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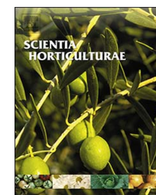

Research Paper

Varietal differences in polyphenol contents, antioxidant activities and their correlations in table grape cultivars bred in Japan

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ABSTRACT

To enhance the knowledge of polyphenol content variability in table grape breeding, the polyphenol content and other fruit quality traits (berry weight, skin color, L* value, anthocyanin content, soluble solids content and titratable acidity) were assessed during the ripening process, and varietal differences in polyphenol contents and antioxidant activities among table grape cultivars bred in Japan were examined. Based on the ripening patterns of an array of individual fruit quality traits, 90 d after full bloom was selected as the optimum time to evaluate the polyphenol content because no remarkable developmental changes were occurring. An analysis of variance showed that genotypic variance was high in the polyphenol content in association with small or negligible variance levels associated with ripening times, genotype × ripening time interaction, among vines within genotype, and vine × ripening time interaction. The repeatability value of the polyphenol content was high, as were the correlation coefficients between the values for each year and the means of 3 years. The polyphenol content was greater in the black- and red-skinned cultivars than in white-skinned cultivars. A linear positive correlation was observed between the polyphenol content and antioxidant activity. Increased polyphenol contents in grape berries will enhance the economic value of newly released cultivars of fresh and processed grapes. In future table grape-breeding projects, colored cultivars should be considered as promising breeding stocks because of their high polyphenol contents.

1. Introduction

Fruit quality traits are crucial factors that influence fruit crop breeding programs. The most commercially important fruit traits of table grapes (*Vitis* spp.) are the berry ripening time, berry weight, soluble solids content (SSC) and titratable acidity. Moreover, commercial acceptance of table grape cultivars depends largely on the balance of SSC and titratable acidity (Winkler et al., 1974). However, there is an increasing interest in functional components of grape berries, such as resveratrol (Jang et al., 1997; Tao et al., 2004), anthocyanin (Frankel et al., 1998; Jing et al., 2008), gamma-aminobutyric acid (Di Cagno et al., 2010), and polyphenol (Orak, 2007) content, which may promote human health. In particular, the demand for naturally occurring phenolic compounds has increased in grape berries and their processed products because of the antioxidant effects on the human body (Macheix et al., 1990; Palomino et al., 2000; Singleton, 1982).

Antioxidant compounds absorbed from the human diet may have roles in preventing cardiovascular and chronic diseases that result from oxidative stress (Joshipura et al., 2001). Oxidative stress is induced by free radical attacks on cellular components by active oxygen species. Antioxidants, which scavenge active oxygen, are considered important for reducing the initiation and progression of these diseases. Fruit and vegetables are excellent sources of antioxidant phytochemicals, such as polyphenols, carotenoids, vitamins and thiol compounds. The polyphenols are hypothesized to function as antioxidants directly, or to influence the production of other antioxidant compounds, in the body. Thus, the improvement of fruit quality and increase in phenolic compound levels in grape berries will enhance the economic value of newly released fresh and processed grape cultivars.

Grape-breeding programs have existed on a worldwide level since the early 20th century (Reisch and Pratt, 1996). A large proportion of the grapes produced in Japan is consumed as table grapes, but another proportion is used to make wine (Shiraishi, 2006). In general, improvements in table grape cultivars have focused on berry weight, SSC and titratable acidity using genetic and environmental variations associated with these traits (Firoozabady and Olmo, 1987; Sato et al., 2000; Shiraishi et al., 2012; Wei et al., 2002; Wolpert et al., 1980). However, the potential for the genetic manipulation of the polyphenol content, except anthocyanins, in grape berries remains largely unknown (Liang et al., 2009).

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To enhance the knowledge of polyphenol content variability in table grape breeding, (1) the changes in polyphenol content, together with other fruit quality traits (berry weight, skin color, L* value, anthocyanin content, SSC and titratable acidity) of grape berries during ripening process, were monitored to determine an optimum sampling time and vine replication, and (2) examined varietal differences in polyphenol contents and antioxidant activities among table grape cultivars bred in Japan were assessed.

2. Materials and methods

2.1. Experiment 1(Optimum sampling time for, and vine replication of, the polyphenol content)

Four table grape cultivars (*Vitis labruscana* × *Vitis vinifera*) commercially grown over Japan were chosen. Cultivars of black-skinned 'Kyoho', 'Pione', 'Suzuka', and white-skinned 'Shine Muscat' were grown at Fukuoka Agricultural and Forestry Research Center, Chikushino, Fukuoka in Japan (33°50′N and 130°57′E) in 2012. Three six-year-old vines per cultivar, grafted on Kober 5BB rootstock were planted in an east–west orientation with 1.0-m spacing within rows in a greenhouse. Each vine was trained on a horizontal trellis system and spur-pruned. The soil type was sandy loam and was uniformly fertilized on an annual basis with 80 kg nitrogen (N) ha^{-1}. The vineyard was micro-sprinkle irrigated and ∼4200 L of water (105 L per d, 40 d in total) was applied over the experiment.

The clusters were dipped in a solution of gibberellic acid (Kyowa Hakko Bio., Tokyo, Japan) at 25 mg L^{-1} and forchlorefenuron (Kyowa Hakko Bio.) at 10 mg L^{-1} during late bloom for seedless induction and berry enlargement. Late bloom was judged using phenological stage 25 (80% of flower caps fallen) as defined by Eichhorn and Lorenz (1977). The numbers of berries were thinned to 30 to 35 berries per cluster. The crop level was adjusted to approximately 1.6 kg m^{-2} on the horizontal canopy surface area. For each cultivar, d after full bloom (DAFB) was noted as the day on which 50% of the flower caps had fallen (stage 23: Eichhorn and Lorenz, 1977). Fruit sampling commenced at 60 DAFB, and subsequent samplings were conducted at 70, 80, 90 and 100 DAFB. Six clusters for each vine were numbered per sampling date, and six berries were collected randomly from the clusters. To minimize the berry-thinning effect resulting from continuous sampling during the growing season, a different numbered cluster within the vine was selected at each sampling date.

The statistical fixed-effect model that we adopted to express the phenotypic value was as follows: $P_{ijkc} = \mu + G_i + R_k + GR_{ik} + V_{ij} + VR_{ijk} + E_{ijkc}$ (Table S1), where P_{ijkc} indicates the phenotypic value of the cth fruit of the jth vine of the ith genotype at the kth ripening time; μ indicates the overall mean; G_i indicates the effect contributed by the ith genotype; R_k indicates the effect of the kth ripening time; GR_{ik} indicates the interaction between the ith genotype and the kth ripening time; V_{ij} indicates the effect of the jth vine of the ith genotype; VR_{ijk} indicates the interaction between the jth vine of the ith genotype at the kth ripening time; and E_{ijkc} indicates the effect of the cth fruit of the jth vine of the ith genotype at the kth ripening time. An analysis of variance (ANOVA) provided the variance associated with genotype (σ_g^2), among ripening times (σ_r^2), genotype × ripening time interaction (σ_{gr}^2), among vines within genotypes (σ_v^2), vine × ripening time interaction (σ_{vr}^2), and among fruits within a vine (σ^2). Data were analyzed using Ekuseru–Toukei 2015 software (Social Survey Research Information, Tokyo, Japan).

2.2. Experiment 2 (Varietal differences in polyphenol contents and antioxidant activities)

In total, 12 table grape cultivars, consisting of 4 white-skinned cultivars ('Rosario Bianco', 'Seto Giants', 'Shine Muscat' and 'Suiho'), 4 red-skinned cultivars ('Sunny Dorche', 'Sunny Rouge', 'Queen Nina' and 'Yoho') and 4 black-skinned cultivars ('Black Beat', 'Pione', 'Shigyoku' and 'Suzuka') were used. These cultivars were grown and evaluated from 2013 to 2015 in a greenhouse at Fukuoka Agricultural and Forestry Research Center, Japan. One seven-year-old (2013) vine per cultivar, grafted on Kober 5BB rootstock, was planted in an east–west orientation with 4.0-m spacing within rows in a greenhouse. Each vine was trained on a horizontal trellis system and cane-pruned. Other cultural practices were as described in *Experiment 1*. Four clusters for each cultivar were harvested at 90 DAFB based on the results of *Experiment 1*, and then 10 berries were randomly collected from the clusters.

The polyphenol content data were subjected to a two-way ANOVA that factored in the genotype (cultivar) and year using an additive model (Table S2), as follows: $P_{ij} = \mu + G_i + Y_j + E_{ij}$, where P_{ij} indicates the phenotypic value of the ith genotype in the jth year, μindicates the overall mean, Gi indicates effect of the ith genotype, Y_j indicates the effect of the jth year, and E_{ij} indicates the random effect of the ith genotype in the jth year. An ANOVA provided σ_g^2, σ_y^2, and σ^2, and the repeatability was calculated using the following equation: $\sigma_g^2/(\sigma_g^2 + \sigma_y^2 + \sigma^2)$.

2.3. Measurement of fruit quality traits, polyphenol contents and antioxidant activities

At each sampling, the berries were weighed individually, and then fruit color on the apex of the berry surface of colored cultivars was scored based on a color chart (CC; National Institute of Fruit Science, MAFF, Japan) where 0 = ivy green, 1 = olive green, 2 = raw umber, 3 = burnt umber, 4 = mahogany, 5 = chocolate, 6 = maroon, 7 = sepia, 8 = lamp black, 9 = black and 10 = jet black). The skin colors of colored cultivars were also measured using a chroma meter (CR-300, Minoruta, Tokyo, Japan), and expressed as L* values. All berries were divided longitudinally into two samples. Half of the samples were hand-pressed, and the SSC and titratable acidity of their filtered juice were measured with a calibrated refractometer (model PAL-1; Atago, Tokyo, Japan) and an automatic titrator (model AT-500N; KEM, Kyoto, Japan) using 0.1 N NaOH having an endpoint of pH 7.8 (Shiraishi et al., 2010), respectively. The remaining juice sample was clarified by centrifugation at 5000 × g for 10 min. The resulting supernatant was diluted four times with deionized water and filtered through a 0.45-μm filter. The sugar composition was determined using an HPLC (model LC-10A, Shimadzu, Kyoto, Japan) consisting of a SCL-10A system controller, LC-10AD pumps, a CTO-10A column oven and a RID-10A refractive index detector. The column (SCR-101N, 7.9 × 300 mm, Shimadzu) was operated at 60 °C with water delivered at a flow rate of 0.6 mL min^{-1}. The injection volume was 10 μL.

Skin samples of 0.6 to 1.0 g were macerated in 10 mL of 50% aqueous acetic acid (v/v) for 12 h at 4 °C in the dark, followed by filtration through a No.2 filter paper (Advantec, Tokyo, Japan). The anthocyanin contents were measured by reading the absorbance at 520 nm [mg g^{-1} fresh weight (FW) skin cyanidin-3-monoglucoside equivalent) with a spectrophotometer (UV-260, Shimadzu) under conditions of SCALE 20 nm/cm and SLIT 2 nm. The duplicate skin samples were ground in liquid nitrogen and weighed. The powdered samples (0.5 to 1.0 g) were homogenized with a homogenizer (Polytron PT10-35, KINEMATICA AG, Luzemerstrasse, Switzerland) in 40 mL of 80% methanol (v/v). The homogenate was centrifuged at 5000 × g for 10 min. The supernatant volume was increased to 50 mL with 80% methanol. The polyphenol contents were determined using the Folin–Denis method. Briefly, 1.0 mL of extract solution was mixed with 7.5 mL of distilled water. Then, 0.5 mL of Folin–Denis reagent was added and mixed thoroughly. After 3 min, 1.0 mL of saturated Na_2CO_3 was added, and the mixture was allowed to stand for 1 h in the dark. The absorbance was measured at 725 nm (mg g^{-1}FW skin catechin equivalent). Antioxidant activities (mmol L^{-1} Trolox equivalent) of the grape skin extracts derived from the polyphenol extraction were determined in 2015 using a total antioxidant status detection kit (Randox

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