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Polyphenolic diversity and characterization in the red–purple berries of East Asian wild *Vitis* species

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ABSTRACT

Grapes (*Vitis* spp.) produce diverse polyphenolic compounds, which are phytochemicals that contribute to human health. In this study, the polyphenolic profiles of the red–purple berries of two wild grape species native to Japan, *Vitis ficifolia* and *V. coignetiae*, and their interspecific hybrid cultivars were investigated and compared with the profiles of *V. vinifera* and *V.* × *labruscana* cultivars. Proanthocyanidins (PAs) were present at lower concentrations in both skins and seeds of wild grape species and their hybrid cultivars than those in *V. vinifera* cultivars. They also differed in their composition, consisting mainly of epicatechin in wild grape species, but containing considerable amounts of both epigallocatechin in the skins and epicatechin gallate in the seeds of *V. vinifera*. In contrast, *V. ficifolia* varieties and their hybrid cultivars accumulated high concentrations of diverse anthocyanins, and whose compositions of anthocyanins and flavonols differed between species in their degree of modification by glucosylation, acylation, methylation and B-ring hydroxylation. Principal component analysis (PCA) indicated that the polyphenolic constituents clearly separate *V. vinifera* and *V.* × *labruscana* cultivars from the wild grape species as well as between wild grape species, *V. coignetiae* and *V. ficifolia*. Intermediate compositions were also observed in the hybrid cultivars between these wild grape species and *V. vinifera*.

1. Introduction

Grapes are horticultural crops that accumulate diverse phenolic compounds, such as flavonoids (anthocyanins, proanthocyanidins (PAs) and flavonols), hydroxycinnamates and stilbenes. Flavonoids are one of the most abundant and important subgroups of phenolic compounds, potentially contributing to human health through their antioxidant, antimicrobial and anticarcinogenic characteristics (Jackson, 2000). They are also contributors to organoleptic qualities of grapes and foods made from them, such as wine (Cheynier, 2005).

Each class of flavonoid (anthocyanin, PA and flavonol) is biosynthesised via enzyme reactions branching from the common flavonoid pathway that starts from the conversion of phenylalanine to cinnamic acid and downstream derivatives, and which accumulate mainly in grape berry skins and seeds (Adams, 2006). Anthocyanins are responsible for the red and purple colours that accumulate in the berry skins of red varieties during ripening. Their aglycones are glucosylated at the C3 position or at both the C3 and C5 positions, and some are further acylated at the C6″ position of the glucose moiety with functional groups such as acetyl, *p*-coumaroyl and caffeoyl groups **(1–26)** (Fig. 1A). These modifications affect the color properties of anthocyanins, as well as their stability against light and heat (Hrazdina et al., 1970; Jackman and Smith, 1996; Yokotsuka and Singleton, 1997). Flavonols are pale yellow pigments that act as co-pigments of anthocyanin in red wine (Boulton, 2001). They are found in berry skins mainly as glycosides of quercetin **(27–29)** and myricetin **(30)** (Fig. 1B; Mattivi et al., 2006). PAs, which are polymers of flavan-3-ol units, such as catechin (C) **(32)**, epicatechin (EC) **(33)**, epigallocatechin (EGC) **(34)** and epicatechin-3-*O*-gallate (ECG) **(35)**, are abundant flavonoids that are present in both the skins and seeds (Fig. 1C) and are responsible for the bitter and astringent properties of red wine (Vidal et al., 2003). The subunit composition of PAs can be determined using an analytical method that involves acid depolymerisation in the presence of excess phloroglucinol (phloroglucinolysis) (Kennedy

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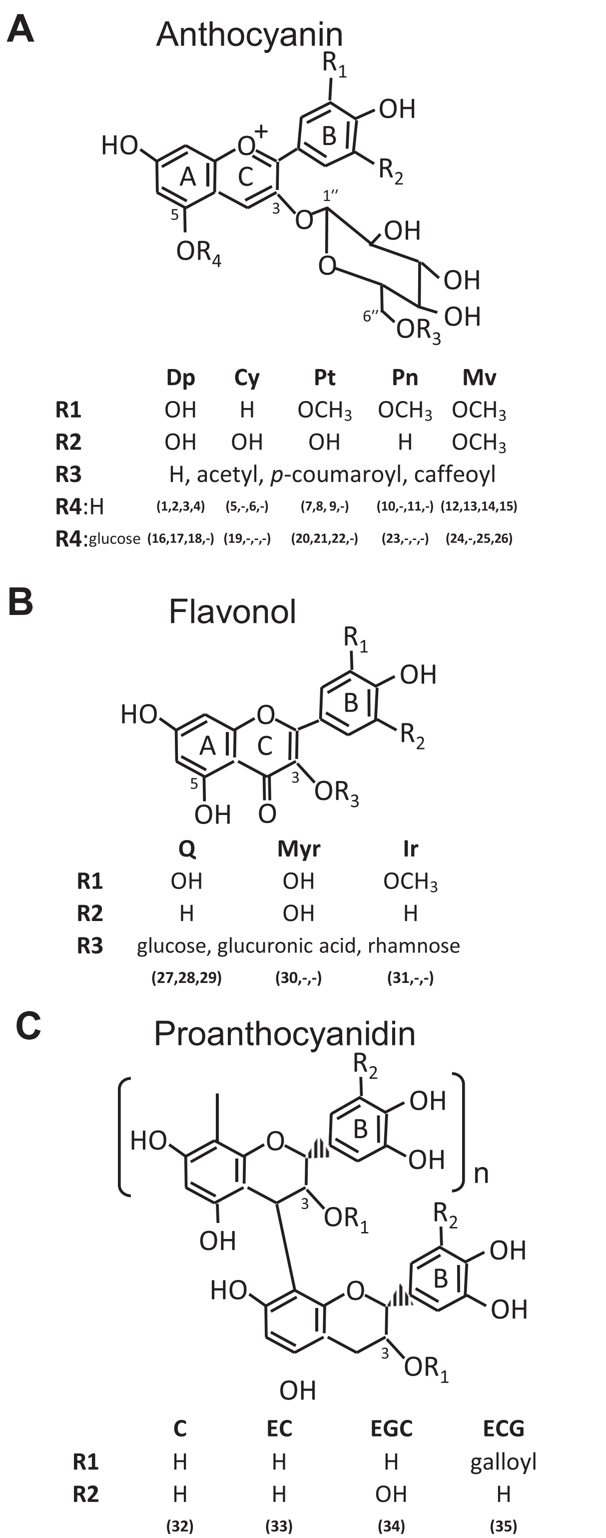

Fig. 1. The chemical structures of the (A) anthocyanins, (B)flavonols and (C) proanthocyanidins detected in the grape accessions used in this study. The number of anthocyanin with H, acetyl, *p*-coumaroyl, or caffeoyl group at R3 position is shown in parentheses in this order. Similarly, the number of flavonol with glucose, glucuronic acid, or rhamnose at R3 position is shown in parentheses in this order. A dash means corresponding chemical was not detected. Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; Q, quercetin; Myr, myricetin; Ir, isorhamnetin; C, catechin; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin-3-*O*-gallate.

and Jones, 2001). Based on the mean degree of polymerisation (mDP), grape PAs appear to be highly polymerised, and this is supported by observations using chromatographic methods such as normal-phase HPLC (Koyama et al., 2007). Differences in PA composition potentially influence wine sensory properties as shown by the decline in overall astringency of wine with decreasing mDP and the rise in the coarseness of wine with increased galloylation and decreased trihydroxylation of the B-ring (Vidal et al., 2003). Skin PAs are characterised by the presence of EGC **(34)** units and have a higher mDP and a lower proportion of galloylated subunits than seed PAs, which are thought to confer a pleasant organoleptic character.

It has been reported that the concentrations and composition of flavonoids in grapes are under genetic control (Mazza, 1995; Huang et al., 2012; Ban et al., 2014; Costantini et al., 2015; Malacarne et al., 2015). Thus, although various environmental and agronomical conditions, such as vintage, production area and viticultural practices, affect their total concentration to some extent (Downey et al., 2006; Jackson and Lombard, 1993), their composition within a cultivar is relatively stable. Consequently, both anthocyanin and flavonol profiles have been used for the taxonomic classification of similar cultivars (Mattivi et al., 2006; Ortega-Regules et al., 2006; Figueiredo-González et al., 2012). Few studies, however, have investigated whether the PA profiles of grape skins and seeds can be used to distinguish cultivars, especially those with a high (>3) degree of polymerisation (Lago-Vanzela et al., 2011; Narduzzi et al., 2015).

European grapes (*Vitis vinifera*) are the most widely cultivated grape species in the world (Alleweldt et al., 1990). However, the grapes of this species are highly susceptible to fungal diseases and so they have failed to adapt well to the wet climate in Japan. Consequently, hybrid cultivars between the American hybrid cultivar *V.* × *labruscana* and *V. vinifera* were created (Kawase, 1996), of which Muscat Bailey A and Black Queen are commonly used for red wine making in Japan.

The genus *Vitis* also includes uncultivated wild species, several of which (e.g. *V. amurensis*, *V. coignetiae* and *V. ficifolia*) are native to East Asian countries (Li et al., 1992). These wild grapes are adapted to the growing conditions in Japan and possess unique characteristics in terms of their resistance to diseases and undesirable environmental conditions (e.g. low and high temperature). Thus, these wild species are considered to be important genetic resources for the varietal improvement of cultivated grapes such as *V. vinifera*, and several interspecific hybrid cultivars have been developed. However, these wild resources have not been sufficiently explored for the improvement of fruit quality-related traits of cultivated grapes, partly due to a lack of knowledge about their potential contribution to this, including their polyphenolic compositions. Mochioka et al. (1995) successfully classified the 10 wild grape accessions native to Japan using the profiles of partially identified anthocyanins in their berry skins. However, there is only limited information about the composition of other classes of phenolic compounds in these wild grapes and their hybrid cultivars (Poudel et al., 2008).

Therefore, in this study, the polyphenolic profiles of the red–purple grape berries of two wild grape species native to Japan (*V. ficifolia* and *V. coignetiae*) and their hybrid cultivars were comprehensively investigated, and compared with the profiles of *V. vinifera* and *V.* × *labruscana*. The aim was to elucidate the PA profiles and the anthocyanin and flavonol compositions in the skins and seeds of wild grapes to distinguish the species and characterise their potential for improving the quality of wine grapes.

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