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Summary

The anthocyanins of the tetraploid blue-black grapes, ‘Pione’, ‘Izunishiki’ and ‘Black Olympia’ were extracted with 1% HCl-methanol, and were purified by the lead acetate method. The individual pigments were separated by thin-layer chromatography. They were identified by their *Rf* values, molybdate shift and color reactions in the visible and ultra-violet regions. The co-existence of mono- and diglucosides, and the presence of *p*-coumaric acyl groups were indicated in all cultivars. The pigments in ‘Black Olympia’ consisted of eight anthocyanins as follows: delphinidin-, petunidin- and malvidin-3-monogluco- and acylated with *p*-coumaric acid, and malvidin-3,5-diglucoside and acylated with *p*-coumaric acid. ‘Izunishiki’ contained ten anthocyanins, those of ‘Black Olympia’ plus petunidin- and delphinidin-3,5-diglucosides with *p*-coumaric acids. ‘Pione’ contained fourteen anthocyanins, the same ten as ‘Izunishiki’ plus cyanidin- and peonidin-3-monoglucosides, and petunidin- and delphinidin-3,5-diglucosides. The aglycone level pigments, in decreasing order of concentration, were malvidin, delphinidin, petunidin, cyanidin and peonidin, in ‘Pione’.

In ‘Izunishi’ and ‘Black Olympia’, the aglycones were in about the same order, but cyanidin and peonidin were lacking.

Introduction

Grapes which are used fresh as food or table decoration are generally called table or dessert grapes, and for them an attractive appearance is required, such as large size of berry and bright color(3). ‘Kyoho’ grape, a very large-sized cultivar, and other tetraploid grapes have been used to obtain 4× progeny, such as ‘Pione’ and ‘Izunishiki’ in Japan. These tetraploid cultivars were bred by Ikawa and others, and have contributed extensively to viticulture and grape commerce. In particular, ‘Pione’ (‘Kyoho’ × ‘Cannon Hall Muscat’, a blue-black hybrid cultivar) and ‘Izunishiki’ (another seedling of the cross between ‘Kyoho’ and ‘Cannon Hall Muscat’, a blue-black and the largest-fruited cultivar) are extremely high quality grapes. ‘Black Olympia’ (‘Kyogei’ × ‘Kyoho’, a blue-black hybrid cultivar) has black skins when ripened also at high temperature.

This work was carried out for the identification of anthocyanin pigments in black skinned berries of the three tetraploid grape cultivars.

Materials and Methods

Ripe grapes of ‘Pione’ were harvested from the vineyard of the Fukuoka Agricultural Research Center, ‘Izunishiki’ from Kyushu University in Fukuoka, and ‘Black Olympia’ from Kurume-city in Fukuoka, 1984. In all cases, the clusters of grapes were brou-
ght immediately from the vineyards to the laboratory and were tested. The methods of sample treatment and isolation of anthocyanins were the same as in a previous report(6).

Photodensitometry of separated anthocyanin spots on thin-layer chromatoplates: The optical densities of the separated anthocyanin spots were measured by reflection densitometry at 535 nm with a Shimadzu dual-wavelength chromatogram scanner, Model CS-910, which combines two methods, the dual-wavelength and spot beam zigzag scanning. The dual-wavelength was set at 700 nm for compensating the background level.

Results and Discussion

Figure 2 is a tracing of a two-dimensional thin-layer chromatogram of the anthocyanins extracted from 'Pione'. The pigments separated on the chromatoplate appeared clearly. The fourteen spots are the anthocyanins which appeared on all thin-layer chromatograms repeated five times. Pigments 14 and 19 exhibited fluorescence, indicating that sugar residues might be attached to the 5-position in ring A of the anthocyanidins(4). Pigments 1, 2, 3, 6, 7, 11, 12, 16 and 17 shifted from bright to dull color when the plate was sprayed with molybdate reagent. The test enables a general differentiation to be made.

Fig. 2. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Pione' grape skins.

Fig. 3. A two-dimensional thin-layer chromatogram of the anthocyanidins in 'Izunishiki' grape skins.

Fig. 4. A two-dimensional thin-layer chromatogram of the anthocyanidins in 'Black Olympia' grape skins.
among the derivatives of the cyanidin, delphinidin and petunidin groups, which show a positive color change, and those of the peonidin and malvidin groups, which show no color change (1). Figures 3 and 4 show tracings of spots separated from 'Izunishiki' and 'Black Olympia', respectively.

Table 1 indicates the $R_f$ values of the anthocyanins in the examined grape cultivars in two solvent systems, $i$BAW and $A$HW, color reactions in visible and ultra-violet light, and molybdate shift.

Based on the position of the pigments on the two-dimensional chromatograms and on color reactions, the presence of fourteen anthocyanins is expressed. Their identifications based on $R_f$ values and color reactions are showed in Table 2, and the anthocyanin constitution of each cultivar is summarized in Table 3. Table 4 shows the aglycone constitution of the three tetraploid grape cultivars.

The aglycone constituents of 'Pione' are malvidin, delphinidin, petunidin, cyanidin and peonidin, and those of 'Izunishiki' and 'Black Olympia' are the same five ones minus cyanidin and peonidin.

The present study indicates that the grape color of 'Black Olympia' consisted of monodiglucosides and the three aglycone level
pigments, malvidin, petunidin and delphinidin. The presence of \(p\)-coumaric acyl groups in the pigments is also indicated.

'Izunishiki' contains ten anthocyanins, those of 'Black Olympia' plus petunidin-3,5-diglucoside acylated with \(p\)-coumarate and delphinidin-3,5-diglucoside acylated with \(p\)-coumarate.

There are fourteen anthocyanins in 'Pione', the same ten as in 'Izunishiki' plus cyanidin-3-monoglucoside, peonidin-3-monoglucoside, petunidin-3,5-diglucoside and delphinidin-3,5-diglucoside.

These tetraploid blue-black grape cultivars, hybrids of 'Kyohō' and 'Kyogei' or 'Cannon Hall Muscat', are most useful for table grapes when cultivated under structures in Japan. Paticularly, 'Pione' and 'Izunishiki' are deep blue-black and owe their high quality to their pollen parent, V. vinifera cultivar 'Cannon Hall Muscat'. Based on their anthocyanin constitution, the deep blue-black skin colors of these grape cultivars seem to be promoted by the presence of malvidin-derived anthocyanins.

Color development of grapes has some problems. First, the skin color of the blue-black hybrid cultivars tends to be lighter at high temperature after veraison of berries. Therefore, it is necessary to study the processes of anthocyanin biosynthesis during maturation of grape berries at high temperature conditions. And it must be established whether the distribution of grape pigments is constant after veraison and during ripening, or whether the monoglucosides appear first and the acylated pigments follow.

Second, the color development of grapes infected with virus is generally insufficient for marketability. It is necessary to clarify the mechanism of this effect.

**Literature Cited**

4. **Harbone, J. B.** 1967. Comparative bioche-
ANTHOCYANIN PIGMENTS IN THE BLUE-BLACK TETRAPLOID GRAPE CULTIVARS


摘 要

4 倍体の黒色系ブドウ品種 ‘ブラック・オーリンピア’, ‘ピオーネ’, ‘伊豆錦’ (Vitis vinifera L. × V. labrusca L.) における アントシアニン色素について

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‘伊豆錦’には10種のアントシアニンが存在し、‘ブラックオリンピア’に含まれる8種のアントシアニンに、ペチュニン、デルフィニンの3,5-ジグルコシドのアシル化色素が加わった色素構成であった。

‘ピオーネ’は14種のアントシアニンを含み、‘伊豆錦’にシアニジン、ペオニンの3-モノグルコース、ペチュニン、デルフィニンの3,5-ジグルコシドが加わった色素構成であった。

‘ピオーネ’中のアグリコンは、マルビジン、デルフィニン、ペチュニンの順に減少し、少量のシアニジン（5%）、ペオニン（4%）が存在した。‘伊豆錦’と‘ブラックオリンピア’のアグリコンは同一で、シアニジン、ペオニンを欠いていた。