Optimising colour and tannin composition of grapes and wine



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSP 06/02**

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Research Organisation: **CSIRO Plant Industry**

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Title: Optimising colour and tannin composition of grapes and wine

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1. Abstract

Grape flavonoids including anthocyanins and tannins contribute to the colour and taste of wine. The influence of temperature and irrigation on grape flavonoid composition was investigated to determine how fruit composition could be managed in the vineyard. Genes in the flavonoid biosynthetic pathway were identified and provide tools to monitor pathway activity and determine how the pathway is controlled in grapes. Improved methods of transforming grapevines were developed for functional testing of these genes. Experiments were initiated to determine the pathway to tannin synthesis in grapes and the contribution of several enzymes at branch points in the pathway. Key regulatory genes that determine pathway activity were identified, characterised and functionally tested by changing their expression in grapevine tissues.

2. Executive Summary

- The effect of increased temperatures on the flavonoid composition of Shiraz grapes was investigated in collaboration with SARDI researchers at Nuriootpa. Heat was applied to bunches with blowers or to the vine canopy using under-vine tents or chambers at different times through the growing season. Short interval heat applications during the season did not change flavonoid content at harvest. Increased night temperatures, applied after fruit set, hastened berry development and advanced the onset of ripening. However, by the time fruit was harvested there were no significant changes in anthocyanins. We concluded that greater changes in temperature, treatment for longer periods, or more elevated night temperatures may influence anthocyanin accumulation and should be further investigated.
- The influence of irrigation on grape flavonoid composition was also investigated in a trial on Cabernet Sauvignon growing in the Riverland. Decreased irrigation reduced anthocyanins and tannins in the skin, while having no effect on seed tannins.
- The roles of the three grapevine chalcone synthase (CHS) genes in flavonoid synthesis were elucidated. All three genes were found to have a role in flavonoid synthesis in grapes but they are differentially expressed during berry development and in the different tissues of the grapevine. The grape *VvCHS1* gene is primarily involved in flavonoid synthesis in vegetative tissues and very young berries, while *VvCHS2* and *VvCHS3* appear to play a role in anthocyanin synthesis.
- The grapevine homolog of a gene identified as having a potential role in tannin synthesis in Arabidopsis plants (TDS6) was identified and characterised. The grapevine TDS6 gene (*VvTDS6*) was expressed in grape skin and seed tissues during berry development and ripening, suggesting it may have a role in both anthocyanin and tannin synthesis. The expression of the *VvTDS6* gene is regulated by the tannin regulators VvMYBPA1&2, suggesting a role in tannin synthesis in grapes. However the exact function of this gene has still to be elucidated and it may play a pivotal role in flavonoid synthesis.
- Two additional MYB-type transcription factors that play a role in controlling flavonoid synthesis in grapevine were characterised. The grapevine gene *VvMYB5b* was expressed in both skins and seeds during berry development and ripening and activates genes involved in the early steps of the pathway as well as genes specifically involved in tannin synthesis. Another transcription factor,

VvMYBF1, was found to activate the grape flavonol synthase gene, the key step in flavonol synthesis. This transcription factor gene was expressed in flowers and very early in berry development, when flavonols are synthesised.

- A hairy root transformation system was established for grapevine, using *Agrobacterium rhizogenes,* to functionally test flavonoid pathway genes. Whilst the system had reasonable transformation efficiency, variation in the growth of different root cultures from the same transformation gave inconsistent results and we concluded that this was not the ideal vehicle for testing genes in complex secondary metabolic pathways.
- Stable grapevine transformation utilising *Agrobacterium tumefaciens* is a difficult and time-consuming method but it can give rise to independent transgenic lines that can regenerate normal grapevine plants, allowing assessment of gene function in a range of tissues. The stable grapevine transformation system was of very low efficiency, generating very small numbers of independent transgenic lines after 1-2 years of intensive work. We have systematically re-evaluated the system and made a number of improvements, significantly improving the efficiency and reducing the time taken to produce transgenic embryos and to regenerate plants.
- The grapevine gene encoding the transcription factor VvMYBA1 controls anthocyanin synthesis in grapes. Transgenic grapevines with altered expression of this regulatory gene have been developed to functionally evaluate how the pathway is controlled. Over-expression of the gene encoding VvMYBA1 resulted in plants that accumulated high levels of anthocyanins in most tissues, including berry flesh. Grapes from transgenic vines containing gene constructs designed to decrease expression of the MYBA genes had decreased levels of anthocyanins and ranged in berry colour from black through rose to white.
- Tannin synthesis is also being studied using transformed grapevines with altered expression of key genes involved in tannin synthesis. Plants have been regenerated with gene constructs to decrease expression of the grapevine gene *VvANR,* which is believed to be important for the formation of epicatechin subunits. Grapes from these transgenic vines had increased levels of anthocyanins and decreased levels of total tannin and epicatechin, but the levels of catechin and epicatechin gallate were also decreased. Further analysis of these transgenic grapevines is continuing to determine how the pathway operates and the potential to modify tannin composition.

3. Background

The GWRDC recently commissioned an extensive review of recent tannin research in which was conducted by Professor Geoff Scollary under project GWR 0905. The excellent report produced from that review provides detailed information on tannins including chemistry, biosynthesis, analysis, extraction into wine etc. This report is available from the GWRDC website at:

http://www.gwrdc.com.au/webdata/resources/files/Tannin_Review_final.pdf

3.1 Flavonoids in Plants

Plants produce more than 4,000 different flavonoid compounds which have a range of physiological functions in the plant. The flavonoids include anthocyanins, condensed tannins and flavonols and these compounds are important contributors to wine quality. Flavonoids have also been shown to have significant health benefits for humans and have received much attention in recent times due to their ability to act as antioxidants in food and beverages.

Chemically, the flavonoids are all derived from a basic structure containing a flavan skeleton:

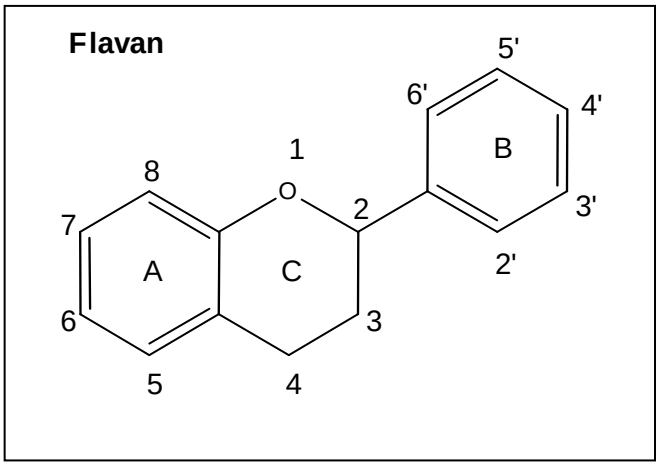


Figure 3.1. Chemical structure of the flavan skeleton.

The biosynthesis of flavonoids is the culmination of two secondary metabolic pathways, the shikimate pathway and the phenylpropanoid pathway. The phenylpropanoid pathway synthesises flavonoids from carboxylated acetyl-CoA (malonyl-CoA) and the amino acid phenylalanine, which is produced in the shikimate pathway. The primary products of the phenylpropanoid pathway are generally considered to be anthocyanins but numerous other diverse end products are formed including lignin, aurones, flavones, isoflavonoids, flavonols and tannins. A stylised phenylpropanoid pathway is shown below:

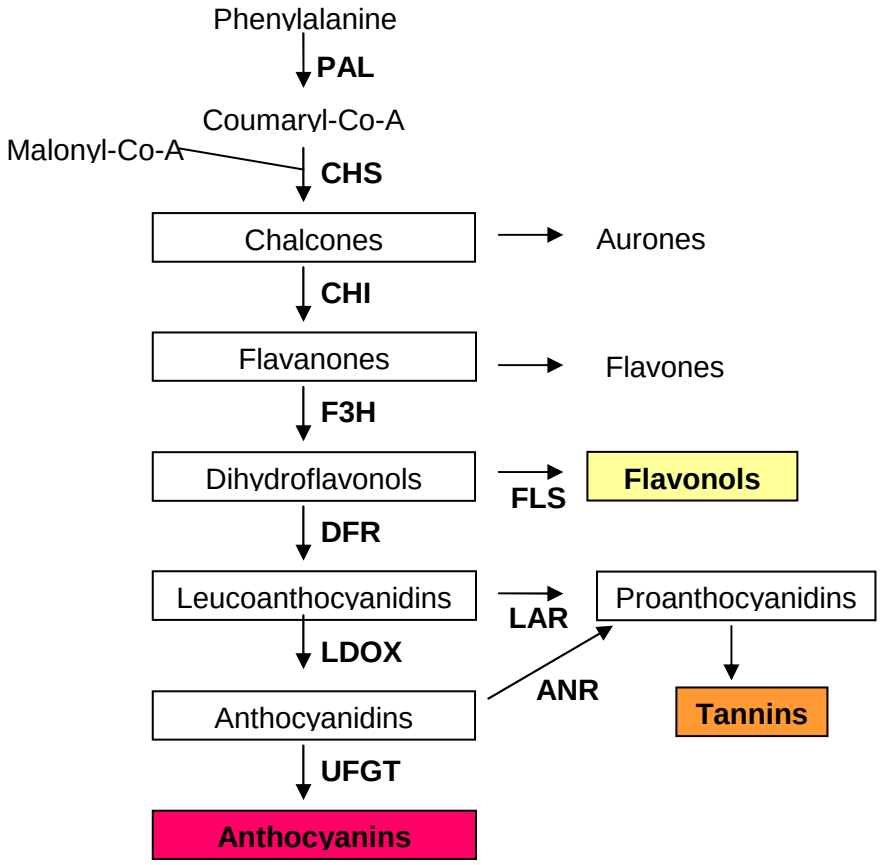


Figure 3.2. Schematic representation of phenylpropanoid pathway.

The scheme indicates major products, intermediates and biosynthetic enzymes:

PAL - phenylalanine ammonia lyase
CHS – chalcone synthase
CHI - chalcone isomerase
F3H – flavanone-3-hydroxylase
FLS – flavonol synthase
DFR – dihydroflavonol reductase
LAR – leucoanthocyanidin reductase dioxygenase
LDOX – leucoanthocyanidin
ANR - anthocyanidin reductase
UFGT – UDP glucose flavonoid 3-*O*-glucosyltransferase

The key diversification of the pathway arises from hydroxylation of the B-ring of the flavan skeleton and subsequent addition of sugars and other groups, leading to a wide range of different flavonoid compounds in each of the major classes. Plants produce thousands of different flavonoid compounds and even in one species, a wide range of different flavonoid compounds are often produced.

3.2 Flavonoids in Grapes

The most abundant flavonoids in grapes and in wine are tannins, anthocyanins and flavonols. Grape tannins and anthocyanins are both important fruit quality components that contribute to the colour and taste of red wines. Tannins contribute to the mouthfeel of wines but they also form pigmented polymers in association with the anthocyanins to provide the stable pigments required to give red wine its colour. While the role of flavonols in wine quality is not yet certain, they can act as copigments for anthocyanins and may have a role in stabilising colour during winemaking and ageing.

Anthocyanins are probably the most studied phenolic component of grapes because of their contribution to the colour of red wines. Anthocyanins occur in the vacuoles of dedicated cells in the epidermal layers of the skin of red grapes and in the pulp of red fleshed varieties. The anthocyanins in grapes are the –3-O-monoglucosides, –3-O-acetylglucosides –3-O-p-coumaroylglucosides of cyanidin, petunidin, peonidin, delphinidin and malvidin. Malvidin is the major anthocyanin in red grapes, with malvidin glycosides accounting for around 60% of total pigment.

Several studies have been made of flavonols in grapes focussing on the induction of flavonols by UV light and the apparent correlation between this phenomenon and the quality of grapes and subsequent wines (Downey et al., 2003b; Ristic et al., 2007). The main flavonols in grapes are the glycosides of quercetin, although glycosides of myricetin and kaempferol have also been detected, albeit at significantly lower levels. Flavonols occur in the upper epidermis of the grape, consistent with a role in UV screening and have not been reported in the seeds, or flesh of the berries.

Grape tannins are predominantly condensed tannins (also called proanthocyanidins). Chemically, condensed tannins are polymers, each made up of a number of different phenolic subunits joined together like a chain. In grapes, the tannin polymers are a mixture of different subunit types and polymer lengths. These tannin polymers can be extracted from the grapes and chemically hydrolysed into their individual subunits (Kennedy et al., 2000). By analysing these components, it is possible to measure how large the polymers were and to determine the composition of the tannins ie the different types of subunits that make up the polymer chains. Both the skin and seeds of grapes contain significant amounts of tannin and in Shiraz grapes, about 75% of the chemically extractable tannin is found in the seeds with the remainder in the skin. The type of tannin polymer in grape seeds is different to that in skins. Grape skins contain longer polymers, on average around 20-30 subunits in length in ripe fruit, whereas the tannins in grape seeds are normally shorter, averaging 4-6 units long (Downey et al., 2003a). The type of subunits making up the tannin polymers in skins and seeds is also subtly different. These differences in tannin polymer length and subunit composition probably determine the extractability and mouthfeel properties of the tannins.

In the seeds and skins of grapes, the flavan-3-ols catechin, epicatechin and epicatechin-gallate are the common monomers. The flavan-3-ols form the terminal subunit of the condensed tannin polymers, which range from dimers to polymers containing up to 30 subunits. In the grape seeds, flavan-3-ols and condensed tannins are found in the seedcoat. The levels of catechin and epicatechin are reputedly similar with lesser amounts of epicatechin-gallate. In addition, all of the B-type dimers and the C2 trimer have been reported in grape seeds at varying levels. The dominant stereochemistry of grape seed oligomers and polymers is epicatechin with the ratio epicatechin:catechin increasing from 2:1 to 12:1 with increasing polymerisation with catechin relatively more abundant as the terminal subunit unit. The difference between seed and skin tannins is

primarily the presence of the trihydroxylated, prodelphinidins in the skin and a higher degree of polymerisation. The dominant flavan-3-ol in grape skin is catechin with the trihydroxylated products, gallocatechin, epigallocatechin and epigallocatechin-gallate detected in addition to catechin, epicatechin, epicatechin-gallate. In skins, only the dimers B1 to B4 are commonly reported, although trace amounts of the B5 dimer which is one of the most prevalent in the seed have also been reported (Downey et al., 2003a; Hanlin and Downey, 2009). In addition, proanthocyanidins in grape skin form complex associations with other cellular components, including cell wall polysaccharides, lignins and proteins (Hanlin et al., 2010).

3.3 Biosynthesis of Flavonoids in Grapes

Anthocyanins, flavonols and condensed tannins are all produced via the flavonoid biosynthetic pathway (Figure 3.2). Some of the steps are catalysed by 'shared enzymes' which are required for the general pathway, whereas specific steps lead to production of the different classes of flavonoids: UFGT leads to anthocyanin production, FLS results in formation of flavonols, and LAR and ANR result in formation of catechin and epicatechin, the monomers that initiate formation of the tannin polymers.

The grapevine genes that direct production of the enzymes that carry out the shared steps (CHS, CHI, F3H, DFR and LDOX) and the last step (UFGT) which is only required for anthocyanin synthesis, have been known for some time. Expression of these genes was determined during grape berry development to study how the flavonoid pathway is regulated in grapes. The UFGT gene was expressed in Shiraz grapes only after veraison, when anthocyanins are being made in the skin (Boss et al., 1996a). In white grapes, and in the flesh of Shiraz berries, UFGT was not expressed suggesting this is a key gene for accumulation of anthocyanins in grapes (Boss et al., 1996b). As expected, the shared genes in the flavonoid pathway were also expressed in the skin of Shiraz grapes after veraison as they are part of the anthocyanin production line. However, these shared genes were also found to be expressed prior to veraison, when UFGT was not expressed and no anthocyanins were being made. These shared genes were highly expressed early in berry development, just after flowering, and it was suggested that this activity was related to the pathway being switched on to produce the colourless flavonoids, such as flavonols and tannins (Boss et al., 1996a).

The FLS gene encodes the enzyme that is the branch point to producing flavonols in grapes. In grapevines, the concentration of flavonols is highest in flowers and small developing berries suggesting they are synthesised very early in berry development. After fruit set, the concentration of flavonols in grapes declines as the berries expand and by harvest the grapes contain much lower concentrations of flavonols. On a per berry basis, the total amount of flavonols increases during ripening suggesting a second period of synthesis after veraison. Measurements of the expression of the FLS gene give a better picture of flavonol synthesis during berry development. In both Chardonnay and Shiraz grapes, FLS was highly expressed around flowering and then the expression this gene decreased to low levels by veraison before increasing again in the last 3-4 weeks of ripening (Downey et al., 2003b). This indicates that there are two distinct periods of flavonol synthesis in grapes and explains why the shared genes of the flavonoid pathway are expressed early in berry development, even though no anthocyanins are being synthesised.

There are two important phases of tannin development in grapes: tannin accumulation, between flowering and veraison, and tannin maturation, after veraison when the berries ripen. Grapes contain high concentrations of tannin in developing fruit suggesting that

tannin is synthesised early in fruit development. More recent studies of tannin accumulation in Shiraz grapes indicate that the tannin concentration is highest in the 1-2 weeks before flowering, suggesting that tannin is being synthesised before the fruit has even set. This was supported by the expression patterns of the grape LAR and ANR genes, which were both expressed before flowering (Bogs et al., 2005). Once the flower has been fertilised, fruit growth starts and the berry increases in size dramatically between flowering and fruit set. The tannin concentration of grapes was maintained during this phase suggesting continued synthesis of tannin to keep up with the growth of the fruit. The expression of both the LAR and ANR genes in grapes increased in the 1-2 weeks after flowering, indicating that this is the most active period of tannin synthesis and that both pathways to forming the tannin initiating subunits are involved.

After berry set it is possible to follow tannin synthesis separately in the skin and seeds of the grape berry. In the skin, tannin levels increased after berry set reaching a maximum 1-2 weeks before veraison. Expression of the two tannin genes declined during this time and by veraison the level of expression of these genes was very low. This indicates that tannin synthesis in the skin of the developing grape berry is most active between flowering and fruit set, and is completed before veraison. The pattern of tannin synthesis in seeds was different to that in skin. In the seeds, the levels of tannin increased after fruit set, reaching a maximum 1-2 weeks after veraison. Expression of the tannin genes also increased during this time and peaked at veraison then declined to very low levels by 2-4 weeks after veraison. Tannin synthesis in seeds continues after veraison and this coincides with the maturation of the seed coat and its change in colour from green to brown. In both seeds and skin, there is no tannin synthesis during the later stages of berry ripening, when extractable levels of tannin are actually declining.

The tannin concentration in a number of fruits declines during fruit growth and ripening so the fruit becomes more palatable to herbivores that will ingest the fruit and aid in dispersing the mature seeds. In part, the decline in tannin concentration in fruits during development is just the result of fruit growth. If tannin synthesis occurs very early in fruit development and then stops, the tannin that has been made is diluted out as the fruit grows. However, the total amount of tannin per fruit may also decline, suggesting that tannin is degraded or becomes less available. Tannins are relatively robust chemically and it is generally accepted that tannins are not chemically broken down to any great extent during fruit ripening; they just become more bound up within the fruit tissues and are less easily extracted. In grapes, many studies have observed a significant decrease in tannins after veraison indicating that the tannins are becoming modified in such a way that they are no longer readily extracted. This has a parallel in fruit quality assessment by winemakers, who are often looking for tannins to ‘ripen’ or ’soften’ in the later stages of berry ripening. It is not clear what causes this decrease in extractable tannin. Continual growth of the tannin polymers to produce longer chains could decrease their extractability but measurements of the mean degree of polymerisation, a measure of chain length, indicates that the seed and skin tannins do not get appreciably longer during the tannin maturation phase. It seems more likely that the tannin polymers become chemically conjugated to other compounds in the berry during the maturation phase.

It is now clear that tannin synthesis in grapes occurs during the tannin accumulation phase, between flowering and veraison, whereas anthocyanin synthesis occurs only after verasion in the berry skin, as indicated diagrammatically below:

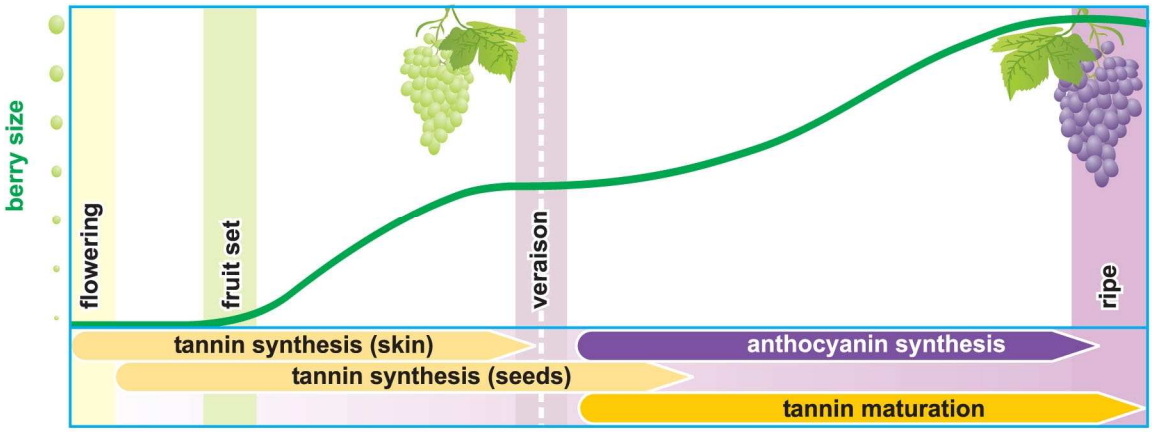


Figure 3.3. Anthocyanin and tannin production during grape berry development and ripening. Berry size is illustrated by the green curve showing the two phases of berry growth between fruit set and veraison, and after veraison when the berries are ripening. Tannin synthesis starts very early in berry development and continues until veraison in skin and for 1-2 weeks after veraison in seeds. Tannin maturation, resulting in decreased extractability of the tannins, occurs during ripening. Anthocyanin synthesis occurs in the skin of red grapes after veraison, and after tannin synthesis is complete.

Both of the pathways that make the tannin initiating subunits are active in grapes but tannin synthesis occurs independently in the skin and in the seeds of grape berries. In the skin, tannin synthesis is completed before veraison whereas anthocyanin synthesis in red grapes only starts after veraison. Thus the flavonoid pathway is regulated so that tannins are made in the skin before veraison; after veraison the pathway is switched to production of anthocyanins. In seeds, tannin synthesis occurs up until 1-2 weeks after veraison when the seeds are fully mature. No further synthesis occurs after this but the tannins become progressively less extractable during the tannin maturation phase, as the grapes ripen.

3.4 Regulation of Flavonoid Biosynthesis in Grapevines

The phenylpropanoid pathway involves complex secondary metabolism, with a large number of sequential and related reactions leading to the final products. The structural pathway genes that encode the enzymes that catalyse these reactions are controlled by regulatory genes, also called transcription factors.

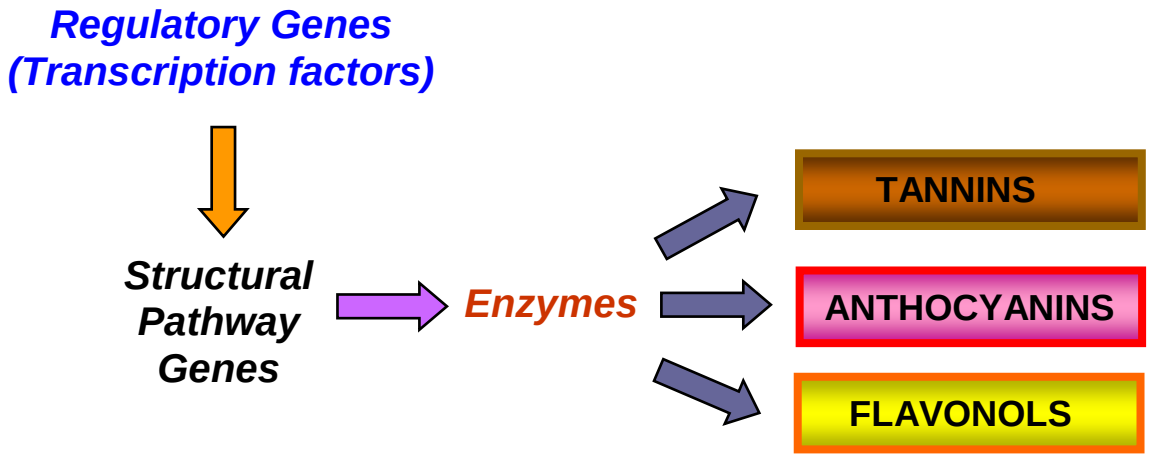


Figure 3.4. Regulation of the flavonoid pathway.

To understand how the pathway is regulated, and to ultimately manipulate the pathway to improve grape composition, it will be necessary to identify the transcription factors that regulate the structural genes of the pathway. A number of these transcription factors have now been identified in grapes and it is clear that a regulatory complex comprising MYB, bHLH and WD40 type transcription factors is involved in regulating the flavonoid pathway.

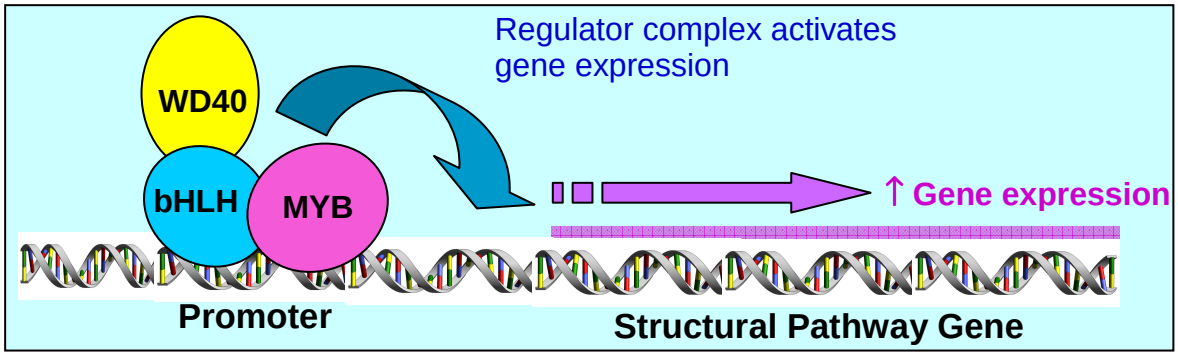


Figure 3.5. Regulatory complexes of MYB, bHLH and WD40 transcription factors regulate the flavonoid pathway in grapevine.

Of these transcription factors, the MYB genes appear to have a major controlling influence and several MYB genes that regulate different parts of the pathway have now been identified. MYBA genes regulate the synthesis of anthocyanins in grapes and control the expression of structural genes encoding enzymes in the last steps of the pathway. White grapes do not produce anthocyanins because mutations in their MYBA genes result in their failure to express genes encoding enzymes that catalyse the final steps in anthocyanin synthesis (Kobayashi et al., 2004; Walker et al., 2007). In red grape cultivars VvMYBA1 and VvMYBA2 regulate anthocyanin synthesis by controlling *UFGT* expression in the skin from veraison onwards while white grape cultivars have mutations that inactivate both genes. Since these mutations are the same in all of the white grape cultivars studied from divergent groups within *Vitis vinifera*, it is probable all white grapes have arisen from a single ancestor (Walker et al., 2007). However somatic mutations such as deletions of the colour locus have been also observed in colour sports of Cabernet Sauvignon and within the Pinot family (Walker et al., 2006).

Two tannin regulators (MYBPA1 and MYBPA2) (Bogs et al., 2007; Terrier et al., 2009) have been identified and they appear to control expression of both LAR and ANR, as well as some of the shared genes in the pathway. Expression of the MYBPA genes in grapes correlates with tannin synthesis and constitutive expression of these genes in Arabidopsis resulted in increased tannin levels.

Two regulators of the early shared genes in the pathway have also been identified (MYB5a and MYB5b) (Deluc et al., 2006; Deluc et al., 2008). The scheme below indicates the involvement of different MYB genes in regulating flavonoid biosynthesis in grapevines.

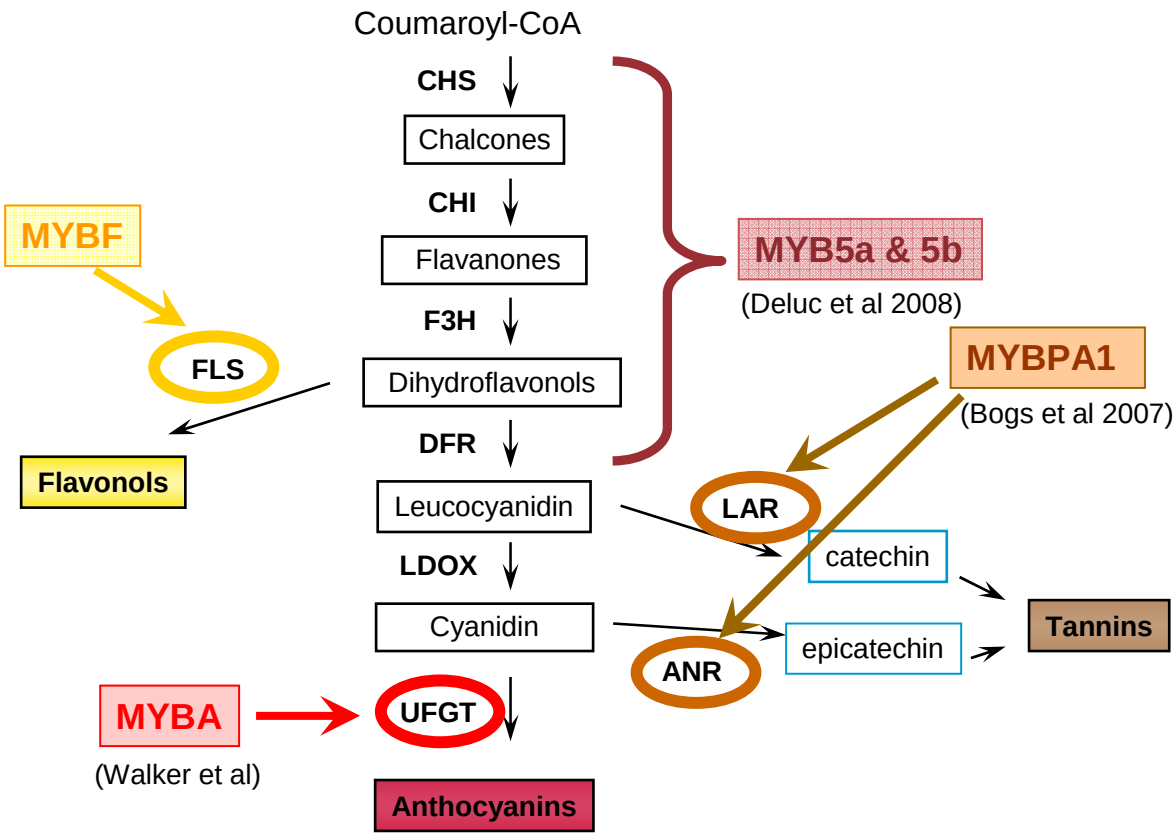


Figure 3.6. Regulation of the flavonoid pathway in grapevine by MYB transcription factors.

4. Project Aims and Performance Targets

The aims of this project were:

1. Characterise pathways leading to anthocyanin and tannin accumulation in grapes
2. Identify genes that determine anthocyanin and tannin composition of grapes
3. Identify regulatory genes that control the synthesis of flavonoids in grapes
4. Investigate environmental factors that determine flavonoid composition in grapes
5. Determine the effects of viticultural management on flavonoid composition
6. Investigate factors that determine extractability of grape colour and tannins

Outputs and Performance Targets 2006-07

Outputs	Performance Targets
1. Project established	Contract signed (Aug 06), staff appointed (Nov 06)
2. Trial sites established	Suitable sites identified and experimental plan completed (Nov 06), samples collected (Apr 07)
3. Analytical techniques	Anthocyanin and tannin analysis methods refined (Jan 07)
4. Flavonoid genes	Genes controlling tannin synthesis identified (Jun 07)
5. Communication to stakeholders	Annual Progress Report (Feb 07)

Outputs and Performance Targets 2007-08

Outputs	Performance Targets
1. Trial sites established	Suitable sites identified and experimental plan completed (Aug 07), samples collected (Apr 08)
2. Flavonoid analyses	Trial site & glasshouse vine samples measured (Jan 08)
3. Gene constructs	Gene constructs completed for transformation (Jan 08)
4. . Flavonoid genes	Genes regulating tannin synthesis identified (Jun 08)
5. Communication to stakeholders	Annual Progress Report (Feb 08), Industry journal article (Jun 08)

Outputs and Performance Targets 2008-09

Outputs	Performance Targets
1. Trial sites established	Suitable sites identified and experimental plan completed (Aug 08), samples collected (Apr 09)
2. Flavonoid analyses	Flavonoids measured in trial site samples and from glasshouse vines (Apr 09)
3. Flavonoid genes	Genes controlling flavonoid composition identified (Jun 08)
4. Transgenic grapevines	Gene constructs completed for transformation experiments (Jan 08), transformation of grapevines completed (May 09)
5. Communication to stakeholders	Annual Progress Report (Feb 09), Final Report (Jun 09)

Outputs and Performance Targets 2009-10

OUTPUTS	PERFORMANCE TARGETS
1. Trial sites established	Suitable sites identified and experimental plan completed (Aug 09), samples collected (Apr 10)
2. Flavonoid analyses	Flavonoids measured in trial site samples and from glasshouse vines (Apr 10)
3. Flavonoid genes	Genes controlling flavonoid composition identified (Jan 10)
4. Transgenic grapevines	transformation of grapevines completed (May 10)
5. Communication to stakeholders	Mid year Progress Report (Feb 10) Final Report (Jun 10)

Note that Output #1 for 2009/10 was deleted by agreement with GWRDC (Letter from GWRDC dated 17/03/2010).

7. Outcomes & Conclusions

Well irrigated Shiraz vines do not show any reduction in flavonoid composition when subjected to short regimes of increased temperature. Combinations of reduced water with heat events are likely to be the cause of fruit damage experienced in the last few years. However this aspect needs to be studied to understand the interaction between these environmental factors and berry quality characters, including flavonoid synthesis.

We tested two transformation methods and have concluded that hairy root methods are not reliable. Improvement to the stable transformation techniques has made this method an excellent way to test the role of genes in grapevine.

Transgenic vines with altered colour have been generated and are now being used to determine the difference between flavour and aroma compounds in red and white wine.

The role of one of the tannin synthesis genes, *ANR*, unpredictably influences catechin synthesis illustrating how little we understand about tannin synthesis in grape.

8. Recommendations

The flavonoid pathway results in the synthesis of anthocyanins, flavonols and tannins in grapes. These compounds are major contributors to wine style, particularly for red wines, and influence the colour, taste and mouthfeel of wines. Whilst much can be done to enhance these key characteristics during winemaking, the levels of these compounds in grapes and their extractability are critical to the final outcome. Our research has provided a fundamental understanding of the pathway and provided tools to investigate how viticultural factors and environmental variables influence grape flavonoid composition. These tools need to be employed to determine how targeted viticultural management can be used to optimise the levels and extractability of flavonoids to achieve desired wine styles. In the longer term, identification of key genes controlling the pathway will provide the potential to develop novel winegrape cultivars with improved flavonoid composition through smart breeding using marker assisted selection, or by production of transgenic grapevines.

We also endorse the recommendations made by Professor Scollary at the end of his Tannin Review.

In particular a major area of research should be to elucidate the mechanisms that regulate the formation of condensed tannin polymers. To this end we have been generating transgenic grapevines with constructs to silence tannin synthesis genes to gain a better understanding of tannin synthesis and how to manipulate tannin composition in grapes.

Another area identified by Prof. Scollary is the relationship between environmental factors and gene expression to identify the link between condensed tannin production and environmental stress. Although we have attempted to identify environmental factors altering tannin synthesis, our results suggest that the best information will be gained by doing experiments combining environmental factors such as water stress and temperature. Vineyard trials examining the physiological effects are essential to this

strand of the elucidation of tannin synthesis. Linking gene expression to environmental factors would provide tools for better vineyard management.

Lastly, we have made an enormous contribution to the successful transformation of grapevine. Maintaining and sharing this capability with other Australian research groups could give them a competitive edge over the rest of the world, thus providing information to assist the Australian industry.

9. Appendix 1 : Communication

Refereed Conference Proceedings

Walker M, Bogs J and Robinson SP (2008). Altering flavonoid composition in grapevine. *8th International Symposium on Grapevine Physiology & Biotechnology*, Adelaide 2008.

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10. Appendix 2 : Intellectual Property

The following patent resulted from work originated in the previous GWRDC project (CRV 99/16a) which was continued through this project.

SP Robinson, MA Walker and J Bogs, 2009.
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12. Appendix 4 : Staff

The following staff were engaged on the Project. All worked at the CSIRO Plant Industry laboratories on the Waite Campus.

Name	Position	Time	Dates
Dr Mandy Walker	Team Leader	30%	July 2006 - Sept 2010
Dr Simon Robinson	Program Leader	30%	July 2006 - Sept 2010
Dr Nilangani Harris	Postdoctoral Research Fellow	100%	May 2007 - June 2010
Ms Debra McDavid	Research Projects Officer	50% 80%	July 2006 - June 2009 July 2009 – June 2010
Ms Caroline Sarneckis	Research Projects Officer	100%	Feb 2007 – June 2007
Dr Sam Brooke	Research Projects Officer	100%	Aug 2007 - Mar 2010
Ms Lauren Hooper	Research Projects Officer	100%	Sep 2006 – June 2010
Ms Amy Koschella	Research Projects Officer	100% 100%	Nov 2008 – Aug 2009 Feb 2010 – May 2010

13. Appendix 5 : Budget Reconciliation

The End of Project Financial Statement has been provided separately.

14. Appendix 6 : Confidential scientific results

Information contained within this appendix is not for publication with the report and will be provided separately.