Biosynthesis of Flavonoids in Grapevines (Vitis vinifera L.)

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Abstract

Flavonoids in grapes contribute to wine quality and to the health benefits derived from grapes and wine. This project sought to examine the spatial and temporal distribution, as well as the composition, of the flavan-3-ols, proanthocyanidins and flavonols in winegrapes throughout berry development. In addition, we sought to explore how modifying bunch exposure to light would impact upon flavonoid biosynthesis in the grape berry by comparing the flavonoid composition of shaded and exposed grapes. The expression of key genes in the flavonoid biosynthetic pathway was also investigated to understand how the pathway is regulated during berry development.

The flavan-3-ol and proanthocyanidin composition of both seeds and skin of grapes was determined by reversed-phase HPLC after acetone extraction and acid-catalysis in the presence of excess phloroglucinol. The main period of proanthocyanidin accumulation in grape seeds occurred immediately after fruit-set with maximum level observed around veraison. Over two seasons there was variation in both the timing and content of proanthocyanidins in seeds. In skin, proanthocyanidin accumulation occurred from fruit set until 1-2 weeks after veraison. Proanthocyanidin subunit composition was different in seeds and skin and changed during berry development. The mean degree of polymerisation of the tannin polymers in skin was higher than in the seeds at all stages of berry development. Proanthocyanidin levels in both seeds and skin decreased between veraison and harvest. Additional proanthocyanidin subunits were released when the residues remaining after acetone extraction were subjected to direct acid-catalysis in the presence of phloroglucinol. In the seeds, these accounted for much of the post-veraison decrease, but not in grape skin. At harvest, 75% of extractable berry proanthocyanidin
was in the seeds. Accumulation of proanthocyanidins in the seeds appears to be independent of that in the skins but in both tissues, synthesis occurs early in berry development and maximum levels are reached around veraison.

Flavonols were identified in the skin of developing berries, buds, tendrils, inflorescences, anthers and leaves of the grapevine. The dominant flavonols were quercetin glucosides with trace amounts of kaempferol glucosides detected in Shiraz flowers but not in developing berries. Flavonols were high in flowers and decreased toward berry-set then remained relatively constant through berry development. Total flavonols per berry were low until pre-veraison then increased during berry development, particularly before ripening, in Chardonnay and during ripening in Shiraz. Two genes encoding the enzyme flavonol synthase (FLS) were isolated from Shiraz flowers. The first of these, VvFLS1, was expressed in leaves, tendrils, pedicels, buds and inflorescences as well as in developing grapes. Expression was highest between flowering and fruit set then declined, increasing again during ripening coincident with the increase in flavonols. Expression of VvFLS2 was much lower than for VvFLS1 and did not change during berry development. The results indicate that two distinct periods of flavonol synthesis occur in grapes, the first around flowering and the second during ripening of the developing berries.

To explore the influence of bunch exposure on grape and thereby wine quality a shading treatment comprising opaque boxes was applied to bunches of Shiraz grapes prior to flowering. The boxes were designed to maintain airflow while excluding light, to minimise changes in temperature and humidity. The effects of light exclusion on berry development, flavonoid accumulation and the expression of genes involved in flavonoid biosynthesis were assessed throughout berry development. There was no significant effect of shading on sugar accumulation and in two of the three seasons studied there was
no effect on berry weight. Chlorophyll concentration was much lower in the shaded fruit, which appeared pale yellow until veraison. The fruit coloured normally in the shaded bunches and in two of the three seasons there was no significant change in anthocyanin content. Expression of the gene encoding UDP-glucose flavonoid-3-O-glucosyl transferase (UGGT), a key gene in anthocyanin biosynthesis, increased after veraison and was similar in both shaded and exposed fruit. Anthocyanin composition was altered in the shaded fruit, which had a greater proportion of the dihydroxylated anthocyanins, the glycosides of cyanidin and peonidin. Shading had no significant effect on the levels of condensed tannins in the skin or seeds of ripe fruit. Shading significantly reduced the levels of flavonols in the grape skin. In the exposed fruit, flavonol concentration was highest around flowering then declined as the berries grew, but there was an increase in flavonols per berry during ripening. When the boxes were applied before flowering, shaded fruit had much lower levels of flavonols throughout berry development and at harvest the level of flavonols were less than 10% of that in exposed fruit. A gene encoding flavonol synthase (FLS) was expressed at flowering and during ripening in exposed grapes but its expression was greatly reduced in shaded fruit. The results indicate that shading had little effect on berry development and ripening, including accumulation of anthocyanins and tannins, but significantly decreased flavonol synthesis.

This research has contributed to our knowledge of grapevine biochemistry and physiology, particularly in relation to the biosynthesis of flavonoids. The pattern of accumulation of flavonoids has been determined in the seeds, skin and flesh of grapes during berry development. These investigations have included study of both the content and composition of the flavonoids, condensed tannins and anthocyanins during berry development. The results indicate that while anthocyanins, tannins and flavonols are all products of the flavonoid pathway, they are synthesised at different times during
development and in different tissues of the berry. These observations are consistent with the pattern of flavonoid pathway gene expression reported here and elsewhere and indicate that viticultural management to improve fruit quality needs to consider the different timing of synthesis of these important compounds.

This research has also revealed much about the influence of bunch exposure on flavonoid metabolism in grape berries. While there were significant yet subtle shifts in the composition of anthocyanins and proanthocyanidins, only the synthesis of flavonols was shown to be light dependent. Whether flavonoid accumulation or composition can be effectively managed through viticultural practices, to what extent they can be modified or manipulated, and how such changes might impact upon winemaking are all fields ripe for future investigation.

**Keywords:** Grapevine, *Vitis Vinifera* L., Shiraz, Chardonnay, flavonoid, anthocyanin, condensed tannin, proanthocyanidin, flavan-3-ol, flavan-3,4-diol, flavonol, gene expression, flavonol synthase, BANYULS, LAR, chlorophyll, phloroglucinol, acid catalysis, berry development, seeds, skin, leaves light, shading
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