ryuhō' and 'Honey Red' were harvested in 1983 at the Fukuoka Agricultural Research Center in Fukuoka.

The methods of sample treatment, isolation of anthocyanins and measurement of the optical densities of the separated spots were the same as in previous reports (Shiraishi *et al.* 1986, 1988).

RESULTS AND DISCUSSION

Figure 1 shows the parentage of the red tetraploid grapes. Each of them could be easily distinguished by its characteristic color. Figs 2 to 6 show two-dimensional

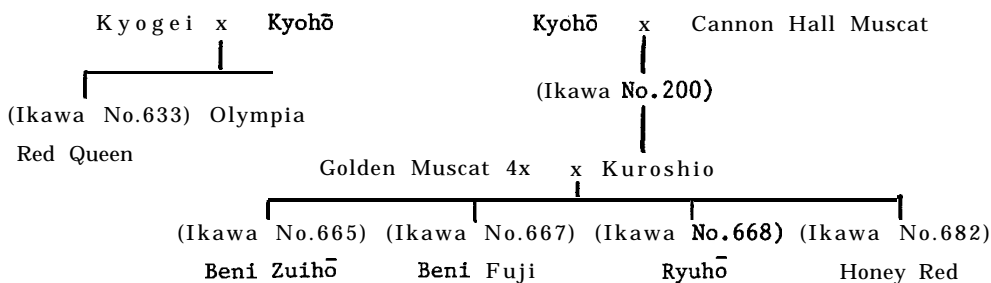


Fig. 1. Parents of the red tetraploid grape cultivars.

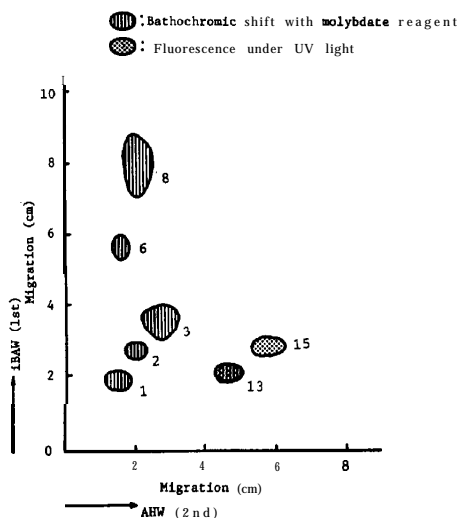


Fig. 2. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Red Queen'.

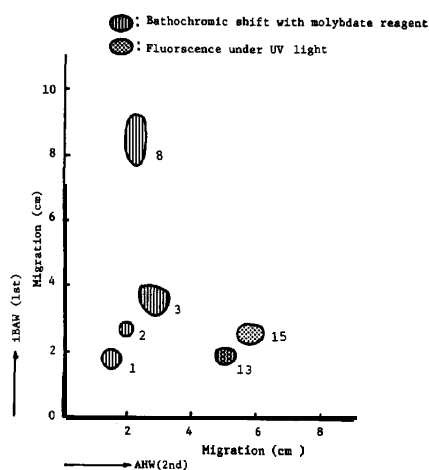


Fig. 3. A two-dimensional thin-layer chromatogram of the anthocyanins 'Olympia'.

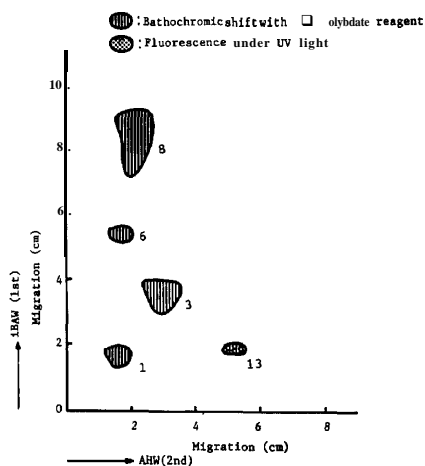


Fig. 4. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Ryuhō'.

thin-layer chromatograms of the anthocyanins extracted from 'Red Queen', 'Olympia', 'Ryuhō', 'Beni Zuihō' and 'Hony Red', respectively. Pigments 13 and 15 exhibited fluorescence, indicating that sugar residues might be attached to the 5 position in ring A of the anthocyanin (Harbone 1967). Pigments 1, 2, 3, 6 and 13 changed color when sprayed with molybdate reagent, indicating the derivatives of the cyanidin, delphinidin and petunidin groups.

Table 1 lists the R_f values of the anthocyanins in the grape cultivars examined in two solvent systems, a iBAW and AHW, and color reactions, visible and ultra-violet light and molybdate reagent shift.

As listed in Table 2, eight anthocyanins were identified from its position of the

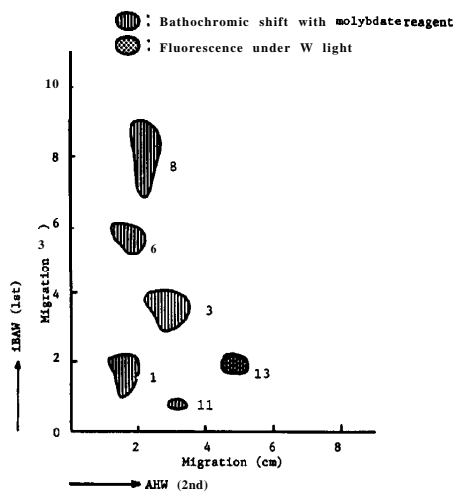


Fig. 5. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Beni Zuihō'.

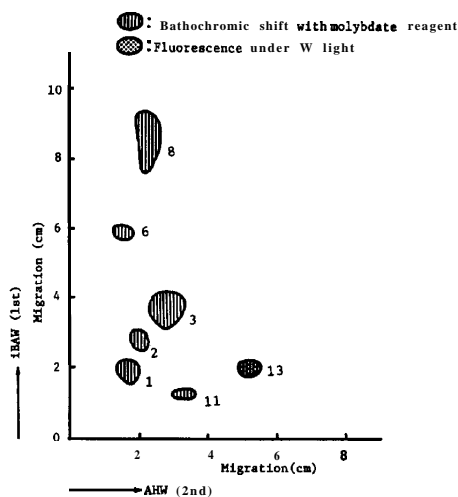


Fig. 6. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Honey Red'.

pigments on chromatograms, R_f values and color reactions, and the anthocyanin constitutions of each cultivars are summarized in Table 3. Table 4 shows the aglycone constitutions of the six cultivars.

Aglycones identified in 'Red Queen' and 'Olympia' were cyanidine, delphinidin, peonidin and petunidin. Those in 'Beni Fuji' and 'Honey Red' were cyanidin, delphinidin, the last one was in small amounts. 'Beni Zuihō' and 'Ryuhō' contained only two aglycones, cyanidin and delphinidin. The major aglycone in these cultivars was

Table 1. Comparison of *Rf* values and colors of individual anthocyanins in examined grapes

Pigment No.	<i>Rf</i> value ($\times 100$) at 25°C		Color in		Molybdate shift
	iBAW	AHW	visible light	UV light	
1	11	8	Purple	Dull purple	+
2	17	11	Reddish purple	Dull purple	+
3	21	14	Red	Dull purple	+
6	41	8	Purple	Dull purple	+
8	48	14	Red	Dull purple	+
11	5	20	Purple	Dull purple	+
13	14	34	Red	Fluor. red	+
15	20	38	Pink	Fluor. pink	

Fluor.: Fluorescence

Table 2. Identification of the anthocyanins in examined grapes.

Pigment No.	Identification	Abbreviation
1	Delphinidin-3-monoglucoside	(DI-3G)
2	Petunidin-3-monoglucoside	(Pt-3G)
3	Cyanidin-3-monoglucoside	(Cy-3G)
6	Delphinidin-3-monoglucoside acylated with <i>p</i> -coumarate	(DI-3G-Cm)
8	Cyanidin-3-monoglucoside acylated with <i>p</i> -coumarate	(Cy-3G-Cm)
11	Delphinidin-3, 5-diglucoside	(DI-3, 5DG)
13	Cyanidin-3, 5-diglucoside	(Cy-3, 5DG)
15	Peonidin-3, 5-diglucoside	(Pn-3, 5DG)

Table 3. Percentages of constituent anthocyanins in the skins of examined grapes.

Pigment No.	Abbreviation of Anthocyanin	Estimated percent of total anthocyanin content					
		Red Queen	Olympia	Ryuhō	Beni Zuihō	Beni Fuji	Honey Red
1	DI-3G	9	12	13	18	14	12
2	Pt-3G	7	5			4	9
3	Cy-3G	22	26	26	25	16	27
6	DI-3G-Cm	7		9	14	7	7
8	Cy-3G-Cm	33	32	45	32	40	30
11	DI-3, 5DG			8	3	5	6
13	Cy-3, 5DG	9	11		9	14	9
15	Pn-3, 5DG	13	15	—			

cyanidin, and next important was delphinidin or peonidin. Peonidin was minor or absent. Malvidin was entirely lacking in these cultivars.

An attractive appearance such as large berry size, bright color or unusual shape are needed for table or dessert grapes (Einset 1975). The grape cultivars examined in this study are tetraploids, having large-sized berries with red skin color. The genetics

Table 4. Aglycone constituents of the red tetraploid grapes.

Aglycone	Estimated percent of total aglycone content					
	Red Queen	Olympia	Ryuhb	Beni Zuihō	Beni Fuji	Honey Red
Delphinidin	16	12	30	35	26	25
Petunidin	7	5			4	9
Cyanidin	64	69	70	66	70	66
Peonidin	13	15				

of grape skin color have been reviewed recently by Durquety and Barritt *et al.* Durquety (Durquety 1957) proposed two gene pairs governing anthocyanin development : G controlling diglucosides or G controlling monoglucosides, and 0 being specific for diphenols. Barritt *et al.* (Barritt *et al.* 1969) proposed two pairs of genes with epistatic action, of which B was a dominant gene for black fruit, and R was a dominant gene for red, and white-fruited grapes were considered to be recessive for both genes.

Crosses between, white-and black-fruited cultivars are expected to produce only white-or black-fruited progeny (Barritt *et al.* 1969 : Snyder *et al.* 1952 : Wellington 1939). Though 'Red Queen' and 'Olympia' are hybrids of 'Kyogei' (White) and 'Kyohō', and 'Ryuhō', 'Beni Zuihō', 'Beni Fuji' and 'Honey Red' are hybrids of 'Golden Muscat 4x' (white) and 'Kuroshio' (black), they are all red. Gene pairs proposed by Barritt *et al.* could not account for red progenies, satisfactorily. These six cultivars contained high amount of cyanidin and lacked malvidin. Methylation of anthocyanins may be related to dull skin color of red grapes (Ribérea-Gayon 1958). 'Ryuhō' and 'Beni Zuihō', which did not contained methylated anthocyanins, petunidin and peonidin, are fresh red compared to 'Red Queen' and 'Olympia', which contained methylated anthocyanins and show dull red. 'Beni Fuji' and 'Honey Red', which are red or fire-red, also lacked peonidin and contained a minor amount of petunidin.

The results show that the red skin color seems to depend mainly on the presence of cyanidin and on the lack of malvidin. In breeding of red tetraploid cultivars, parents should be restricted to those which contain high amounts of cyanidin and delphinidin, and lack methylated anthocyanidin, malvidin, petunidin and peonidin.

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