（様式第１３号）

学 位 論 文 要 旨

氏名: 末廣　優加

題目: Studies on physiological changes in skin during maturation and characterization of related genes in ‘Shine Muscat’ (*Vitis labruscana* Bailey × *V. vinifera* L.)
（ブドウ‘シャインマスカット’果実の成熟に伴う果皮の生理学的変化とその関連遺伝子に関する研究）

‘Shine Muscat’ (*Vitis labruscana* Bailey × *V. vinifera* L.) is a popular yellow-green-skinned table grape cultivar from Japan. The objective of this thesis is to characterize maturation in yellow-green-skinned grapes. Whereas the maturation of grape berries, particularly black-, purple-, and red-skinned cultivars, has been studied in detail, that of yellow-green-skinned grape berries is not understood well. Skin browning occurs at the maturation stage just before harvest, in ‘Shine Muscat’. Browning reduces the market value of the grapes considerably. To make progress in resolving the occurrence of skin browning, a better understanding of berry maturation in yellow-green-skinned grape cultivars is needed. In addition, the optimal time for harvesting of yellow-green-skinned cultivars is difficult to determine because the external appearance of the grapes changes little during maturation. Understanding the mechanism of ripening, which is an important event for fruit development, can be useful information for cultivation to produce high quality fruit. Internal and external factors, including phytohormones, light, temperature, and plant water status, have major roles in the maturation process. Therefore, I focused on phytohormones and examined physiological changes and skin browning during maturation at the molecular level.

1. Skin browning and expression of *PPO*, *STS*, and *CHS* genes in ‘Shine Muscat’ grape

‘Shine Muscat’ is susceptible to berry skin browning, which occurs at the maturation stage just before harvest. Tiny reddish-brown blotches appear on the surfaces of berries and considerably decrease the grapes’ market value. Although the causes and mechanisms of browning are unknown, I hypothesized the involvement of polyphenol compounds and their oxidation reactions. The gene expressions of polyphenol oxidases (*PPOs*), stilbene synthases (*STSs*), and chalcone synthases (*CHSs*), which are key enzymatic genes related to the polyphenol metabolic pathway, were analyzed during berry maturation to examine the molecular basis of browning. Skin browning occurred on several berries in a bunch of ‘Shine Muscat’ grapes from 80 days after full bloom, after which the number of berries with skin browning increased, along with the size of browned areas on the berry surfaces. Increases in the expression of *VvPPO2*, *VvSTS* type B, and *VvCHS1* were associated with skin browning, and *trans*-resveratrol content was also increased in the browned skin, suggesting that biosynthesis and metabolic pathways of phenolic compounds were activated during browning. Specific upregulation of *VvPPO2* expression compared with *VvPPO1* was observed. The promoter sequence of *VvPPO2* contains more *Myb*-binding and W-box motifs than does that of *VvPPO1*.

2. Recombinant expression, purification, and characterization of polyphenol oxidase 2 (*Vv*PPO2) from ‘Shine Muscat’

The specific upregulation of *VvPPO2* gene expression will play a crucial role in the understanding and management of skin browning in ‘Shine Muscat’ grapes. To further characterize *Vv*PPO2, I investigated the activity of the protein (enzyme) by using purified recombinant protein from artificially expressed *VvPPO2* gene in *Escherichia coli*. PPO activity was confirmed in the

mature form (*Vv*PPO2-ΔN), excluding the chloroplast transition site. The molecular size under the denaturing conditions was estimated to be 60 kDa, which agrees with the calculated mass of 59.6 kDa. *Vv*PPO2-ΔN was most active at pH 5.0–5.5, the pH in vacuoles of plant cells, and exhibited higher activity with a surfactant (optimum concentration 0.35 mM). Under the optimized conditions, K_m and k_{cat} values of the enzyme for 4-methylcatechol (4-MC) were calculated to be 1.4 mM and 190 s^{-1}, respectively, which resulted in high catalytic efficiency of this enzyme (140 $s^{-1} \cdot mM^{-1}$). In addition, the substrate specificity of caffeic acid and 4-MC (diphenol) was 25 times and 2,385 times higher for than that of *p*-coumaric acid (monophenol), suggesting that *Vv*PPO2 has diphenolase, rather than monophenolase, activity.

3. Effects of abscisic acid/ethephon treatments on berry development and maturation in the yellow-green-skinned 'Shine Muscat' grape

Abscisic acid (ABA) and ethylene are well-known phytohormones that are involved in the maturation of grape berries and other fruits. I treated 'Shine Muscat' grape clusters at the veraison stage with spray application of ABA or ethephon. These treatments produced darker coloration and increased the *trans*-resveratrol and flavonol contents of berry skins. The ABA and ethephon treatments significantly increased the severity of skin browning. Changes in the expression of genes involved in polyphenol biosynthesis and oxidation were correlated with increases in polyphenols and the severity of browning in berry skins. Regardless of treatment, the expression of *VvACO2* and *VvYUC1*, which are involved in ethylene and auxin biosynthesis, respectively, was upregulated in berries with brown skins. ABA treatment at the veraison stage altered the expression of several genes involved in phytohormone biosynthesis and signaling more significantly than ethephon treatment. Although ABA treatment also increased berry size, the effect of ethephon treatment on berry maturation was similar to, or greater than, that of ABA treatment. In berry skins, *VvACO3* expression increased significantly in response to ABA treatment. Overall, the changes in gene expression produced by ABA and ethephon treatments differed. Therefore, different mechanisms may regulate the physiological responses to ABA and ethephon, although both treatments accelerate berry maturation.

4. Effects of gibberellic acid/cytokinin treatments on berry development and maturation in the yellow-green-skinned 'Shine Muscat' grape

Several studies have been conducted to examine the effects of gibberellic acid (GA) and cytokinin (CK) application on berry development. However, the detailed mechanisms underlying their physiological effects on berry maturation after the veraison stage are not understood. I investigated the effects of GA and CK treatments from the veraison stage through subsequent developmental stages. GA/CK treatment at the veraison stage significantly altered berry appearance at harvest. Berry maturation was delayed by treatments. Both treatments resulted in enlarged berries and the suppression of increased sugar content. Chlorophyll in the berry skin was less decomposed after GA/CK treatment, and the occurrence of skin browning at the maturation stage decreased with decreased *VvPP2Cs* expression. The treatments reduced the expression levels of phytohormone-related genes, particularly those of *VvGID1* and *VvCHKs*, which are involved in GA and CK signaling, respectively. These similar changes in gene expression patterns suggest the presence of phytohormonal crosstalk and a common expressional regulatory mechanism. *VvACO2* and *VvYUC1* expressions were increased significantly in samples with skin browning, regardless of treatment, indicating the involvement of the ethylene and auxin biosynthesis pathways in skin browning.

The present study investigated berry maturation in 'Shine Muscat', with a special focus on skin browning and effects of phytohormones. The investigation revealed the functions of genes involved in complex biological reactions in yellow-green-skinned grape berry, as well as physiological changes in the skin during maturation. I believe the results provide useful information for understanding the maturation of yellow-green-skinned grapes. Further research on phytohormonal crosstalk and plant physiology, molecular biology, and genetics will further elucidate the mechanisms of grape berry ripening.