RESEARCH ARTICLE

MYB24 orchestrates terpene and flavonol metabolism as light responses to anthocyanin depletion in variegated grape berries

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Short title: MYB24 controls specialized metabolism

One-sentence summary: MYB24 controls metabolic responses in skin sections of variegated grape berries lacking anthocyanin to cope with high-intensity and UV light stress, promoting terpene and flavonol accumulation.

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Abstract

Variegation is a rare type of mosaicism not fully studied in plants, especially fruits. We examined red and white sections of grape (*Vitis vinifera* cv. 'Béquignol') variegated berries and found that accumulation of products from branches of the phenylpropanoid and isoprenoid pathways showed an opposite tendency. Light-responsive flavonol and monoterpene levels increased in anthocyanin-depleted areas in correlation with increasing *MYB24* expression. Cistrome analysis suggested that MYB24 binds to the promoters of 22 terpene synthase (*TPS*) genes, as well as 32 photosynthesis/light-related genes, including carotenoid pathway members, the flavonol-regulator *HY5 HOMOLOGUE* (*HYH*), and other radiation response genes. Indeed, *TPS35, TPS09,* the carotenoid isomerase gene *CRTISO2* and *HYH* were activated in the presence of MYB24 and MYC2. We suggest that MYB24 modulates ultraviolet and high-intensity visible light stress responses that include terpene and flavonol synthesis and potentially affect carotenoids. The MYB24 regulatory network is developmentally triggered after the onset of berry ripening, while the absence of anthocyanin sunscreens accelerates its activation, likely in a dose-dependent manner due to increased radiation exposure. Anthocyanins and flavonols in variegated berry skins act as effective sunscreens but for different wavelength ranges. The expression patterns of stress marker genes in red and white sections of 'Béquignol' berries strongly suggest that MYB24 promotes light-stress amelioration but only partly succeeds during late ripening.

IN A NUTSHELL

Background: Since plants made the move to land, they have had to adapt to exposure to high-intensity light and ultraviolet radiation. Anthocyanins that accumulate in epidermal cells shield plant tissues from radiation; the absence of these red/purple pigments negatively affects photosynthesis and other physiological processes. We identified and compared pigmented and unpigmented sections of rare, variegated grapevine (*Vitis vinifera*) berries to see whether different light responses were occurring in red and white skin sections.

Question: What are the causes and effects of berry color variegation? Does a central genetic switch contribute to the abundance of phenylpropanoids and isoprenoids in variegated berries? Can we integrate computational analyses and experimental evidence to disentangle these questions?

Findings: White variegation in red-skinned grapevine fruits is caused by the presence of non-functional alleles of MYBA1/A2 transcription factors that naturally control anthocyanin biosynthesis. The absence of these pigments enhances a ripening-dependent regulatory network mediated by MYB24 that promotes protection against ultraviolet and high-light intensity stress. In response, white skin sections accumulate higher levels of antioxidant monoterpenes and UV-shielding flavonols; however, these compounds only partially ameliorate the detrimental effects of excessive radiation. Genes related to carotenoid metabolism, photosynthesis and other light signaling responses are bound and directly regulated by MYB24. By conducting *in silico* and *in vitro* analyses and using field-grown grapevine plants, we demonstrate that MYB24 orchestrates different specialized metabolism pathways in berry skins in response to increased levels of radiation caused by pigment depletion.

Next steps: Future research should be oriented towards identifying the regulators of MYB24 in order to determine the full regulatory network of MYB24-controlling light responses and late fruit-ripening processes.

Introduction

Plants have many specialized metabolites that are pigments. Most of them accumulate as a result of activity of the phenylpropanoid/flavonoid (anthocyanin) and isoprenoid (carotenoid) pathways, two of the most studied specialized metabolic pathways in plants. Their presence in flowers and fruits has allowed plants to co-evolve with insects and seed dispersers, producing the plethora of color hues and tones found in nature. Beyond this purpose, these compounds also filter harmful excessive radiation and provide accessory photosynthetic capacity, among other roles (Winkel-Shirley, 2002).

As pigments, anthocyanins and carotenoids can be used as reliable markers in forward genetics approaches for the identification of genes underlying their biosynthesis. Based on this advantage, extensive progress in understanding the control of flavonoid pigment synthesis has come from studying the combinatorial interaction between activating and repressive sets of R3- and R2R3-MYBs, beta helix-loop-helix (bHLH) and tryptophan-aspartic acid repeat (WDR) regulators (Albert et al., 2014). WRKY transcription factors also interact with these components (Verweij et al., 2016). This 'MBW-W' complex (Lloyd et al., 2017) is essential for accumulating anthocyanins and acidifying vacuoles, which promote color changes due to the oxidation/reduction of anthocyanin hydroxyl groups.

Besides flavonoid-regulating MYBs, a few isoprenoid MYB regulators have been identified to date, in *Mimulus lewisii* (Sagawa et al., 2016), kiwifruit (*Actinidia deliciosa*) (Ampomah-Dwamena et al., 2019), tomato (*Solanum lycopersicum*) (Wu et al., 2020), *Freesia hybrida* and *Arabidopsis thaliana* (Yang et al., 2020). The overexpression of mimulus *RCP1* in a reduced carotenoid pigmentation (*rcp1*) mutant background restored the production of these pigments and surprisingly decreased anthocyanin production by down-regulating the expression of *PELAN*, which encodes a MYB activator of anthocyanin biosynthesis. This opposite relationship between anthocyanins and isoprenoids has been observed on very few occasions and in some cases with contradictory verdicts (e.g., in tomato) (Long et al., 2006).

In the case of grapevine (*Vitis vinifera*), white grape varieties seem to have higher carotenoid contents compared to dark-skinned cultivars that accumulate anthocyanins in their berry skins (Bunea et al., 2012). Also, terpenes (a group of volatile isoprenoids giving rich aromas) such as linalool accumulate to higher levels in pink-skinned fruits compared to dark red or black cultivars (Cravero et al., 1994). Another study reported that sesquiterpenes have an antagonistic effect on the accumulation of

anthocyanins in grape (Zheng et al., 2021). Altogether, different balances or opposite relationships between anthocyanins and certain isoprenoids seem to exist in the grape berry. Whether this relationship depends on transcriptional regulation remains uncertain.

Grapes constitute a rich source of specialized metabolites and thus represent an interesting model to compare the abundance of metabolites from different pathways, particularly as they are all quantitatively influenced by the environment. In the case of pigmented cultivars, anthocyanins begin to accumulate at the onset of ripening (i.e., the widely used French term *véraison*) in epidermal and subepidermal cell layers that constitute the berry skin (corresponding to L1 and L2 cell types), or also in the flesh (L2) in the case of '*teinturier'* cultivars. Their accumulation increases in response to light and ultraviolet radiation (Matus et al., 2009, 2017) but declines at high temperatures (Mori et al., 2007). Mono- and sesquiterpene levels vary greatly amongst grape cultivars, although a vast majority accumulate at the end of the ripening stage and are highly influenced by temperature (Wen et al., 2015), light (Zhang et al., 2017), UV-B (Carbonell-Bejerano et al., 2014) and water deficit (Savoi et al., 2016). Finally, carotenoid levels decline progressively throughout berry skin development (a sharp decrease occurs at veraison) (Young et al., 2012), but this tendency is arrested and inverted with high radiation levels (Joubert et al., 2016).

The activation of the anthocyanin pathway in the fruit of grapevine depends on the allelic conditions of the *R2R3-MYBA1* and *MYBA2* regulators located at the berry color locus (Kobayashi et al., 2004). One of the most frequent white-skin phenotypes results from the insertion of the *Gret1* retrotransposon in the 5' untranslated region (UTR) of *MYBA1* (Kobayashi et al., 2004) and the concomitant non-synonymous single-nucleotide mutations in the *MYBA2* coding region (Walker et al., 2007). Due to its transposable nature, the insertion/excision of *Gret1* can often occur, leading to somatic mutations in a single cell. If these occur and proliferate in meristematic tissue, pigment mutants can arise as bud sports. Vegetative propagation is widely used for grapes; therefore, bud sports and their novel traits can be selected and retained by breeders. In addition, if somatic mutations occur in restricted cell lineages, variegated phenotypes can be observed (Foster and Aranzana, 2018). Berry color depletion and reversion are often observed in vineyards; however, mosaicism or chimerism (i.e., forms of variegation) are somewhat rare in grapevine, and their molecular study remain limited.

Here, we describe the occurrence of natural berry color variegation in the black-skinned grape cultivar 'Béquignol Noir'. We compared red and white berry skin sections to understand the origin and consequences of this color alteration. The use of a likely isogenic background (the only difference being the allelic composition at the berry color locus) provides compelling evidence of the inverse

accumulation of specific phenylpropanoids and isoprenoids in response to MYBA1/MYBA2 inactivation and establishes a transcriptional association between these two different specialized metabolic pathways in plants. We show that variegation transcriptionally promotes the accumulation of a battery of different specialized metabolites that could filter radiation and/or control oxidative damage. Our results point to MYB24 as an important modulator of this response.

Results

Variegated berries lose the capacity to accumulate anthocyanins in white skin sections due to inactivation of MYBA1 and MYBA2

The grape cultivar (cv.) 'Béquignol' is found in Bordeaux and southwest regions of France, and has a high predisposition for producing bud sports. This varietal group is composed of three recognized somatic variants: the red/black-skinned cv. 'Béquignol Noir (B. Noir)', the pale colored cv. 'Béquignol Gris (B. Gris)' and the unpigmented 'Béquignol Blanc (B. Blanc)' (Supplemental Figure S1A). In addition to these variants, cv. 'Béquignol Noir' vines present an infrequent pigment alteration in some of their berries, occurring in around 53% of the clusters and in about 4% of the berries within those clusters. These berries exhibit uneven skin pigmentation throughout all ripening stages, with small-to-large white or red stripes. In some other cases, half-colored berries are also found (Figure 1A and Supplemental Figure S1B).

We compared transverse sections of variegated berries, sampled at 5 weeks after veraison (5WAV) by optical microscopy (Figure 1B-D). As seen in both cv. 'B. Noir' unvariegated and pigmented variegated berries, skins consist of several layers of anthocyanin-accumulating cells. Large and vacuolated subepidermal cells accumulating both reddish and purplish anthocyanin vacuolar inclusion (AVIs) were discernable in pigmented sections. By contrast, anthocyanins were absent in L1 and L2 cell layers of the white skin sections of unvariegated berries. In the variegated berries, the boundaries between pigmented and unpigmented areas were clearly distinguishable. As surveyed by HPLC quantification, anthocyanin derivatives were only present in pigmented berry skins. These were similar in terms of total abundance and the relative proportion of di/tri-hydroxylated forms in both unvariegated berries of cv. 'B. Noir' and the red skin sections of variegated berries (minor abundance changes were affected by vintage even though the proportions of each derivative were not affected; Supplemental Figure S2).

The color of the grape berry skin relies on the allelic condition of a major locus on chromosome 2 that harbors the genes encoding the anthocyanin-promoting transcription factors (TFs) R2R3-MYBA1 and MYBA2. Thus, we investigated whether these TFs could explain this variegation phenotype. The

inactivation of *MYBA1* through the insertion of the *Gret1* retrotransposon in the promoter/5'UTR (Kobayashi et al., 2004) and non-synonymous single-nucleotide polymorphisms in the *MYBA2* coding region (Walker et al., 2007) account for the un-pigmented phenotype of most white-skinned cultivars known to date. Reversions from non-functional to functional alleles largely occur by excision of *Gret1* in the form of somatic mutations that occur independently in L1 or L2 layers of floral meristems within buds. Since different plant organs and tissues are derived from the L1 and L2 meristem layers, we conducted a cell layer-specific molecular characterization of the berry color locus. Eleven molecular markers in genomic DNA extracted from L1+L2 (berry skins and leaves) and L2 (berry pulp and roots)-derived organs were assessed from all somatic variants of the 'Béquignol' family (Figure 2A). This haplotype structure analysis revealed a recessive homozygous (hm) L2 layer configuration in 'B. Blanc' and 'B. Gris', both bud sports of 'B. Noir' that in turn present a heterozygous (hz) functional allele in this cell layer. This suggests that mutations in L2 are rather common in bud sports and, based on other studies, are probably more widespread than somatic mutations in L1 (Ferreira et al., 2018b). The white skin layer of the variegated berry shows an hm haplotype for the white allele, corroborated with the null expression of *MYBA1* and its target *UFGT1* (Figure 2B). Despite this genetic makeup being identical to that of 'B. Blanc', the variegated-red skin area matches the genetic profile of non-variegated 'B. Noir' berries.

Considering the two genetic scenarios of L1 cells in 'B. Noir', we developed two models for the rise in variegation that can even occur concomitantly. The first considers a somatic recombination event occurring in the L2 cell layer generating the hm white haplotype. This is subsequently incorporated into the L1 layer by displacement, providing a 'patch' presence of non-functional loci. The second model proposes that somatic mutations occur independently in both layers, where cells with non-functional alleles would gradually overlap between layers, leading to white skin sections. The L2 to L1 displacement resembles the sequential model proposed for explaining the formation of cv. 'Shalistin' (Walker et al., 2006), while independent mutations occurring in L1 and L2 leading to non-functional alleles have been suggested in the formation of cv. 'Pinot Blanc' and 'Pinot Gris' (Vezzulli et al., 2012). In all these cases, however, the mutated cells occupy the entire layer, leading to periclinal chimeras.

Lack of anthocyanins and loss of MYBA1/A2 activity correlate with expression changes in phenylpropanoid and isoprenoid pathway genes and additional R2R3-MYB transcription factors

We compared the transcriptomes of variegated red and white skin sections of cv. 'Béquignol Noir' using Operon oligonucleotide microarrays (with approximately 15,000 genes represented, Supplemental Data Set S1). The analysis was conducted on grape skins at the mid-ripening stage of 5WAV (Supplemental

Data Set 2) and showed 807 genes that are significantly differentially expressed in response to anthocyanin depletion (Supplemental Data Set 3), including 454 red-skin up-regulated genes (RUGs, all with fold change ≥ 1.3) and 353 white-skin up-regulated genes (WUGs, all with fold change ≥ 1.3). Gene set enrichment analysis (GSEA, Supplemental Data Set 4) and category enrichment analysis (MapMan Wilcoxon test $p<0.01$, Supplemental Data Set 5) showed that RUGs were enriched in phenylpropanoid pathway terms, including lignin and flavonoid (anthocyanin) biosynthesis and response to heat processes. Meanwhile, many photosynthesis-related terms (e.g., light reaction, photosystem I and II, chlorophyll metabolic processes) and the carotenoid pathway were significantly enriched within WUGs (Figure 3A and B, Supplemental Figure S3). WUGs were also associated with other isoprenoids (e.g., terpenes, gibberellins) and response to abiotic stimulus (e.g., light and radiation) (Figure 3A). In the RUG and WUG lists, several differentially expressed transcription factor genes were present, mostly belonging to the R2R3-MYB family. *MYBA1* and the shikimate/stilbene pathway regulator *MYB15* (Orduña et al., 2022) were induced in red skin areas, while the flavonol-regulator *MYBF1* (Czemmel et al., 2009), the stomata-opening regulator *MYB60* (Galbiati et al., 2011), and a still-uncharacterized *MYB24*-homologue were induced in anthocyanin-devoid sections (Figure 3C).

Taking advantage of the large amount of public transcriptomic data available for *Vitis* sp. and the gene co-expression analyses generated with this data (Orduña et al., 2022, Orduña et al., 2023) and presented in our Vitis Visualization (VitViz) platform (https://vitviz.tomsbiolab.com/) (Navarro-Payá et al., 2022), we explored the potential gene regulatory mechanisms of the few transcription factor genes differentially expressed among WUGs (*MYB24*, *MYBF1* and *MYB60*). We constructed aggregate gene co-expression networks (GCNs) with condition-dependent (flower/fruit; 35 SRA studies, 807 datasets/runs) and -independent (all organs; 131 SRA studies, 2767 datasets) data (Supplemental Data Set 7) and analyzed them by GSEA (Supplemental Data Set 8). In the condition-dependent data, MYB60-GCN was enriched in 'cell periphery and plasma membrane' terms but these were not present in the WUGs-GSEA. Instead, MYBF1-GCN and MYB24-GCN were highly enriched in 'photosynthesis-related' and 'terpene synthase activity' terms, respectively, thus overlapping with the enriched terms found in the variegated WUGs (Figure 3A). The GCN of MYB24, the only uncharacterized TF from this list, contained several specialized metabolic genes related to phenylpropanoid, benzenoid and terpenoid compounds and also to phytohormone (e.g., jasmonic acid, gibberellin) and fatty acid metabolic pathway genes. Several functionally characterized genes involved in the synthesis of mono and sesquiterpenes were present. Among these, a very high correlation was found particularly with TPS35 (VIT_12s0134g00030), an *in vitro* characterized monoterpene synthase (Supplemental Data Set 7*)* (Martin et al., 2010). Our GCN corroborates previous studies showing *MYB24* as co-expressed with terpene synthase genes (Carbonell-Bejerano et al., 2014; Savoi et al.,

2016), but it also suggests a more complex regulatory network composed of light-response, phytohormone and metabolic pathway genes.

MYB24 expression correlates with the transcript accumulation patterns of several members of the terpene synthase family

The grape MYB24 (VIT_14s0066g01090; Vitvi14g01750) is the only R2R3-MYB factor belonging to subgroup 19 in this species (Wong et al., 2016) and is the closest homolog of Arabidopsis AtMYB24, AtMYB21 and AtMYB57, all of which are highly expressed in inflorescences and promote flower maturation in a developmental regulatory network also involving ARF and bHLH transcription factors (Cheng et al., 2009; Qi et al., 2015; Reeves et al., 2012). In concordance with its characterized orthologues, *VviMYB24* shows high expression in flowers, increasing towards the late stage of their development (Supplemental Figure S4A). Additionally, it shows an exponential increase in expression in berry ripening stages towards harvest and post-harvest withering. After reanalyzing public transcriptomic datasets, we identified *MYB24* as being highly expressed in berry skins under two environmental stress conditions: UV-B (in a pigmented cultivar) (Carbonell-Bejerano et al., 2014) and drought (white-skinned cultivar) (Savoi et al., 2016). Re-inspection of RNA-seq data also allowed us to identify a splicing variant (*MYB24.2*) that retained its large intron 2 (>3kb), generating a premature stop codon and leading to an incomplete DNA-binding domain protein. It also lacks a putative transactivation domain located in its C-terminal region, originally described by Liu et al., (2009). The similar transcript profiles of both splicing variants show a clear tissue-specific expression, suggesting the presence of cis-binding elements in the *MYB24* promoter that could account for its restricted expression in flowers and berries. Indeed, MADS-box and auxin response elements (ARE) are present in the -3kb-to-TSS region. These elements are known to give flower-specific expression in Arabidopsis (Reeves et al., 2012) (Supplemental Figure S4B and S4C).

Both condition-independent (all organs) and -dependent (flower/fruit) MYB24-centered networks showed closest relationships with *TPS35/09/10/04* (Supplemental Data Set 7). To identify the key developmental stages of flowers and fruits driving the strong correlation between the expression of *MYB24* and terpenoid pathway genes, we first inspected the cv. ‘Corvina’ expression atlas (Fasoli et al., 2012). Coordinated expression of several *TPS* genes and *MYB24* was most evident in mature flowers and late stages of both berry skin and pulp development (Supplemental Figure S5). We also conducted RT-qPCR expression analyses in different stages of flower and fruit development in both high and low terpene-accumulating cultivars (cv. ‘Gewürztraminer’ and ‘Viognier’, respectively). *MYB*/*TPS* expression levels were higher in cv. ‘Gewürztraminer’, independently of the stage considered. Among

all *TPS* genes surveyed, *MYB24* was tightly co-expressed with *TPS35*, as well as with the sesquiterpene synthase genes *TPS10*, *TPS14* and *TPS07* in both cultivars. *TPS09* was annotated only in VCost.v3, so it was not possible to find it in the 'Corvina' atlas. *MYB24* and *TPS13* expression was correlated only in the high-terpene accumulating cv. 'Gewürztraminer' (Supplemental Figure S6).

MYB24 binds to a set of terpene synthase genes and flavonol regulators, promoting these pathways in response to anthocyanin depletion in berry skin

As grape MYB homologues seemed abundantly co-expressed with terpene synthases, we interrogated whether MYB24 could potentially regulate these genes. We performed DAP-seq to examine the *in vitro* binding of affinity-purified MYB24 protein to genomic DNA in cv. 'Cabernet Sauvignon' (O'Malley et al., 2016). Based on peak calling and motif discovery, MYB24 DAP-seq general statistics were similar to those previously described for Arabidopsis R2R3-MYBs (Bartlett et al., 2017). The number of peaks were quite similar between the two phased haplotype references (6,869 and 6,170 for the CS08 primary (p) and p+haplotig contigs, respectively). *De novo* discovered motifs from sequences under the 600 most-enriched peaks were nearly identical for both analyses, showing the core 'ACCTAAC' consensus motif (Figure 4A). This motif is consistent with an AC-element consensus sequence (ACC[A/T]A[A/C][T/C] or ACC[A/T][A/C/T][A/C/T]) previously associated with subgroup 19 MYBs in Arabidopsis (Kelemen et al., 2015). The AC-element has also been found in nectary gene promoters (TCACCTAA(C/A)) that are bound by a S19 MYB gene (LxS-MYB305) in ornamental tobacco (*Nicotiana langsdorffii* X *N. sanderae*) (Liu et al., 2009).

The 6,170 identified binding sites were assigned to their closest gene feature in the CS08 genome, reaching a total of 5,021 bound genes (Supplemental Data Set 9). Peaks were widely distributed throughout upstream, downstream, and inside-gene regions, but a higher proportion was observed close to transcriptional start sites (TSS; Supplemental Figure S7). From all peaks, we found 33 different regions in close proximity to 22 terpene synthase genes (73% in their upstream regions; Supplemental Data Set 10). *TPS* promoter-preferential binding sites were found to be specific to this subgroup 19 (S19) member and not, for instance, to Subgroup 6 anthocyanin-promoting MYBs such as *Vitis* MYBA1, A6 and A7, nor to Arabidopsis MYB113 (Figure 4B, Supplemental Figure S8). We searched for the identified AC-element in these 22 *TPS* genomic regions, observing an enrichment for a subgroup of *TPS* genes, revealing a preferential binding of MYB24 to *TPS* promoters (Figure 4C). We remapped the DAP-seq reads in the 12X.2 PN40024 reference genome to ascertain the identity of each *TPS*-bound gene, identifying the MYB24 co-expressed *TPS35/09/10* (GCN; Figure 4D) and *TPS13* (RT-qPCR) genes. MYB24 also binds within the promoters of 32 photosynthesis or light-related genes and ten genes

related to isoprenoid/carotenoid metabolism (Supplemental Data Set 11), including genes encoding farnesyl diphosphate synthase (FPS), geranylgeranyl pyrophosphate synthetase 1 (GGPPS1), carotenoid isomerase (CRTISO2), cis-zeta-carotene isomerase (Z-ISO), 9-cis-epoxycarotenoid dioxygenase (NCED6), zeaxanthin epoxidase 1 (ZEP1), and lycopene epsilon cyclase (LCYE).

MYB24 is clearly a nucleus-localized transcriptional activator, as shown by MYB24:E-GFP localization and Gal4DBD:MYB24 activity assays in agroinfiltrated *Nicotiana benthamiana* cells and yeast, respectively (Supplemental Figure S9). Thus, in order to establish a list of high confidence targets (HCTs) among MYB24-bound genes, we overlapped the DAP-seq data (binding up to -5kb from TSS; Supplemental Data Set 12) with MYB24 co-expression data (networks from Supplemental Data Set 7 that also include MYB24-bound *TPS* genes that contain MYB24 as part of their own GCN; Supplemental Data Set 13) and with reanalyzed transcriptomic datasets from ripening berries where *MYB24* was up-regulated, namely in response to UV-B radiation (NimbleGen microarray data; Supplemental Data Set 14) (Carbonell-Bejerano et al., 2014), in light versus shade treatments (Illumina RNA-Seq; Supplemental Data Set 15) (Sun et al., 2017) and in response to water deficit (Illumina RNA-Seq; Supplemental Data Set 16) (Savoi et al., 2016). Three genes were present in all list sets: *TPS35*, a putative jasmonate O-methyl transferase gene (*JOMT*) and a zinc knuckle putative transcriptional regulator gene (*ZnKn*). Twelve MYB24-bound genes were present in at least three datasets, including a UDP-glycosyl transferase gene of unknown function (*UGT-like1*), a thioesterase gene, and a S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase gene (*SAMT*) (Supplemental Figure S10 and Supplemental Data Set 17).

As *VviTPS35* and *VviTPS09* are high-confidence targets, we tested the transcriptional regulation of these genes by VviMYB24 with a dual luciferase assay in *N. benthamiana* agroinfiltrated leaves. Arabidopsis AtMYB24 and AtMYB21, which play a role in flower maturation, must interact with bHLH factors (AtMYC2/5) in order to succeed in these roles (Qi et al., 2015). As expected, the activation of the *VviTPS35* promoter (1.66kb upstream of the ATG) required co-infiltration with *AtMYC5* (Supplemental Figure S11). We used AtMYC5's sequence to search for its homologues in grapevine (Toledo-Ortiz et al., 2003), identifying the ubiquitously expressed *VviMYC2* (*VvibHLH007*) as the closest homologue (Supplemental Figure S12A and S12B). The activity of the *VviTPS35/09* promoters was also dependent on the co-infiltration of *VviMYC2* (Figure 4E). A bimolecular fluorescence complementation (BiFC) assay demonstrated the interaction of VviMYB24 with AtMYC5 and VviMYC2 in *N. benthamiana* agroinfiltrated leaves (Supplemental Figure S12C).

The binding and reciprocal co-expression observed for several *TPS* genes (Supplemental Figure S13),

together with the direct activation of *TPS35* and *TPS09* by MYB24, implied that terpenes should differentially accumulate whenever *MYB24* increases its expression, i.e., in late-flowering and -berry ripening developmental stages, in white skin sections of cv. 'Béquignol' variegated berries and in response to radiation and drought. In fact, this is what we observed; first, high *MYB24*-terpene correlations were found during flower/fruit development in both high and low terpene-producing cultivars (Supplemental Figure S14). Also, by reanalyzing and integrating the results of two metabolomics/transcriptomics studies (Savoi et al., 2016, 2017), we found that *MYB24* expression was highly correlated with the accumulation of berry monoterpenes (geraniol, nerol, linalool, and alpha−terpineol) in response to drought (Supplemental Figure S15). We further explored this relationship in the variegated samples by dissecting white and red skin sections, quantifying gene expression throughout all ripening stages, and using targeted and untargeted GC-MS metabolomics at the harvest stage (9WAV). *MYB24*, *TPS35* and *TPS09* showed very similar expression profiles, with an exponential behavior increasing towards the late stages and increasing their expression in the white skin sections in at least one time point (Figure 4F). Among volatile compounds with higher accumulation in white-variegated berry skin sections, we identified the monoterpenes citronellol (in both targeted and untargeted assays), geraniol, nerol and alpha-terpineol (Figure 4G, and Supplemental Figure S16).

We suggest 418 additional putative MYB24 targets based on the overlap of DAP-seq data with at least one of the four considered datasets (mapped to the PN40024 12X.2 assembly and associated to its VCost.v3 annotation; Supplemental Data Set 17). In addition to an enriched 'terpenoid-related' term, MapMan pathway analysis of these HCTs also showed enrichment of jasmonate, abscisic acid and demethylation-related terms (Supplemental Figure S17 and Supplemental Data Set 18). HCTs also include the light-responsive flavonol-pathway regulators *HY5 HOMOLOGUE* (*HYH*; Loyola et al., 2016) and *MYBF1* (Czemmel et al., 2009), and several photosynthesis-related genes. VviHY5/HYH regulate several early light responses, including flavonol accumulation via activation of the regulator *MYBF1* and metabolic pathway genes such as *FLAVONOL SYNTHASE 1* (*FLS1*) and the flavonol glycosyl-transferase genes *GT5/6* (Loyola et al., 2016; Czemmel et al., 2017). MYB24 binds to the *HYH* promoter at around -0.92kb from the TSS (Figure 5A) and *MYBF1* at -3.6kb upstream of the start codon (Supplemental Data Set 12). A dual luciferase assay in *N. benthamiana* agroinfiltrated leaves demonstrated that the activity of the *HYH* promoter (1.53kb upstream of the ATG) was enhanced by co-infiltration of *VviMYB24* and *VviMYC2* (Figure 5B). It was not possible to amplify an approximately 4Kb promoter to test *MYBF1* activation. However, and as expected, *HYH*, *MYBF1*, and their target *FLS1* and *GT5* were induced in the white skin sections of variegated berries throughout berry ripening, as shown by RT-qPCR (Figure 5C). Analysis of the flavonol contents of white skin sections quantified at 5WAV showed increased levels of the glycosylated forms of quercetin and kaempferol compared to red

skin sections. These flavonol types are known to accumulate in berries in response to UV radiation (Martínez-Lüscher et al., 2014). In particular, the contents of two flavonols, quercetin-3-glucoside (q-3-glc) and q-3-glc-6-ac, in the white skin sections of the variegated berries were triple the amount found in pigmented skin samples. Quercetin-3-gal and kaempferol-3-glc were also more abundant in the variegated white skin sections (Figure 5D). The integration of the two previously included drought studies also showed a high correlation of *MYB24* with kaempferol and quercetin glucosides (Supplemental Figure S15).

Finally, we observed that among the isoprenoid/carotenoid MYB24-bound genes, seven genes were high confidence targets, with all of their binding sites within 5kb from the TSS (Supplemental Data Set 17). Among these genes, we quantified the expression of carotenoid pathway genes, including *CRTISO2*, *LCYE*, *Z-ISO*, and *ZEP1*, and found that all of them were upregulated in white skin sections of variegated berries (Supplemental Figure S18A and S18C). Also, the activity of the *CRTISO2* promoter (2kb upstream of the start codon) was strengthened by the co-infiltration of *VviMYB24* and *VviMYC2* (Supplemental Figure S18B). Although carotenoids were not quantified in these skins, it is highly probable that *MYB24* upregulation also promotes the accumulation of carotenoids in white variegated skins.

MYB24 expression is modulated by light exposure in a dose-dependent manner

As the absence of MYBA1/A2 is concomitant with higher levels of *MYB24* transcripts in variegated berries, we first hypothesized that *MYB24* expression could be negatively regulated by MYBA1. However, the notion of the direct regulation of anthocyanin-related MYBs from Subgroup 6 (i.e., *MYBA1*, *A7* and *A6*) was discarded by inspecting our DAP-seq data, as *MYB24* was not found among MYBA-bound genes. MYBA1 could still indirectly repress *MYB24* expression through a still unknown transcriptional repressor, or alternatively, as MYBA1 triggers anthocyanin production, *MYB24* expression could be negatively influenced by the accumulation of these pigments that are known to filter sunlight in plant tissues (Stapleton and Walbot, 1994).

MYB24 is highly induced by radiation, as found in the reanalyzed 'UV-B responsive' (Carbonell-Bejerano et al., 2014) and 'light vs shade' (Sun et al., 2017) transcriptomic datasets. We confirmed that this light responsiveness occurred throughout most ripening stages by performing RT-qPCR in berry skin samples obtained from light exclusion, UV-B filtering and UV-B irradiance treatments conducted in cv. 'Cabernet Sauvignon' plants grown in the field and greenhouse (Matus et al., 2009; Loyola et al., 2016; Czemmel et al., 2017). In all cases, light and UV-B positively influenced *MYB24* expression, a response mirrored by *TPS35* (Supplemental Figure S19). We further explored data obtained from the

field experiments (i.e., full light versus shade and full light versus UV filtering) to investigate the potential impact of different light qualities on *MYB24* expression. After scaling the expression data and interpolating using polynomial regression models, we identified both developmental and environmental factors affecting *MYB24* behavior at late ripening stages (Figure 6A-B). *MYB24* expression seems to be primarily driven by development (i.e., ripening) and UV-B radiation, and to a lesser extent by visible light (Figure 6B). However, the contribution of both light factors seems to increase towards the latest ripening time-points.

The light-responsive behavior of *MYB24* drove us to further inspect whether the differential abundance of anthocyanins could influence the response of *MYB24* to light, and if this could explain its lower expression in the red skin sections of 'Béquignol' berries. We inspected gene expression levels in response to light at two depths within the berry pericarp (i.e., skin and pulp) in the cv. 'Gamay' and its '*teinturier'* (red-flesh) somatic variant cv. 'Gamay Fréaux', which is characterized by the accumulation of anthocyanins in the flesh/pulp starting at veraison (Guan et al., 2016). As expected, shade reduced the expression of *MYB24*, *TPS35* and *HYH* in both tissues in several post-veraison time points surveyed (Figure 6C and Supplemental Figure S20). Additionally, we observed an influence of tissue and cultivar, suggesting a positional effect on the expression of *MYB24* that resembles a light dosage response (i.e., inner pulp tissue being less light responsive than skin). This effect was more evident at the late stage (i.e., 4WAV), when *MYB24* displayed its highest expression (Supplemental Figure S20) and when the contribution of light factors increased (Figure 6B). Furthermore, when considering the levels of anthocyanins in these samples, we observed a clear negative correlation between *MYB24* expression and the abundance of these pigments at ripening stages when independently analyzing skins and pulps (Figure 6D), corroborating a known sunscreen effect of anthocyanins. Lower anthocyanin content could also explain a diminished *MYB24* expression in 'Gamay Fréaux' compared to 'Gamay'.

Different light shielding properties of anthocyanins and flavonols are in tune with a temporal delay in UV- and high light-stress protection in white sectors of variegated berries

The expression of *MYB24* in cv. 'Béquignol' and cv. 'Gamay' and its negative correlation with anthocyanins prompted us to explore if different radiation levels were influencing red and white variegated sectors, as white skins do not accumulate anthocyanin sunscreens but do accumulate higher amounts of UV-shielding flavonols. The anthocyanins malvidin and delphinidin glucosides and the flavonols quercetin and kaempferol glucosides largely accumulate in red and white variegated sectors, respectively (Supplemental Figure S2, Figure 5D). We thus studied their light transmission properties as a proxy of their capacity to filter harmful radiation (Figure 7A). Through an optical setup, we compared

different light spectra ranging from UV-C (200-280 nm), UV-B (280-315 nm), UV-A (315-400 nm) and visible (400-700 nm) to infrared (IR; 780-900 nm). Flavonols and anthocyanins were shown to absorb (i.e., filter) a wide fraction of UV and visible light, respectively. Flavonols showed null capacity in filtering visible light, while anthocyanins absorbed UV wavelengths to a lesser extent compared to their own ability to filter visible light (Supplemental Figure S21).

The clearly distinct optical filtering properties of flavonols and anthocyanins tested here suggest that variegated skins experience different levels of excessive visible and UV light irradiance depending on the metabolites that accumulate. Based on the *FLS1* gene expression pattern in 'Béquignol' during different stages of development (Figure 5C), and knowing that flavonols more highly accumulate in white skins (Figure 5D), we hypothesized that the protective role of these compounds was acquired gradually, reaching a maximum of protection only at very late stages. We thus checked the expression of high light stress-responsive (*HPR1*, *ACD1*, *SEN1*, *PHOT2*), UV-B responsive (*sigE*, *BBX22*, *FOT6-4*, *FOT1*), and stress-protection (*SEP2*, *UVR1*, *SOD*, *LPP1*) related genes. With the exception of the dark-inducible *SEN1*, which showed the expected increased expression in red skins, all light-related genes were significantly up-regulated in white variegated skins (Figure 7B). This behavior was also observed for MYB24 high-confidence targets *ELIP1* (*EARLY LIGHT-INDUCIBLE PROTEIN 1*), *OHP2* (*LIGHT STRESS-RESPONSIVE ONE-HELIX PROTEIN* 2), and *PRK* (*PHOSPHORIBULOKINASE*), all of which respond to and act in light signaling (Supplemental Figure S10 and S22; Pastore et al., 2013; Harari-Steinberg et al., 2001; Sharma and Hall, 1992). Additionally, most protection-related genes were unresponsive at the latest stage, supporting the notion that even when protective compounds accumulate, the cells in the white skin areas are still stressed at early and middle time points and that this stress seems to cease at the end of ripening.

Discussion

Vitis vinifera 'Béquignol Noir' is a red-skinned grape cultivar that produces a rather small proportion of variegated berries with uneven skin pigmentation. This is a stable phenotype that, albeit unreported in the literature, has been observed with persistence over all seasons surveyed in a field collection for at least 67 years. In this study, we show that anthocyanins exclusively accumulate in pigmented skin sections of variegated berries, presenting similar derivative diversity and quantity compared to unvariegated 'B. Noir' berries, as quantified in many other red-skinned cultivars (Castellarin and Di Gaspero, 2007).

As largely described for many different grape cultivars and their somatic variants, the depletion of anthocyanins usually results from deletions or mutational-induced inactivation of *MYBA1/A2*

transcription factor genes (Ferreira et al., 2018b). As presented here, the white skins of variegated cv. 'Béquignol Noir' berries showed a genetic configuration resembling that of white cultivars, even though the rest of the plant was heterozygous for *MYBA1*/*A2* functional alleles. Variegated tissues, as a form of mosaicism, can be described as periclinal or sectorial chimeras. Here, the uneven and 'patchy' arrangement of red and white sections we observe in 'Béquignol' variegated berries resembles a 'non-patterned sectorial chimera' where, as described by (Frank and Chitwood, 2016), it is common to find highly active transposons moving in and out of pigment-related genes, like in the case of the *MYBA1* gene becoming unfunctional due to *Gret1* retro-transposition. This quite rare event differs from the 'B. Gris' periclinal chimera, where one cell layer is genetically distinct from the other as a result of mutation propagation throughout the entire layer.

Sectorial chimeras mostly occur due to spontaneous or induced mutations at early stages of embryo development when there is a minimum number of cells in the meristem cell layers. However, the variegated phenotype in 'Béquignol Noir' may arise at different developmental stages and as a mixture of different events, including L2 to L1 displacements and independent mutations occurring at L1 and L2 as cell group-specific *Gret1* retro-transposition events. This would explain the presence of uneven patches together with extended variegated sectors (e.g., covering half a berry).

Cells within a layer tend to move into other layers as cell division proceeds due to errors in the cell division plane (Marcotrigiano, 1997). This supports the idea that L2 meristematic cells with unfunctional *MYBA1/A2* alleles would further invade, gradually and heterogeneously, into the epidermal cell layer (L1). This invasion of L1 cells by L2 unpigmented sub-epidermal cells has been suggested to explain the phenotype of cv. 'Shalistin', from cv. 'Malian' (Walker et al., 2006), and could occur more frequently than the independent mutation in L1 and L2 cells at the same location. Either way, further studies should address the cause of the high transposon instability observed in the 'Béquignol' family compared to other cultivars.

The increased accumulation of flavonols in the white skins of our study subject (Figure 5D) allowed us to hypothesize that anthocyanin-devoid sections responded more intensely to sunlight. The roles of anthocyanins in radiation filtering and oxidative damage protection have been well-documented in several plant species. In addition, here we show that grape anthocyanins are capable of filtering both UV and visible light with different capacities, depending on their wavelength (Figure 7A). Correspondingly, the decrease or complete depletion of anthocyanins in the skin epidermis negates the protective advantages endowed by these pigments acting as sunscreens (Stapleton and Walbot, 1994). When these pigments are absent, as in the case of white skin sectors of variegated berries, sunscreen-depleted cells

respond to ensure alternative mechanisms for light stress protection. In this scenario, epidermal layers of berry skins are sheltered from the effects of excessive light and ultraviolet radiation by accumulating flavonols. This response to excessive light represents one of the fastest metabolic responses to environmental light-related stresses ever described in plants (Agati and Tattini, 2010), as they also play important roles as antioxidants in photoprotection.

However, the flavonols tested here do not seem to protect cells from high visible light stress, at least via filtering (Figure 7A). This is corroborated by the induction of high-intensity light (HL) stress genes in white skin sectors, even at the latest time-points (Figure 7B). It has been demonstrated that HL-driven signaling leads to massive transcriptome changes in Arabidopsis involving central transcription factors such as AtBBX30 (Huang et al., 2019), which includes the dynamic regulation of genes encoding photoreceptors and phytochrome-interacting factors and genes associated with phytohormones, photosynthesis and the phenylpropanoid pathway. Under HL conditions, excess absorbed energy is produced and multiple reactive oxygen species (ROS) accumulate. Plants acclimate and protect themselves from these effects by protecting the Photosystem II reaction centers against photodamage and activating antioxidant mechanisms (Goss and Lepetit, 2015; Ruban, 2016; Apel and Hirt, 2004). Other coping mechanisms include variations in the composition of the photosynthetic apparatus (Walters, 2005, Schöttler and Tóth, 2014) and leaf optics (Knapp and Carter, 1998). In the absence of HL-stress ameliorating anthocyanins, it is thus logical to think that plants trigger the accumulation of other metabolites (e.g., terpenes, flavonols, and carotenoids) to promote protection. Additionally, the higher expression of UV and stress-related marker genes in the middle ripening stages of cv. ‘Béquignol’ also suggest that white skin sectors are stressed due to the effects of ultraviolet radiation despite them having already initiated the accumulation of UV-shielding flavonols (Figure 5D and 7B).

Our results suggest that the accumulation of isoprenoids, in particular monoterpenes, is also triggered by MYB24 in response to higher radiation caused by anthocyanin depletion. The potential roles of monoterpenes in dealing with oxidative stress have been previously suggested, i.e., to directly mitigate ozone levels and scavenge ROS, leading to decreased oxidative damage and improved thermotolerance (Lee et al., 2015; Zuo et al., 2017). Although previous studies in grapevine showed that terpene levels increase in response to radiation (Carbonell-Bejerano et al., 2014; Friedel et al., 2016), less is known about how terpene synthases are transcriptionally activated by light. We determined that MYB24 is a key regulator of this response. The contents of the monoterpenols terpineol, citronellol, geraniol and nerol increased significantly in the non-pigmented sections of variegated berries (Figure 4G). We cannot discard the notion, however, that depending on the cultivar, the accumulation of other terpenes could be regulated by MYB24. Also, we cannot rule out the possibility of a single TPS producing different

terpenes *in planta*, despite being validated *in vitro* for just one specific compound. For instance, cv. 'Gewurztraminer' produces beta-ocimene in high correlation with *TPS35* and *MYB24* expression, while 'Béquignol' does not accumulate this specific compound despite *TPS35* being up-regulated. Considering that terpene synthases have been proven to be promiscuous in many species including grape (e.g., Martin and Bohlmann, 2004) and that SNPs in a *TPS* gene can lead to different products (Smit et al., 2021), it is possible that TPS35 produces different terpenes depending on the cultivar.

The infrequent variegation phenotype observed in this study provided an ideal framework for studying the genetic control of both flavonoid and isoprenoid metabolism. Our transcriptomics and RT-qPCR gene expression analyses of the variegated berries showed that several genes associated with photosynthesis (including photosystem function), carotenoid metabolism and UV/light- and stress-induced responses were significantly induced in white-skin sections (Figure 3C and 7B, Supplemental Figure S18 and S22), in addition to the regulatory and structural pathway genes related to the accumulation of terpenes and flavonols. The activation of these genes upholds the theory that white skins are experiencing increased radiation due to the lack of anthocyanin sunscreens. In an effort to explore how variegated berries may activate terpenoid and flavonol metabolism, due to the uneven distribution of skin anthocyanins during ripening, we searched for gene expression changes in transcription factors potentially governing these responses. Our transcriptomic/metabolomic metanalyses, cistrome data and their validation through several approaches suggested that these changes are governed transcriptionally through the activity of a few transcription factors from the R2R3-MYB (MYB24 and MYBF1) and bZIP families (HYH).

The inspection of MYB24 high confidence targets showed an enrichment of the term 'secondary (i.e., specialized) metabolism terpenoids' (Supplemental Figure S17). In addition, the DAP-seq data of MYB24, its expression behavior in terms of light-responsiveness, and its high correlation with the increase in light-responsive gene expression and the accumulation of metabolites in all the datasets generated and reanalyzed (Supplemental Figure S15 and S19) reveal a major role of this TF in the general light-signaling pathway. For instance, MYB24 binds to genes encoding an early light-inducible protein (*ELIP1*), a one-helix protein 2 (*OHP2*) and a phosphoribulokinase (*PRK*), all of which are related to the light stress response (Pastore et al., 2013; Harari-Steinberg et al., 2001; Sharma and Hall, 1992) (Supplemental Figure S22). Gene ontology analysis of MYB24-bound genes revealed 'RNA biosynthesis-Transcriptional regulation' as a highly significant enriched term (Supplemental Figure S17); in fact, ~7.3% of its target genes encode transcription factors, placing MYB24 in a top hierarchy of transcriptional regulation.

Among MYB24 targets encoding transcription factors, we identified *HYH* and *MYBF1*, both encoding TFs associated with light and UV-B radiation responses in grapevine (Loyola et al., 2016; Czemmel et al., 2017). *HYH* expression seems to be sensitive to the increase in MYB24 levels due to light stress but not much to its increase during development, as *HYH* expression tends to decrease at late ripening. Although we could not demonstrate the MYB24-mediated activation of *MYBF1,* MYB24 could at least indirectly regulate its expression through the induction of *HYH*. HY5 and HYH regulate each other, act as partially redundant central mediators of photomorphogenic responses and are considered to be marker genes of light signaling (Lee et al., 2007; Binkert et al., 2014). Because of their 'very early' behavior, we cannot rule out the possibility of feedback regulation of MYB24 by any of these bZIP regulators. As shown here, the control of flavonol accumulation by MYB24 is indirect, i.e., through the activation of HYH and MYBF1 as intermediate regulators. Despite this, it seems to be a stable process, as *MYB24* was previously identified among several metabolic QTLs segregating with flavonol content in ripe berry skins (Costantini et al., 2015). Additionally, *MYB24* and flavonols showed a high correlation in our integrated analysis of two drought studies (Savoi et al., 2016, 2017).

Our results suggest that MYB24 potentially regulates an additional metabolic pathway. MYB24 binds in close proximity within the promoters of ten genes related to isoprenoid/carotenoid metabolism. The clearest role of MYB24 in modulating light responses through carotenoids is observed with the carotenoid isomerase *CRTISO2* and lycopene beta cyclase *LCYE* genes, which were highly induced in the white skin sections of variegated berries (Supplemental Figure S18A). CRTISO2 promotes the accumulation of trans-lycopene. LCYE produces alpha-carotene from lycopene and is the first committed step in the production of lutein, the most abundant carotenoid (xanthophyll) in photosynthetic plant tissues where it plays important roles in light-harvesting complex-II structure and function (Kim and DellaPenna, 2006; Richaud et al., 2018).

Previous studies have provided evidence for an opposite relationship between terpenes or carotenoids (either the metabolites or their related genes) and anthocyanins, especially when comparing cultivars with different degrees of pigmentation (Cravero et al., 1994; Massonnet et al., 2017; Zheng et al., 2021; Bunea et al., 2012). Our data put MYB24 and its regulatory network in the center of this conjuncture (Figure 8). Anthocyanin depletion results in excessive radiation leading to UV and high-light stress responses in white-skin sections, ultimately boosting *MYB24* and *HYH/MYBF1* expression and leading to the accumulation of di-substituted (i.e., quercetin) and mono-substituted (i.e., kaempferol) flavonols. On the contrary, skin-localized anthocyanins seem to produce a self-shade effect over the mesocarp cells, reducing *MYB24* expression in pulp compared to skins (Figure 5B). The inverse correlation of *MYB24* and *TPS* expression and anthocyanin abundance in berry skin at late stages of ripening is also

demonstrated when comparing pink and dark red cultivars (Santibáñez et al., 2019). Our data strongly suggest that high-light, and a fraction of the non-filtered UV spectra, upregulate *MYB24* expression as a consequence of the lack of epidermal sunscreens. However, we cannot rule out the possibility of indirect repression of *MYB24* by MYBA1 (Figure 8). In fact, *MYBA1-UFGT1* and *MYB24* also showed a negative correlation in the cv. 'Gamay' study (Supplemental Figure S23).

Very few transcription factors are known to control terpene synthesis in model plant species, and even less are known as regulators of more than one metabolic pathway. Previous studies have implied that VviMYB24 is related to terpene and flavonol accumulation (Savoi et al., 2016; Lu et al., 2021), while their roles in carotenoid accumulation have been unclear. Our study demonstrates that MYB24 is a key coordinator of these pathways as part of the UV/high-intensity light-driven signaling response. All these processes respond to the depletion of the anthocyanin sunscreen in variegated berries, thereby contributing to the opposite abundances between isoprenoids and anthocyanins found in fruits.

Materials and Methods

Plant materials and field sampling of grape organs throughout development

Ripening berries belonging to the *Vitis vinifera* cultivars cv. 'Béquignol Noir' (some with variegated berries) and 'Béquignol blanc' were sampled from a grapevine germplasm collection (INRA, France) at five weeks after the onset of ripening/veraison (5WAV) in two consecutive years (for transcriptomic analysis) and every two/three weeks, starting from veraison for RT-qPCR gene expression analysis and volatile aroma compound identification and quantification. Approximately two berries from eight clusters belonging to five plants were used for each biological replicate (n=3). The berries were immediately frozen in liquid nitrogen, peeled after slight thawing, and deseeded in liquid nitrogen, and the skin and pulp were kept separated for later analyses. The samples were ground into a powder in liquid nitrogen using a ball grinder MM200 (Retsch, Haan, Germany) and stored at –80 °C for later analysis. Grape berries were also harvested from cv. 'Gewürztraminer' (at 40, 53, 67, 84, 101, and 116 days after anthesis, DAA) and cv. 'Viognier' (32, 45, 73, 92, and 105 DAA) vineyards located in Oliver (BC, Canada). Forty berries per sample were collected for terpene and transcript analyses; berries were cut off from the cluster at the pedicel level, snap frozen in liquid nitrogen, and stored at -80°C. Open flower samples were collected from two-year-old vines grown in the UBC Horticulture Greenhouse. At each developmental stage, three biological replicates per cultivar were performed. All berries were immediately peeled and deseeded before sample storage. All organs and tissues were frozen in liquid nitrogen and stored at -80 ºC until required for HPLC or GC analyses or RNA extraction.

Microscopy

Sections of ripening berries (at 5 weeks after veraison, WAV) were generated with a vibrating-blade microtome (Microm HM 650V). After cutting the fruit in two at the equatorial zone with a scalpel, the half berry obtained was fixed on a piece of metal placed in the center of a tank-driven microtome. Cross-sections from 60µm to 100µm were cut in water and mounted between a slide and a coverslip with a drop of distilled water. Sections were observed at 10X and 20X under a Zeiss Axiophot microscope, and digitalized pictures were obtained with a spot camera (Diagnostic Instruments).

Phenylpropanoid extraction and HPLC

Aliquots of 200 mg of berry skin powder were freeze-dried for 72 h, and the dried powders (~50 mg) were extracted in 1.0 mL methanol containing 0.1% HCL (v/v). The extracts were filtered through a 0.45 µm polypropylene syringe filter (Pall Gelman Corp., Ann Arbor, MI, USA) for HPLC analysis. Each individual sample was analyzed by HPLC as described (Guan et al., 2016, Rodríguez-Lorenzo et al., 2023). Anthocyanin quantification was performed as described in (Dai et al., 2014). Flavonol identification was carried out by MS and NMR spectrometry (MS-NRMS; Hilbert et al., 2015). Quercetin 3-O-glucoside, quercetin 3-O-galactoside, kaempferol-3-glucoside and myricetin-3-glucoside standards were obtained from Extrasynthese (Genay, France) and used to generate calibration curves.

Molecular marker analysis

Different organs and tissues representing pure L2 (i.e., root and berry pulp) and mixed L1 plus L2 (i.e., leaves and berry skin) cell ontologies were sampled from cv. 'Béquignol' somatic variants, including skin samples from variegated berries. Genomic DNA was extracted using a slightly modified CTAB based extraction procedure as described previously (Lodhi et al., 1994). The haplotype structure was analyzed using nine different microsatellite markers across distal arm chromosome 2 and analysis of *VvMybA1* and *VVMybA2* alleles. *VvMybA1* gene polymorphisms were investigated using the primers a and d3, and PCR amplifications were performed as reported (Lijavetzky et al., 2006) using previously described F2 and R1 primers (Azuma et al., 2008). PCR fragments were separated by electrophoresis in 1.5% agarose gel in TBE buffer, stained with ethidium bromide and photographed under UV light. For the *VvMybA2* gene, one point mutation (SNP) related to berry color, *VvMybA2R44* (Walker et al., 2007), was investigated as described (Carrasco et al., 2015) and analyzed by capillary electrophoresis (ABI PRISM 310 Genetic Analyzer, PE Applied Biosystems, California, USA), and data analysis was performed using Peak Scanner Software 2 version 2.0. Microsatellites used were described in different publications: (VvNTM1, VvNTM3, VvNTM4, VvNTM5 and VvNTM6) (Fournier-Level et al., 2009),

(SC8_0146_010; SC8_0146_010) (Ferreira et al., 2018a) (VMC7G3) (Pellerone et al., 2001), (VVIU20) (Merdinoglu et al., 2005). Amplifications were performed separately for each microsatellite, and size was calculated after capillarity electrophoresis at SECUGEN S.L. (ABI PRISM 310 Genetic Analyzer. PE Applied Biosystems, California, USA). The analysis of all capillarity electrophoresis data was performed using Peak Scanner Software 2 version 2.0.

Transcriptomic exploration of variegation

Microarrays were produced using the *Vitis vinifera* Array-Ready Oligo Set™ version 1 (Operon Biotechnologies, Germany) as described (Camps et al., 2010). Microarrays were scanned using a GenePix 4000B fluorescence reader using GenePix Pro version 4 image acquisition software (Axon Instruments, Canada). Spot quantification and quality control were done using Maia version 2.75 (Novikov and Barillot, 2007). Bad quality spots and those with intensities above 50000 were filtered before further analyses. Data analyses were performed using the R/Bioconductor (Gentleman et al., 2004) package limma (Smyth, 2004). Background correction was done using the normexp method (Ritchie et al., 2007). Array normalization was carried out using the limma function 'normalizeWithinArrays' (Smyth and Speed, 2003) and the method 'printtiploess'. P-Value adjustment was done using the Benjamin-Hochberg method. Genes with expression ratio above 1.6 and adjusted *p*-value below 0.1 were considered to be differentially expressed. For probe mapping, oligonucleotide probes were mapped to the 12X.1 CRIBI V1 annotation of the PN40024 grapevine genome (Jaillon et al., 2007) as in Ensemble Genes (Kersey et al., 2014) release 22. Best matches were considered to be targets. Genome sequences were annotated using best BLAST matches against the uniref100 database, release 15.14 (Suzek et al., 2007) using the qualifiers: homologous to (> 50% alignment identity), similar to (> 70%), weakly similar to (<= 70%), complete (> 98% hit coverage) and partial (<= 98% hit coverage). Functional analyses were done using the MapMan and GSEA Ontology (Thimm et al., 2004; Subramanian et al., 2005) and grapevine mappings made for the microarray employed (Rotter et al., 2009). Significantly affected categories were identified using a Wilcoxon rank-sum test implemented in R (R Development Core Team, 2014). Most significantly affected categories were illustrated using R/Lattice (Sarkar, 2008).

Gene co-expression networks

Two distinct aggregate gene co-expression networks –condition independent and condition dependent GCNs– were constructed from RNA-Seq datasets downloaded from the SRA database. The condition-dependent network only contained datasets extracted from berry tissue from *Vitis vinifera*, while the condition-independent network contained all available *Vitis vinifera* datasets. Networks were

constructed as described in (Orduña et al., 2022). Briefly, the results of 131 and 67 SRA studies were obtained, encompassing 2,766 and 1,615 runs from condition-independent and condition-dependent (berry) samples, respectively (Supplemental Data Set 19 and 20). Each SRA study was individually analyzed to build a highest reciprocal rank (HRR) matrix (Mutwil et al., 2011). To construct the aggregate whole genome co-expression network, the frequency of co-expression interaction(s) across individual HRR matrices was used as edge weights, and after ranking in descending order, the top 420 frequency values for each gene were chosen to build the final aggregate networks. The list of top 420 most highly co-expressed genes (1% of all VCost.v3 gene models) was used to generate individual gene-centered co-expression networks (GCNs). The individual *TPSs* and *MYB24* GCNs extracted from the condition-dependent and -independent networks were analyzed to generate Supplemental Figure S13.

Re-analysis of public RNA-seq datasets

Raw sequencing transcriptome datasets were processed using Trimmomatic, and filtered reads were aligned to the aligned to the 12X grapevine reference genome using HISAT2 as previously described (Vannozzi et al., 2018). Expression of grapevine *MYB24* splice variants (*MYB24.1* and *MYB24.2*) was estimated from HISAT2-aligned BAM outputs using Stringtie with default settings. Transcript abundance is expressed as TPM (Transcripts Per Kilobase Million).

Transactivation assay in yeast

The coding sequence of MYB24 was cloned into the pDEST32 vector by LR recombination to obtain the *Gal4DBD:VviMYB24* construct. The fusion protein vector was transformed into yeast (*Saccharomyces cerevisiae*) strains SFY526 (Wade Harper et al., 1993) and NLY2:SS-38 (Arnerić et al., 2002). Transformants were selected on SD-glucose medium supplemented with -Leu drop-out solution (BD Biosciences). Confirmation of interaction was performed by targeted transformation of the specific constructs using the small-scale yeast transformation protocol as described in the yeast protocol handbook (Clontech). Transformants were grown in YPDA medium to OD_{600} 0.5 – 0.8. Tubes with 1.5 mL of culture medium were centrifuged at 1400 rpm for 30 sec and the supernant was discarded. The pellet was resuspended by pipetting in 300µL of Z-buffer. The solution was frozen in liquid nitrogen and thawed at 37ºC for 30 sec; this process was repeated 3 times. Finally, transcriptional activity was measured using ortho-nitrophenyl-β-galactoside (ONPG) as substrate for β-galactosidase enzyme released from yeast strains SFY526 and NLY2:SS-38 transformed with *Gal4DBD:VviMYB24*. When hydrolyzed, ONPG generates a galactose molecule and an o-nitrophenol molecule, with the latter being quantified using a spectrophotometer at a wavelength of 410nm.

Confocal microscopy

Agrobacterium tumefaciens strain GV3101, previously transformed with the vector pK7FWG2 (35S:VviMYB24-eGFP), was grown in 10 mL of liquid LB supplemented with rifampicin 50 ng / L, gentamicin 25 ng / L and spectinomycin 50 ng / L. Bacteria isolated from the selection medium were resuspended in 4 mL of 10mM MgCl2 for infiltration. *Nicotiana benthamiana* leaves were infiltrated through the abaxial surface with a needleless syringe. The plants were left in the greenhouse for 3 days. Thirty minutes prior to visualization, 1 cm^2 leaf sections were incubated in a 20 mg / mL solution of 4', 6-diamino-2-phenylindole (DAPI). Fluorescence was visualized under a Nikon Confocal Eclipse C2si microscope. A 405 nm laser was used to excite DAPI, and a filter cube with emission at 445/35 (427 - 462nm) was used for its detection. For GFP, a 488 nm laser and a filter cube with emission at 525/50 (500 - 550nm) and 600/50 (575 - 625nm) were used.

DNA affinity purification sequencing (DAP-seq)

Genomic DNA (gDNA) was purified from young grapevine leaves of cv. 'Cabernet Sauvignon' CS08 by crude nuclei isolation (with PVP40) and 20% Sarkosyl-chloroflorm extraction (Thomas et al., 1993). Genomic DNA library and DAP-seq were performed following a published protocol (Bartlett et al., 2017). Briefly, the gDNA sample was sonicated into 200 bp fragments on a Covaris Focus-ultrasonicator instrument, which underwent end repair, A-tailing and adapter ligation to attach Illumina-compatible sequencing adapters to the DAP-seq library. We verified successful adapter ligation by RT-qPCR of the DAP-seq library with primer sequences that anneal to the adapter, as well as gel electrophoresis of the sonicated genomic DNA and the DAP-seq library (Bartlett et al., 2017). *MYB24*, *MYBA1*, *MYBA6* and *MYBA7* were amplified from cv. 'Cabernet Sauvignon' and cloned into the pIX-HALO expression plasmid (TAIR Vector:6530264275) to create an expression vector that contained a HALO-tag in frame at the N-terminus. *MYB113* amplified from Arabidopsis was also used (O'Malley et al., 2016).

Clones were verified by digestion with restriction enzyme XhoI. The MYB-expression vectors were used in a coupled transcription/translation system (Promega) to produce MYB proteins with the HaloTag-fusions. Expression of MYB24 was confirmed by immunoblotting using an anti-HaloTag antibody (Promega). The protein expression reaction was mixed with HaloTag-ligand conjugated magnetic beads (Promega) to pull down the HaloTag-fused TF. The pulled down TF was then be mixed with the DAP-seq library in order for TF-DNA binding to occur. A 400ng library was used in each DAP-seq reaction. The bound DNA was then eluted and PCR amplified to generate sequencing libraries, which were sequenced on an Illumina NextSeq 500 platform (sequencing of libraries was set at 30

million and 1x75bp single-end reads). As a negative control, we performed a DAP-seq experiment with the pIX-HALO expression vector without any ORF insert, accounting for possible non-specific DNA binding in the reaction mixture, as well as copy number variations at specific genomic loci. Sequencing reads were mapped to the CS08 (140X) and the reference PN40024 (12X.2) genome assemblies available at https://cantulab.github.io/data.html. Enrichment of binding events was first evaluated by plotting genome coverage compared to the negative control (Bartlett et al., 2017). To identify "peaks", enriched regions relative to the control that correspond to binding events, the GEM peak caller was used (Guo et al., 2012), which performs simultaneous peak calling and motif refinement. *De novo* motif discovery was performed using 200 bp sequences centered at GEM-identified binding events for the 600 most enriched peaks (Bailey et al., 2009). MYB24 target genes were identified by associating the peak regions to the TSS of the closet genes.

Analyzing the overlap of DAP-seq and transcriptomic datasets

DAP-Seq peaks were converted from cv. 'Cabernet Sauvignon' to their corresponding cv. 'Pinot Noir' IDs after filtering a BLAST output between both genome annotations (98% identity and 98% coverage thresholds). *TPS* genes were manually curated and assigned to its cultivar counterpart based in BLAST and global alignments. Berry skin transcriptomic datasets from UV-B-irradiated (RMA-normalized) samples (Carbonell-Bejerano et al., 2014) were analyzed using PN40024 12X.2 Assembly V1 annotation, and those of drought stressed (raw data downloaded from PRJNA313234 BioProject at SRA) (Savoi et al., 2016) and 'light versus shade' treatments (raw data downloaded from PRJNA661034 at SRA) (Sun et al., 2017) were reanalyzed using PN40024 12X.2 Assembly VCost.v3 annotation. The RMA-normalized microarray data, containing two developmental stages (23º and 26º Brix degrees) and two UV-B conditions, were compared using a one-factor ANOVA (limma package in R). Genes with (adj pvalue < 0.05 for RNA-seq data, < 0.1 for microarray data, and logFC > 0.53) in at least one berry density were considered to be differentially expressed (DE). The Illumina raw sequence reads were trimmed using fastp version 2.0 (the minimum PHRED score accepted for trimming was 20, and reads shorter than 40 bp were discarded). Reads were aligned against the reference PN40024 12X.2 genome assembly using the HISAT2 v2.1.0 aligner with default parameters. Aligned reads were counted with Feature Counts, using the Vcost annotation mapped in 12X.2. Finally, drought and 'light vs shade' DE genes (adj pvalue < 0.05 and logFC > 0.53) were extracted using the limma R package. The overlap between UV-B, drought, and 'light vs shade' DE genes, the MYB24-GCN and the DAP-seq peaks was analyzed using the UpSetplot package in R.

Transient leaf transfection and dual-luciferase reporter assay

The transactivation of the upstream regions of *TPS35*/*09*, *HYH,* and *CRTISO2* by MYB24 was tested using a Dual-Luciferase Reporter Assay (Promega). The promoters/5' UTRs of *TPS35/09*, *HYH*, and *CRTISO2* from 'Cabernet Sauvignon', and the coding sequences of the TF genes *VviMYB24*, *AtMYC5*, *VviMYC2*, *VviMYBA1*, and *VviMYC1* were amplified and cloned into the entry vector pENTR/D-TOPO (Invitrogen) and then transferred by site-specific recombination into the reporter and effector vectors (pPGWL7.0 and pK7WG2.0, respectively) using LR Clonase. The resulting constructs and the reference vector expressing the Renilla luciferase gene (Cavallini et al., 2015) were transferred to *A. tumefaciens* strain EHA105 by heat shock. Dual-Luciferase assays were performed in Agrobacterium-infiltrated *Nicotiana benthamiana* leaves as described in (Espley et al., 2009). The combinations of *VviMYBA1* and *VviMYC1* with the *TPS35*/*09*, *HYH* and *CRTISO2* promoters were used as negative controls (Supplemental Figure S18 and S24). Plants were grown from seeds in a greenhouse with temperature between 30°C and 21°C, relative humidity of approximately 32-50%, and 15 h/9h light/dark cycle, also after agroinfiltration (three days). Light was given by led bulbs (Phillips GP LED production DR/B 150 LB), each one providing a typical photon flux of 210 umol/s, consuming 32W with an efficacy of 3.3 umol/J. Plants were placed 70 cm below the light source receiving an average of 2000 lux, measured using a luminometer (MT-912 Light Meter). Firefly (LUC) and Renilla (REN) luminescence were detected using a GENios Pro TECAN instrument. Statistical analysis of the results of the Dual-LUC assay is provided in Supplemental Data Set 21.

Bimolecular fluorescence complementation (BiFC) assay

The BiFC assays were performed in Agrobacterium-infiltrated *N. benthamiana* leaves as described in (Navarro et al., 2020). Four TF genes (*VviMYB24*, *AtMYC5*, *VviMYC2,* and *VviMYC1*) were amplified and cloned to two binary vectors (35S:NtGFP and 35S:CtGFP). The recombinant constructs were transferred to *A. tumefaciens* strain C58 by heat shock. A single PCR-positive Agrobacterium transformant was inoculated in medium and grown overnight with the corresponding antibiotics. The final culture was centrifuged at room temperature at low speed (4000 rpm). The pellets were resuspended in MMA solution (0.1 M PH=5.6 MES, 1 M MgCl2). According to each combination, bacteria suspensions were diluted and mixed, keeping the OD600 at 0.2 for each construct. The mixture was infiltrated into the abaxial sides of leaves. Plants were grown and kept in the same light conditions (16 h light /25 °C and 8 h dark /22 °C). After 48h, infiltrated samples without leaf veins were collected. Samples were observed under a Zeiss LSM780 AxioObserver confocal microscope at 40X. GFP (Exc 488nm, Emission 490-544nm) and Chlorophyll (Exc 488nm, Emission 680-760 nm) channels were used for visualization.

Nucleic acid extraction and quantification of gene expression by RT-qPCR

Different methods of RNA extraction were used depending on the plant materials. Total RNA was isolated from cv. 'Cabernet Sauvignon' and cv. 'Béquignol' berry skins as described previously (Reid et al., 2006). For cv. 'Gewürztraminer' and cv. 'Viognier', frozen samples were ground to a fine powder in liquid nitrogen using an analytical mill (A11 Basic, IKA). RNA extraction and determination of RNA quality and quantity were performed as described (Wong et al., 2016). The RT reactions were performed with two µg of total RNA, reverse transcribed with oligo(dT)15 in a 20-µL reaction mixture using Moloney murine leukemia virus reverse transcriptase (Promega) or a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific), according to the manufacturer's instructions. RT-qPCR was performed for all genes of interest using specific primers. For cv. 'Béquignol' samples, a Brilliant® SYBR® Green QPCR Master Reagent Kit (Stratagene) and the Mx3000P detection system (Stratagene) were used. In the remaining cases, PowerUp SYBR Green Master Mix (Thermo Scientific) was used with an ABI 7500 Real-Time PCR System (Applied Biosystems). Amplification of the *UBIQUITIN1* gene (99bp, Downey et al., 2003), *ELONGATION FACTOR1* (*EF1;* 91bp) and *ACTIN* (81bp) (Hichri et al., 2010) were used for normalization of relative gene expression. All RT-qPCR biological replicates were run in two or three technical replicates within the same plate. PCR conditions, standard quantification curves for each gene and relative gene expression calculations were conducted as in (Wong et al., 2016). Statistical analysis of the RT-qPCR results is provided in Supplemental Data Set 21. Primers are shown in Supplemental Data Set 22.

Analysis of volatiles in cv. 'Gewurztraminer' and 'Viognier'

Free (non-glycosylated) VOCs were analysed with some modifications (Kovalenko et al., 2021). Five g of frozen grape powder were weight in a 20 ml SPME glass screw cap vial with 1.5 g sodium chloride. Then 4 ml citrate phosphate buffer (pH 5.0) and 100 µl 200 g/L ascorbic acid were added. Finally, 3-Octanol (10ppb final concentration) and d3-Linalool (10ppb final concentration) were added to the sample as internal standards. The free volatile compounds were extracted and analyzed by headspace–solid phase microextraction (SPME) gas chromatography-mass spectrometry (GCMS). A carboxen–divinylbenzene–polydimethylsiloxane SPME fiber was used (No. 57329-U Supelco, Sigma,St Louis, MO, USA). VOC analyses were performed on a gas chromatograph (model 7890A, Agilent, Waldbron, Germany) equipped with a 5975C mass spectrometer detector and GC PAL80 autosampler (Agilent Technologies). The column used was an Agilent J&W Scientific DB-WAX (30m x 0.25mm ID with 0.25um film thickness) with a film thickness of 0.25 mm. Helium was used as the carrier gas at a constant flow of 0.8mL min^{-1}. The capped vials were heated at 40 °C for 20 min to promote the

transference of the compounds from the sample to the headspace. After this step, the SPME fiber was inserted into the sample vial headspace (22mm depth) for a 30 min sample extraction at 40 ℃ and then inserted into the GC injection port at 250 ℃ and kept for 5 min for desorption. The injection port was lined with a 0.75-mm splitless glass liner. The Pulse splitless injection mode was used. The oven temperature program was as follows: 40 ℃ for 4 min, followed by 3 ℃ /min up to 150 ℃, 25 ℃ /min to 230 ℃, 230 ℃ for 10 min. The mass spectrometer was operated in the electron impact mode at 70 eV, scanning from *m*/*z* 33–500. The ion source temperature was 230 ℃ and the quadrupole temperature was 150 ℃. Data processing was carried out by MSD Chemstation (E.02.02.1431, Agilent Technologies Inc.). Only VOC peaks with signal-to-noise ratios greater than 10:1 were considered for data quantification.

The authentic standards used for identification were reported in Kovalenko et al., 2021. Terpenes were identified by comparing the retention times of ion extracted chromatogram (IEC) peaks, with the retention times of their reference standards when available. The retention indexes (RI) were calculated in relation to a mixture of C7–C30 n-alkanes and compared with published work, and by identifying the mass spectra using the Wiley09Nist08 Database (NIST library) (Kovalenko et al., 2021). The volatile concentration of samples was determined based on the calibration curve of the respective authentic standard. Compounds were semi-quantified using the calibration curve of the compound with the closest chemical functionality and structure when authentic standards were not available.

GC-MS analysis of volatiles in cv. 'Béquignol' samples

Samples were prepared and solid-phase extracted (SPE). Three biological replicates of red and white skins of variegated berries were used. Skins were ground in liquid nitrogen and suspended in 4 mL of sodium sulphite solution at (Na_2SO_3, 10 g/l). The supernatant obtained after a 15 min centrifugation (11400 x g at 4 °C) was passed through a glass fibre prefilter and a glass filter. Twenty microliters of a 1 g/L 3-octanol solution were added as external standard to allow for the quantification of the major terpenols. The filtrate was passed through a 1 g phase C18 silica-bonded non-polar column (HF Mega Bond Bond Elut C18, Agilent, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States), previously rinsed with 6 mL of methanol and 2x6 mL of ultrapure water, at a rate of approximately 1 drop/sec. Total fractions of free and bound monoterpenols were eluted with 4 mL of absolute ethanol. This total terpenol extract was diluted in 40 mL of citrate-phosphate buffer (pH 4.5) and the resulting solution was incubated with 50 mg of Rapidase® AR 2000 glycolytic enzyme (DSM Food Specialties Beverage Ingredients P.O. Box 1, 2600 MA Delft, The Netherlands) overnight at 37.5 °C to release the glycosidically bound terpenols. The released terpenols were separated by SPE again on a C18 column

and eluted with 4 mL of dichloromethane after rinsing with water. Extracts were dried in Pasteur pipettes filled with 0.5 g of anhydrous sodium sulphite and concentrated to 500 µl under a gentle nitrogen flux. Twenty microliters of a 1 g/L m-cresol solution were added to each sample as internal control. Samples were stored at –20 °C prior to gas chromatography analysis.

For GC-MS non-targeted analysis, chromatogram files were deconvoluted and converted to ELU format using AMDIS Mass Spectrometry software (http://www.amdis.net), with the resolution and sensitivity set to medium. Chromatograms were then aligned and integrated using Spectconnect (http://spectconnect.mit.edu). All metabolites found in the blank run or believed to have originated from the column bleeding were removed from analysis at this time. After removing contaminant metabolites (volatiles), the IS matrix from Spectconnect was normalized according to the sample weight and 3-octanol surface (external standard). Volatiles were identified by comparing to the NIST 14 standard reference database. Identities of metabolites of interest were then confirmed using authentic standards when available.

Terpenols were quantified by GC-MS targeted analysis. Extracts were analyzed by GC-MS using an Agilent 6890 gas chromatograph equipped with a Gerstel MP2 autosampler and an Agilent 5973N mass spectrometer for peak detection and compound identification. The GC was fitted with a DB-Wax column (30m×0.32mm i.d., 0.5 µm film thickness, Agilent J&W, Agilent Technologies France, 3, Avenue du Canada, 91978 les Ulis France). Helium was used as the carrier gas with a column flow rate of 1.5 mL/min. The GC oven temperature was programmed from 45°C to 235° C, at 2.7°C/min (hold 10 min). The injector was set to 230°C and used in pulsed splitless mode (15 psi for 0.50 min). The MS transfer line and ion source temperatures were set at 270 °C and 230 °C, respectively. The MS was operated in EI mode and positive ions at 70 eV were recorded with a scan range from m/z 30 to m/z 400. Agilent MSD ChemStation software (G1701DA, Rev D.03.00) was used for instrument control and data acquisition. Injection of a solution of n-alkane standards (C7 C35) was also run to calculate linear retention index. Total amounts of alpha-terpineol, citronellol, linalool, nerol and geraniol were determined using linear calibration curves generated from five different concentrations over a range of 0.5–100 ng/µL analyzed in triplicate for each standard. Final concentrations were expressed in microgram/kg of fresh weight. Statistical analysis is provided in Supplemental Data Set 21.

Sunlight/UV-B experiments

Three different sunlight or UV-B radiation experiments were conducted using cv. ‘Cabernet Sauvignon’ commercial plants (either treated in the field or uprooted and transferred to a greenhouse). Experiment 1: low fluence UV-B exposure treatment applied to clusters from nine-year-old potted vines in a UV-free

greenhouse during the 2011-2012 and 2012-2013 growing seasons (n=3), respectively (Loyola et al., 2016). Experiment 2: A UV-B filtering radiation treatment was applied in a commercial vineyard as described previously (Czemmel et al., 2017) during the 2011-2012 growing season (n=4). The filtering treatment consisted of blocking solar UV-B radiation by installing a 100 µm clear polyester film at the positions of grape clusters. Experiment 3: Sunlight reduction treatments were conducted in the field as described previously (Matus et al., 2009) and consisted of full shading of fruits by the plant's own canopy (0% exposure) and full sunlight exposure from veraison onwards, generated by displacement of leaves around the cluster region (100% exposure, n=3). Berry skins were frozen in liquid nitrogen and stored at -80ºC until RNA extraction.

Light shading experiments in cv. 'Gamay' and cv. 'Gamay Fréaux'

Light exclusion was conducted using 23-year-old spur pruned vines, with a density of 1.6 m between rows and 1 m between plants in a germplasm collection vineyard. Eighteen vines were chosen to form three blocks with three vines each, and two similar clusters from two adjacent shoots of each vine were tagged. Opaque boxes were applied to one of the two tagged clusters of each vine from 2 weeks before veraison until maturity for light-exclusion treatment, and the other clusters were exposed under natural light conditions as the control as described previously (Guan et al., 2016). Light-exposed or shaded clusters of both cultivars were sampled weekly from one week after treatment until maturity. Three berries from each cluster and three clusters from three vines per block were sampled for each treatment at each sampling date. Berries were weighed, deseeded, separated into skin and pulp, and the skin and pulp were immediately frozen in liquid nitrogen. The samples were ground into a powder in liquid nitrogen using a ball grinder MM200 (Retsch, Haan, Germany) and stored at –80 °C for later analysis.

Correlation of *MYB24*/*HYH* expression with metabolite composition in berry skins of drought-stressed plants

Transcriptomic data (Savoi et al., 2016, 2017) were remapped to the PN40024 12X.2 assembly and VCost.v3 annotation. Genes with low expression levels were filtered according to the method described in (Chen et al., 2016), and raw counts were normalized to FPKM (Fragments Per Kilobase Million) values. Applying the Weighted Gene Co-Expression Network Analysis (*WGCNA*) R package (Langfelder and Horvath, 2008), the genes were grouped into different clusters. The parameters used were a blockwiseModules with a soft-threshold power value of 30 (fitting a scale free topology network), a deepSplit of 4 and a mergeCutHeight of 0.2, obtaining a total of 28 modules. The correlation between gene or the modules eigengenes (1st principal component) and metabolite quantification was visualized using the pheatmap (https://CRAN.R-project.org/package=pheatmap) R package, performing

a hierarchical clustering using the complete linkage method with Euclidean distance.

Transmission and absorbance analysis of major flavonols and anthocyanins in berries

The optical absorption and transmittance spectra of flavonols (Quercetin 3-β-D-glucoside and Kaempferol-3-glucoside) and anthocyanins (Malvidin chloride, Malvidin-3-galactoside chloride, and Delphinidin 3-O-β-D-glucoside chloride) was measured in a home-built optical setup, described in Liang et al., 2022a, and consisting of a mercury lamp (covering the optical range of 200 to 900 nm), fused silica lenses, reflecting optics objectives, and a visible near-Infrared spectrometer (Ocean Optics Maya2000 Pro). The light was spotted using a 20μm diameter pinhole. HPLC analytical standards of flavonols (Quercetin 3-β-D-glucoside from Sigma-Aldrich and Kaempferol-3-glucoside from HWI group) and anthocyanins (Malvidin chloride from Cayman, Malvidin-3-galactoside chloride and Delphinidin 3-O-β-D-glucoside chloride from Sigma-Aldrich) were prepared in DMSO and diluted to 0.5 mg/ml with DEPC water. One drop of each solution was set on the top of a sapphire (Al2O3) thin slice (with a thickness of 0.5 mm) Absorbance measurements were performed after the solution on the slice was completely dried. The sample-in and sample-out method (Liang et al., 2022b) was used to acquire the spectra, in which the intensity of the light transmitted through the sample [$I(\omega)$] was normalized against the intensity of the light transmitted through an empty area of the sapphire [$I(\omega)$]. The absorption spectra were fitted by Gaussian profiles with results given in Supplemental Figure S21. Data are shown as percentage of its maximum transmission in Figure 7A.

Phylogenetic analysis

The complete protein sequences of Arabidopsis and grape bHLH subfamily 8 genes were used for phylogenetic reconstruction. Flavonoid-related bHLH subfamily 7 members VviMYC1 and AtTT8 were used as outgroup. Multiple sequence alignments (gap open penalty of −2.9) were performed using the MUSCLE algorithm-based AlignX module from MEGA software (Supplemental File 1). Phylogenetic trees were constructed using the maximum likelihood (ML) method, with partial deletion gap treatments. Reliability of tree nodes was evaluated with 2000 bootstraps. Resulting tree (Supplemental File 2) was visualized with FigTree (shown in Supplemental Figure S12).

Statistical analyses

Statistical analyses were carried out using MinitabExpress™ 1.5.3 software. RT-qPCR data in cv. 'Béquignol' were analyzed using a one-way ANOVA to investigate differences between red and white skin areas at each developmental stage. Tukey's *post hoc* test was used to infer statistically significant means ($p < 0.05$). For gene expression analyses in cv. 'Gamay', two-way ANOVA were performed to

either confirm or rule-out interactions between genotypes (treating genotype and light condition together as a single factor) and tissues. Tukey's *post hoc* test was used to deduce differences between means ($p < 0.1$; $p < 0.05$). Means that do not share a letter are significantly different. Volatilome (targeted) and Dual luciferase data were tested for significance using one-way ANOVA followed by Tukey's *post hoc* test. Statistical analysis of metabolite, dual luciferase, and gene expression (RT-qPCR) data is provided in Supplemental Data Set 21.

Accession numbers

Sequence data from this article can be found in the Grapedia portal/Gene Cards app (https://grapedia.org/genes/) under the following VCost.v3 accession numbers: *MYB24* (Vitvi14g01750), *MYBA1* (Vitvi02g01019), *UFGT1* (Vitvi16g00156), *MYBF1* (Vitvi07g00393), *HYH* (Vitvi05g00274), *FLS1* (Vitvi18g02541), *GT5* (Vitvi11g01290), *TPS35* (Vitvi12g00574), *TPS10* (Vitvi18g02449), *TPS09* (Vitvi18g02448), *HPR1* (Vitvi03g00154,), *ACD1* (Vitvi06g00063), *SEN1* (Vitvi04g02160), *PHOT2* (Vitvi03g00272), *sigE* (Vitvi16g01214), *BBX22* (Vitvi12g00543), *FOT6-4* (Vitvi09g00517), *FOT1* (Vitvi02g00475), *SEP2* (Vitvi07g01829), *UVR1* (Vitvi07g01923), *SOD* (Vitvi08g01802), *LPP1* (Vitvi01g01956), *ELIP1* (Vitvi05g00563), *OHP2* (Vitvi18g02961), *PRK* (Vitvi02g00915), *Z-ISO* (Vitvi05g01347), *CRTISO2* (Vitvi12g02046), *LCYE* (Vitvi11g00148), *ZEP1* (Vitvi07g01745), *UBIQUITIN1* (Vitvi16g01364),
EF1(Vitvi12g02055) and *ACTIN* (Vitvi04g01613). Omics data have been treated and uploaded in public repositories according to the FAIR principles, in accordance to the guidelines found at INTEGRAPE website**.** GCN and DAP-seq data are available at the Vitviz platform within the AggGCN and DAPBrowse Apps (http://vitviz.tomsbiolab.com/). DAP-seq (GSE198702) and microarray (GSE241123) data is available at NCBI GEO.

Supplemental Data

Supplemental Figure S1. The three somatic variants of the cv. 'Béquignol' family.

Supplemental Figure S2. Anthocyanin composition in red-skinned variegated and non-variegated cv. 'Béquignol Noir' berry skins at 5WAV.

Supplemental Figure S3. A selection of significantly enriched terms from KEGG and GO ontologies in red-skin up-regulated genes (RUGs).

Supplemental Figure S4. Expression domains of *MYB24* splicing variants.

Supplemental Figure S5. Several grape sesqui- and mono-terpene synthase (*TPS*) genes are highly co-expressed with *MYB24* in late stages of flower and berry development.

Supplemental Figure S6. Expression correlation matrices for MYB24 and isoprenoid/terpenoid genes surveyed in flower and berry developmental samples from high and low terpene-accumulating 'Gewurztraminer' and "Viognier, respectively.

Supplemental Figure S7. Distribution of MYB24 peaks in the cv. 'Cabernet Sauvignon' CS08 v1.0 genome with respect to all transcription start sites (TSSs) of assigned genes.

Supplemental Figure S8. Preferential binding sites in the *Terpene synthase* (*TPS*) promoter are specific to MYB24.

Supplemental Figure S9. MYB24 is a nucleus-localized transcriptional activator *in vivo*.

Supplemental Figure S10. Definition of MYB24 target genes, resulting from the overlap of DAP-seq data (mapped on the PN40024 12X.2 assembly), MYB24 co-expression data (merge of condition-dependent and -independent networks) and up-regulated genes found in UV-B radiation, drought stress, and/or 'light vs shade' transcriptomic studies.

Supplemental Figure S11. Transient expression of *VviMYB24* with Arabidopsis *thaliana AtMYC5* activates the *VviTPS35* promoter.

Supplemental Figure S12. Phylogenetic analysis and expression of MYC2 and its interaction with MYB24.

Supplemental Figure S13. Regulatory and reciprocal co-expression network of MYB24 and the grape terpene synthase family.

Supplemental Figure S14. Quantification of terpenoid volatiles and the expression patterns of *MYB24* and *TPS35* in the high terpene-accumulating cultivar cv. 'Gewurztraminer' and the low terpene-accumulating cv. 'Viognier'.

Supplemental Figure S15. Correlation analysis between specialized metabolites and mRNA abundances deduced from the integration of transcriptomics/metabolomics datasets from two drought-responsive experiments.

Supplemental Figure S16. Gas chromatography–mass spectrometry (GC-MS) untargeted volatile compound analysis in red and white skin sections of the variegated cv. 'Béquignol Noir' berry.

Supplemental Figure S17. A selection of enriched terms from the MapMan pathway enrichment analysis conducted for i) all MYB24-bound genes (DAP-seq), ii) bound genes at -5kb from TSS, and iii) High confidence target genes (HCT, -5Kb-TSS).

Supplemental Figure S18. MYB24 potentially regulates the carotenoid pathway.

Supplemental Figure S19. *MYB24* and *TPS35* expression in response to UV-B irradiation and sunlight/UV-B depletion in greenhouse and field trials.

Supplemental Figure S20. Light responsiveness of the *MYB24*, *TPS35* and *HYH* genes in cv. 'Gamay' and 'Gamay Fréaux' grape berry tissues.

Supplemental Figure S21. The optical absorbance spectra of flavonols and anthocyanins.

Supplemental Figure S22. Expression profile of some MYB24 targets related to light signaling and photosynthesis.

Supplemental Figure S23. Correlations between different genes in the cv. 'Gamay' experiment.

Supplemental Figure S24. MYBA1 has no effect on the activity of the *TPS35*, *TPS09*, and *HYH* promoters.

Supplemental File 1. MEGA alignment used for bHLH phylogeny.

Supplemental File 2. bHLH phylogenetic newick tree file.

Supplemental Data Set 1. All spotted genes in the Operon array.

Supplemental Data Set 2. Operon oligonucleotide microarrays of variegated red and white skin sections.

Supplemental Data Set 3. Differentially expressed genes in anthocyanin-depleted sections.

Supplemental Data Set 4. Gene set enrichment analysis (GSEA) of RUGs and WUGs.

Supplemental Data Set 5. MapMan enrichment analysis (significantly enriched terms are highlighted in color).

Supplemental Data Set 6. Genes selected to construct the heatmap (Fig. 3C).

Supplemental Data Set 7. MYB24/F1/60 co-expression network analysis in condition-dependent (flower/fruit) and -independent (all organs) data (MYB24/F1/60-GCN).

Supplemental Data Set 8. Gene set enrichment analysis of MYB24/F1/60-GCN (MYB24/F1/60-GSEA).

Supplemental Data Set 9. The MYB24 DAP-seq results mapped in the CS08 genome.

Supplemental Data Set 10. MYB24-bound TPS genes list from CS08.

Supplemental Data Set 11. Photosynthesis or light-related and isoprenoid/carotenoid-related genes whose promoter regions were bound by MYB24.

Supplemental Data Set 12. MYB24-DAP results mapped in the PN40024 12X.2 assembly, filtered by peak position (-5kb < distance to TSS < 0).

Supplemental Data Set 13. Co-expression data for MYB24-bound TPS genes that are not in the TOP420 co-expressed list of MYB24 GCN but whose own GCNs include MYB24.

Supplemental Data Set 14. Up-regulated genes in two berry developmental stages (23 º and 26 º Brix) in the UV-B studies.

Supplemental Data Set 15. Up-regulated genes in three berry developmental stages under light versus shade treatments.

Supplemental Data Set 16. Up-regulated genes in two berry developmental stages (68 and 93 DAA) in the drought studies.

Supplemental Data Set 17. List of putative MYB24 target genes corresponding to the Pinot Noir genome.

Supplemental Data Set 18. MapMan analysis of MYB24- all-bound genes, bound genes (-5kb-TSS), and high confidence target genes (HCTs) list.

Supplemental Data Set 19. Condition independent metadata list.

Supplemental Data Set 20. Condition dependent metadata list.

Supplemental Data Set 21. Statistical analysis of the Dual-LUC assay.

Supplemental Data Set 22. List of primers used for RT-qPCR analysis.

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Author contributions

J.T.M., Z.W.D. and F.B. designed the research. C.Z, Z.W.D, T.F, L.O., A.S., A.P., D.C.J.W., C.K., R.L., A.A., B.K., M.M.L., AK.L., D.C., C.M., C.E., G.H., R.F-B., D.C., R.A., P.A-J., P.C., E.D., S-s.C.H, D.E., and M.R.C. contributed reagents or conducted experimental/bioinformatic analyses. C.Z., S.S., S.D.C, G.B.T., S-s.C.H and J.T.M. analyzed data. C.Z. and J.T.M. wrote the paper.

Competing interests

The authors declare no competing interest.

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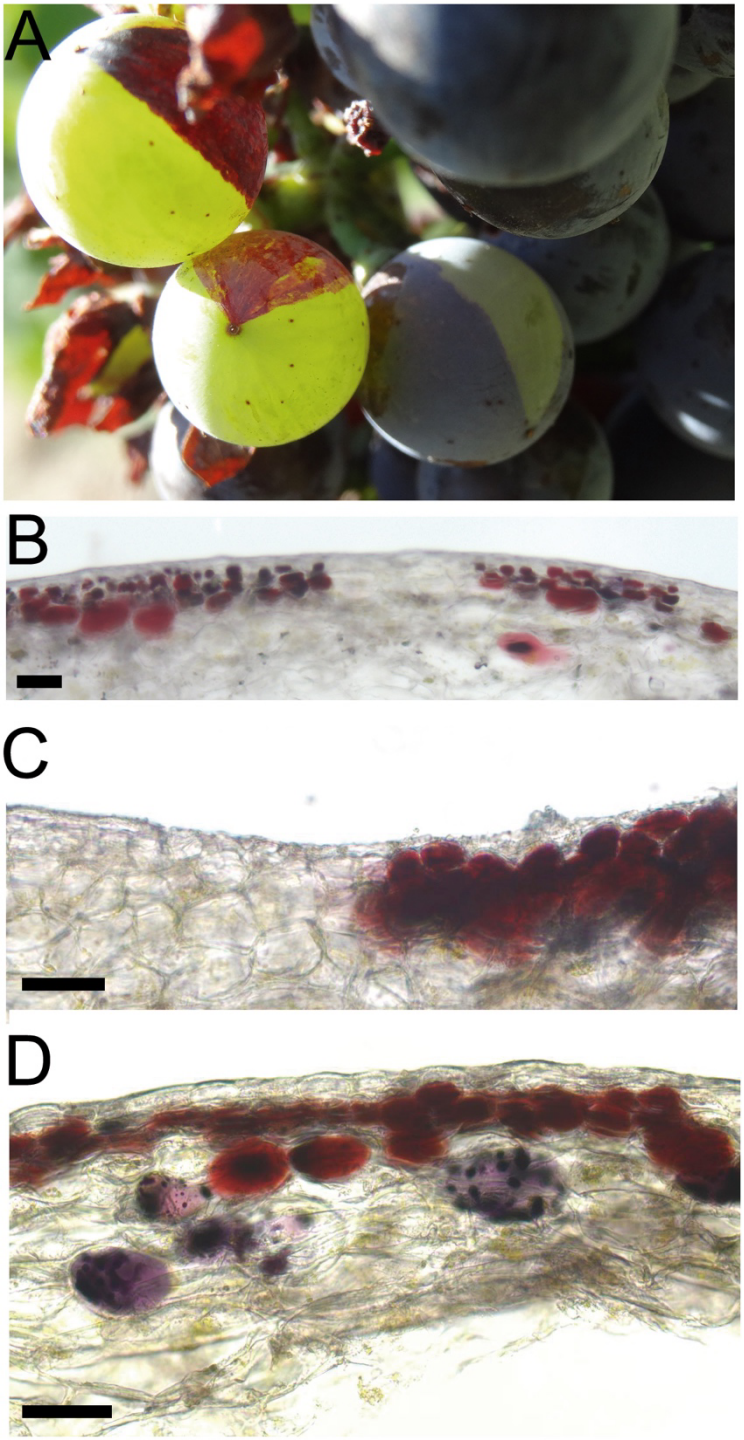

Figure 1. Differential skin pigmentation as a result of anthocyanin depletion in variegated cv. 'Béquignol Noir' berries. (A) Irregular berry skin pigmentation observed in field-grown vines at two weeks after veraison (2WAV). (B-D) Light microscopy images of variegated berry skin cell layers. (C-D) Sub-epidermal cells near color transitions show anthocyanin vacuolar inclusions (AVIs) accumulating either reddish or purplish pigments corresponding to di- and tri-hydroxylated anthocyanin derivatives, respectively (see also Supplemental Figure 2A). Pigment accumulation in variegated berries is seen throughout fruit ripening. Pigment accumulation in non-variegated cv. 'Béquignol (B.) Noir', 'B. Gris and 'B. Blanc' fruits can be seen in Supplemental Figure 1. Bar scale: 5µm.

A

Molecular marker and genomic coordinate on chromosome 2 (in Mb)

Cultivar	Organ	Cell layer	SC8_010		SC8_026		VVNTM1		VvMybA2		VvMybA1		VVNTM3		VVNTM5		VVNTM6		VVNTM4		VVIU20		VMC7G3	
			12674		12970		14149		14181		14248		14288		14325		14330		14384		16539		18270	
B. Noir	Root	L2	121	123	250	256	165	171	T	G	Gret	Non-Gret	274	298	283	289	149	151	208	210	365	368	116	116
B. Noir	Leaf	L1+L2	121	123	250	256	165	171	T	G	Gret	Non-Gret	274	298	283	289	149	151	208	210	365	368	116	116
B. Noir-variegated	Red skin section	L1+L2	121	123	250	256	165	171	T	G	Gret	Non-Gret	274	298	283	289	149	151	208	210	365	368	116	116
B. Noir-variegated	White skin	L1+L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. gris	Root	L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. gris	Leaf	L1+L2	121	123	250	256	165	171	T	G	Gret	Non-Gret	274	274	283	289	151	151	208	210	365	368	116	116
B. gris	Pulp	L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. gris	Skin	L1+L2	121	123	250	256	165	171	T	G	Gret	Non-Gret	274	274	283	289	151	151	208	210	365	368	116	116
B. blanc	Root	L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. blanc	Leaf	L1+L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. blanc	Pulp	L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116
B. blanc	Skin	L1+L2	121	121	256	256	171	171	T	T	Gret	Gret	274	274	289	289	151	151	208	210	365	368	116	116

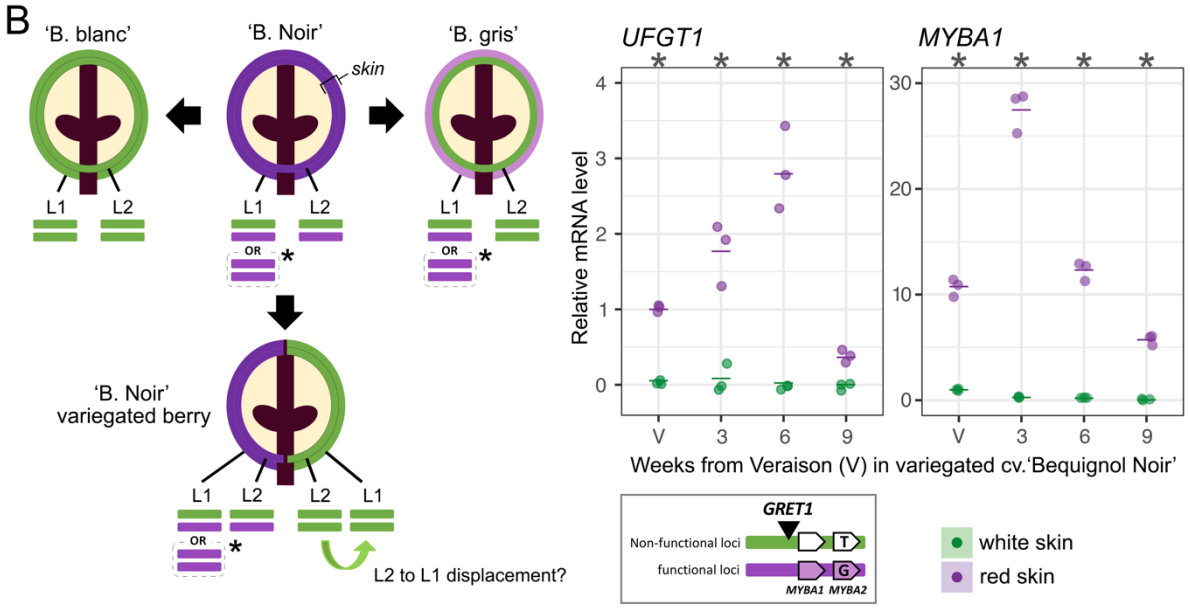

Figure 2. **Haplotype structure analysis of the berry color locus reveals two different genotypes in a single variegated berry of cv. 'Béquignol Noir'.** (A) Genetic profiling of the berry color locus and its surrounding genomic region in color somatic variants, including white, grey, red and variegated berries of 'Béquignol' cultivars. Eight microsatellite markers were assessed across the distal arm of chromosome 2, including allele sequence analysis of the anthocyanin regulators *MYBA1* and *MYBA2* (allele sizes are shown in base pairs). *MYBA1– Gret1* and *MYBA2–T* are the non-functional alleles, while *MYBA1–non-Gret1* and *MYBA2— G* correspond to the functional alleles. Allele size is shown in bp. (B, left panel) Model for the formation of 'B. Blanc', 'B. Gris', and the variegated phenotype from independent somatic mutation events in 'B. Noir'. Structural dynamics at the berry color locus in L1 and L2 meristematic cell layers are indicated for each variant. Asterisks represent an alternative configuration for both alleles in the L1 layer. (B, right panel: expression profiles of *UFGT1* and its target *MYBA1* at different ripening stages in red and white skin sections of variegated berries. Sixteen berries from 8 clusters from five plants were used per biological replicate. Data from three biological replicates are shown (averages as horizontal lines). Asterisks indicate significant differences ($p<0.05$) between skin sections based on one-way ANOVA followed by Tukey's *post hoc test* performed independently for each stage.

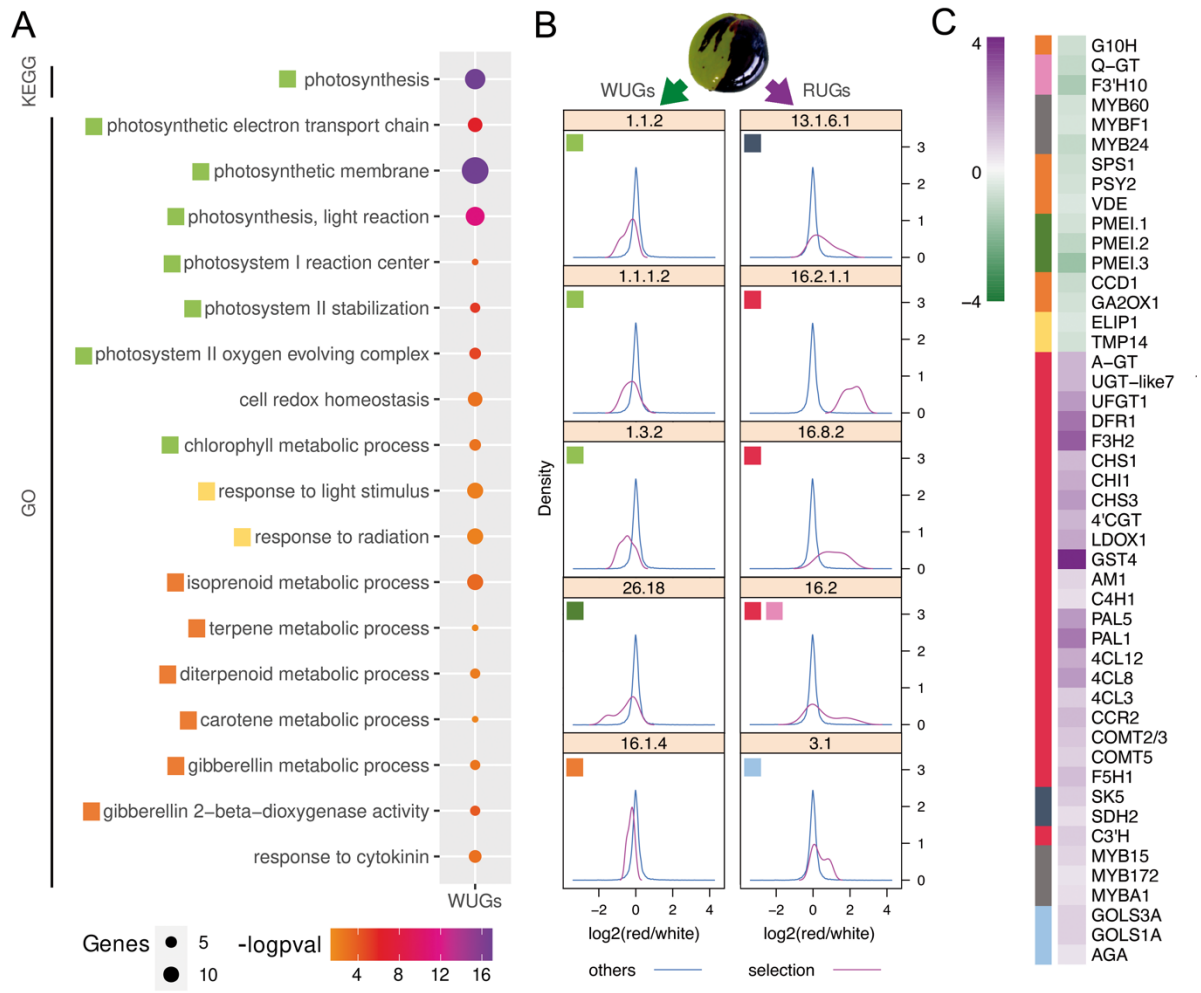

Figure 3. Transcriptomic landscapes of inversely pigmented sections of variegated berries show different pathways perturbed by MYBA1/MYBA2 inactivation. (A) A selection of significantly enriched terms from KEGG and GO ontologies in white-skin up- regulated genes (WUGs). The -logpval scale represents statistical significance on a continuous color scale from orange to purple. The number of WUGs intersecting with each ontology term is indicated by point size. (B) Density plots of functional MapMan ontologies in berry skin transcriptomes illustrating shifts in differential gene expression. Significantly affected categories (bins) were determined based on Wilcoxon test (FDR<0.05). Bins: 1.1.2 light reaction, photosystem I; 1.1.1.2 light reaction, photosystem II (polypeptide subunits); 1.3.2 Calvin cycle, rubisco small subunit; 26.18 invertase/pectin methylesterase inhibitor family protein (misc.); 16.1.4 secondary metabolism, isoprenoids, carotenoids; 13.1.6.1 aromatic amino acid metabolism, chorismate; 16.2.1.1 lignin biosynthesis, PAL; 16.8.2 flavonoids, chalcones; 16.2 secondary metabolism, phenylpropanoids; 3.1 minor CHO metabolism, raffinose family. Other significant categories can be found in Supplemental Data Set 5. (C) Log2 expression ratios of selected genes belonging to significant categories illustrated by colored boxes found in (A) and (B). Gene IDs and expression values are found in Supplemental Data Set 6.

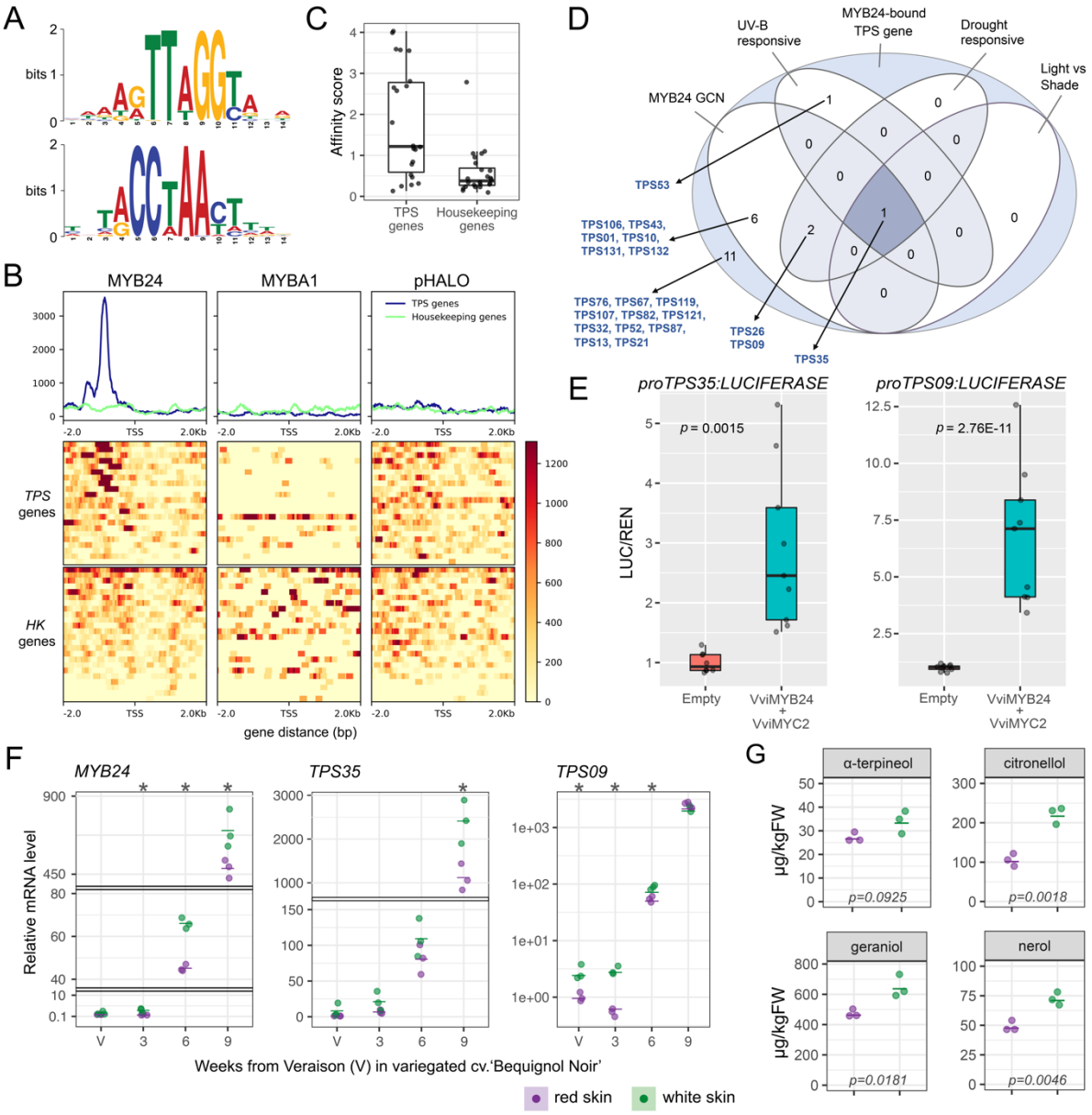

Figure 4. Discovery of genome-wide transcription factor binding sites (TFBSs) by DNA affinity purification sequencing (DAP-seq) identifies *TPS35* and *TPS09* as high-confidence targets of MYB24. (A) Binding motif derived from the 600 most significant peaks of MYB24 DAP-seq, in forward (top) and reverse complement (bottom) directions. (B) Smoothed DAP-seq binding signal at (-2kb, +2kb) from the TSS (*x* axis) in 22 terpene synthase (*TPS*) genes and compared to background housekeeping (HK) genes for MYB24, the anthocyanin regulator MYBA1, and empty vector (pIX-HALO) as a negative control. (C) Average motif score ("affinity") of the MYB24 binding motif across the entire (-2kb, +500bp) region of *TPS* and housekeeping genes. (D) Intersection of *TPS* genes bound and co-expressed with MYB24 in different transcriptomic datasets (GCN= gene-centered aggregated co-expression network). (E) Transient expression of *VviMYB24* with *VvibHLH07/MYC2* activates the *VviTPS35* and *VviTPS09* promoters. *Nicotiana benthamiana* plants were agroinfiltrated with 35S:VviMYB24 and 35S:VviMYC2 constructs either alone or in combination (empty vector used as a negative control) and kept in low light conditions for three days before LUCIFERASE activity quantification. *p* values were calculated based on one- way ANOVA followed by Tukey's post hoc test. (F) *MYB24* and target *TPS* genes expression profiles at different ripening stages in red and white skin sections of

variegated berries of cv. ‘Béquignol Noir’. Sixteen berries from 8 clusters belonging to five plants were used for each sample. Gene expression data from three biological replicates is shown (averages as horizontal lines). Asterisks indicate significant differences (p<0.05) between tissues based on one-way ANOVA followed by Tukey’s post hoc test (performed independently for each developmental stage). (G) GC-MS targeted monoterpene quantifications in red and white skin sections of variegated berries of cv. 'Béquignol Noir' at maturity (9WAV). p values were calculated based on one-way ANOVA followed by Tukey’s post hoc test. For all boxplots the lower border and upper border of the box show the lower quartile and upper quartile, respectively. The line in the box shows the median. Whiskers show Min to Max.

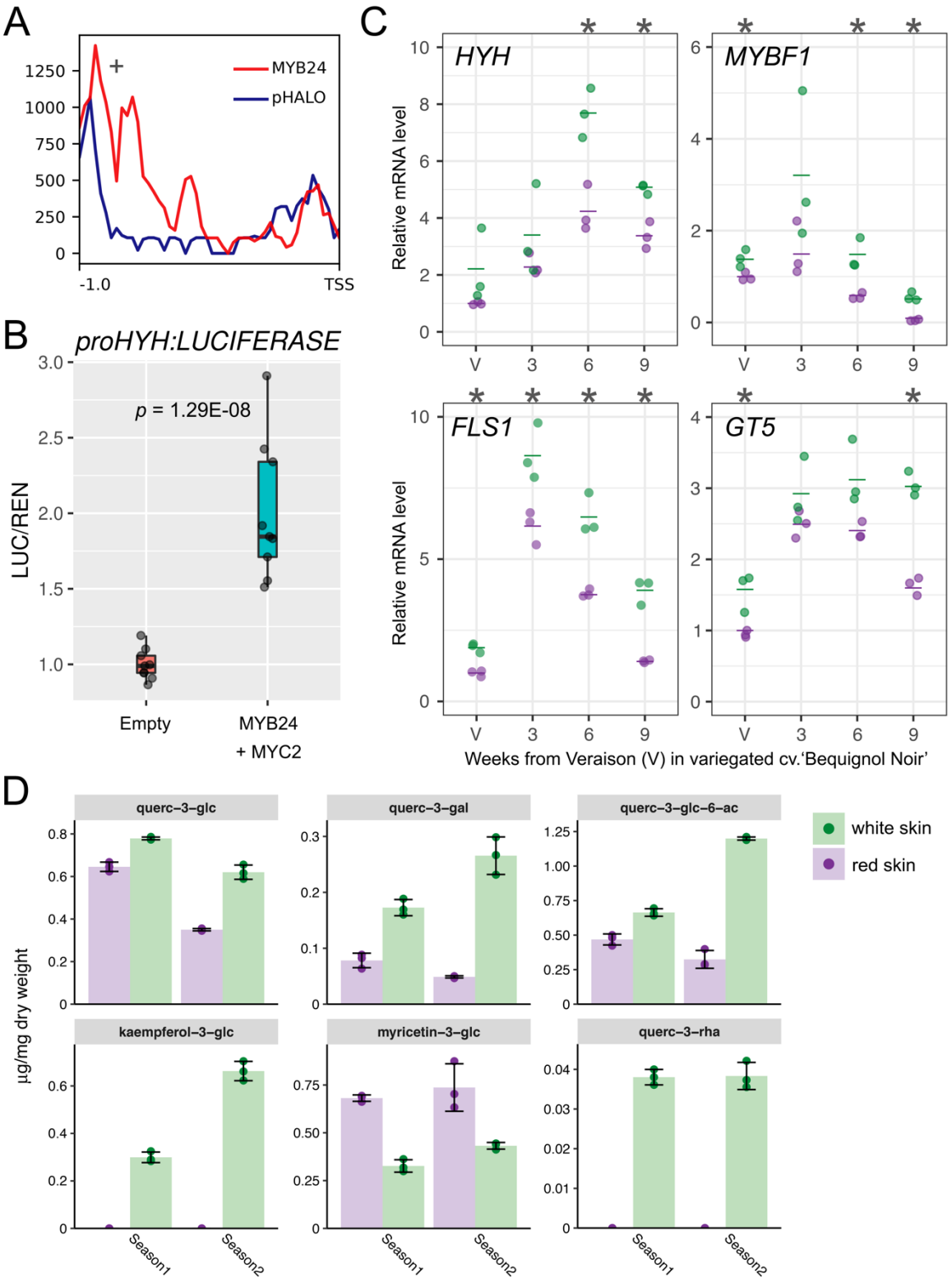

Figure 5. MYB24 promotes flavonol accumulation via the binding and regulation of the flavonol-regulator *HYH*. (A) MYB24 binds to the promoter of the light-early response regulator *HY5* homologue (*HYH*). DAP-seq binding signal (+) at -0.92kb from the TSS (*x* axis), compared to empty vector (pIX-HALO) as a negative control. (B) Transient expression of *VviMYB24* with *VvibHLH07/MYC2* activates the *VviHYH* promoter in *N. benthamiana* leaves. *p* values were calculated based on one-way ANOVA followed by Tukey's post hoc test. (C) The expression profiles of flavonol-related genes (*HYH*, *MYBF1*, *FLS1*, and *GT5*) at different ripening stages in red

and white skin sections of variegated berries of cv. ‘Béquignol Noir’. Approximately 16 berries from 8 clusters belonging to 5 plants were used for each biological replicate. Gene expression data from three biological replicates is shown (averages are shown as horizontal lines). Asterisks indicate significant differences ($p<0.05$) between tissues based on one-way ANOVA followed by Tukey’s *post hoc* test (performed independently for each developmental stage). (D) Flavonol composition in ‘B. Noir’ variegated berry skins at 5WAV at two consecutive seasons (vintages). High performance liquid chromatography (HPLC) quantifications are expressed as µg/mg of dry weight. Quercetin 3-O-(6"-acetyl-glucoside) and quercetin-3-O-rhamnoside are expressed in quercetin-3-O-glucoside (querc-3-glc) equivalents. Standard error bars were calculated from three biological replicates, sampled as described before.

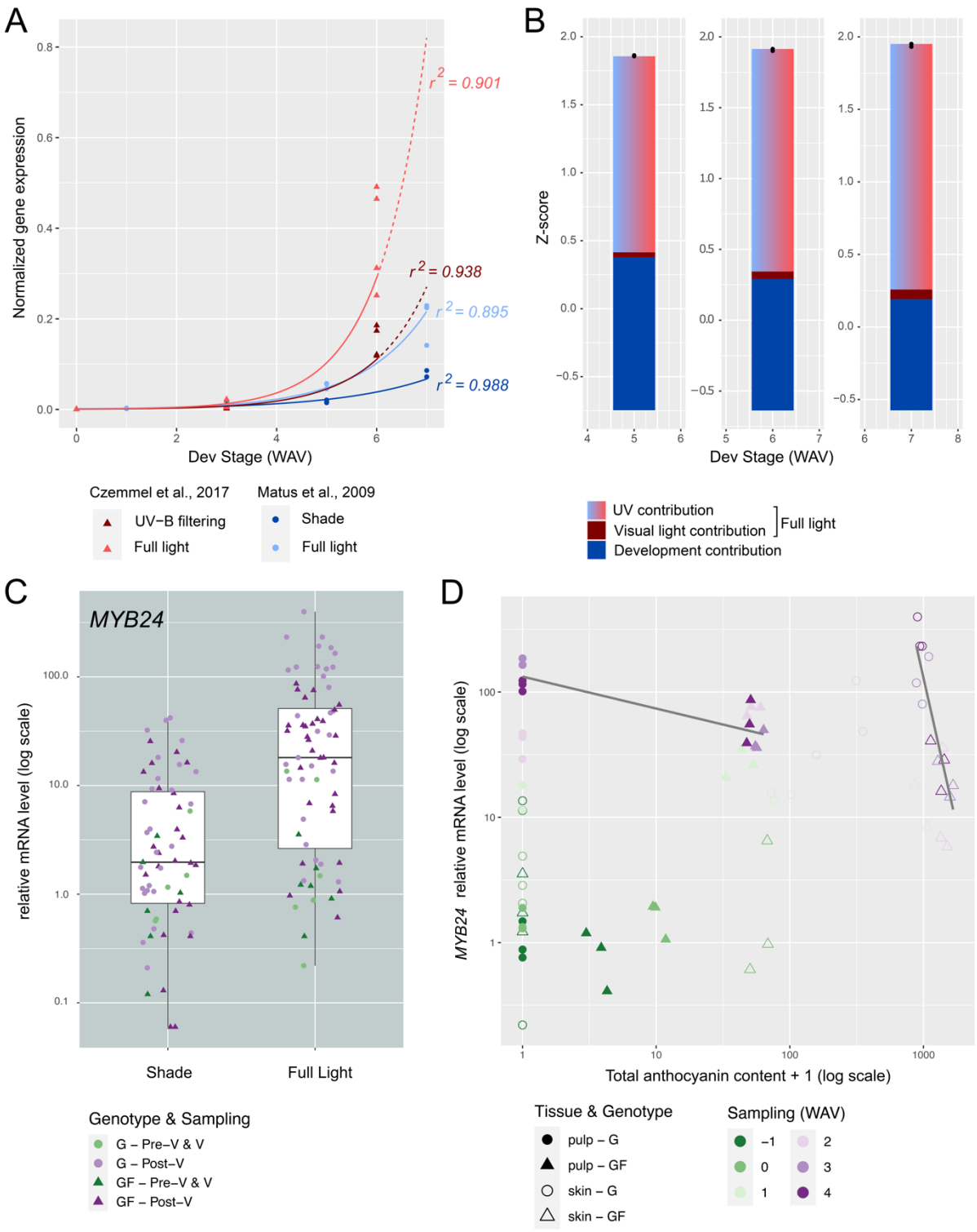

Figure 6. MYB24 responds to different light qualities and is inversely correlated to anthocyanin berry accumulation. (A) Exponential regression model of *MYB24* expression in full shade (Matus et al., 2009) and UV-B filtering (Loyola et al., 2016) experiments conducted in 'Cabernet Sauvignon' vineyards. Data were independently normalized for each experiment. (B) Individual contributions of visible light and UV radiation to overall *MYB24* expression during ripening. Normalized z-score values were calculated for each experiment from (A) and represented at 5WAV and 6WAV (using measured or interpolated values), while predicted values were used for 7WAV (extrapolated using the regression fit curve from A). UV-B contribution is defined as the z-score

difference between full light conditions in any of the two experiments and the z-score value resulting from UV-B filtering. Visible light contribution is defined as the z-score difference between UV-B filtering and shade values. The developmental contribution is the z-score value of shade at the corresponding time-point. Black dots depict full-light Z-score values from both experiments. (C-D) *MYB24* expression increases during development but its negatively influenced by shade, sunscreen (i.e., anthocyanin) accumulation and berry tissue position. Skin and mesocarp gene expression responses to sunlight exclusion were obtained from field trials of cv. 'Gamay (G)' and its '*teinturier*' (red- flesh) somatic variant cv. 'Gamay Fréaux (GF)'. Developmental stages: veraison (V), and pre/post-veraison (Pre/Post-V). A complete fruit sunlight exclusion treatment was imposed by covering grape clusters with opaque boxes (from two weeks before veraison, -2WAV, until maturity) and compared to grape clusters exposed to natural light conditions as a control (100% light incidence).

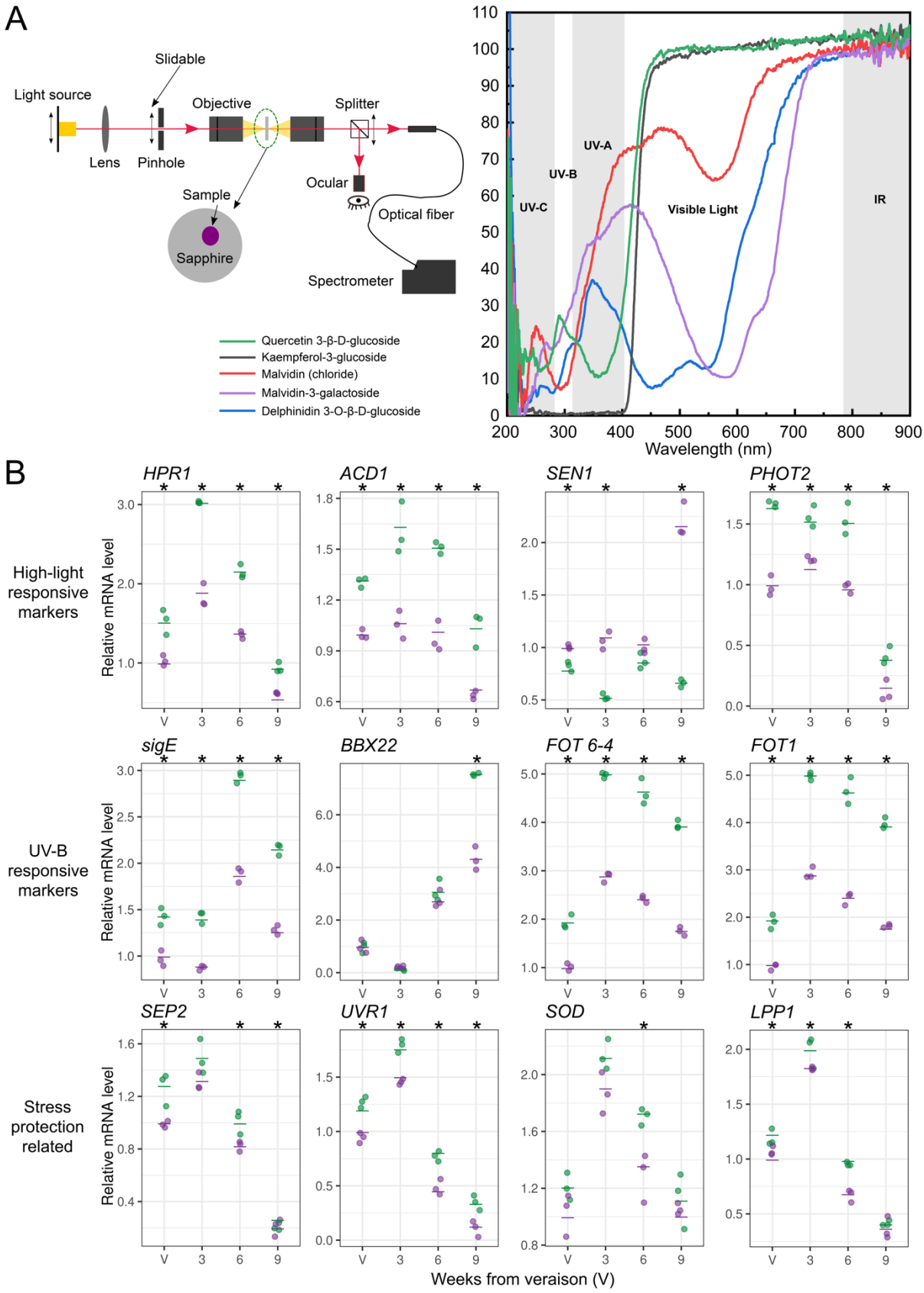

Figure 7. Integration of optical measurements and gene expression profiling shows that white skins of variegated berries are stressed by excessive radiation conditions and that this stress ceases at full maturity. (A) Differential light-shielding properties of berry skin flavonoids. Optical transmittance of common

berry skin flavonols and anthocyanins found in white and red skins of cv. ‘Béquignol’. Left panel: A home-built optical setup (Liang et al., 2022a) equipped with a mercury lamp (covering the optical range from 200 nm to 900 nm), fused silica lenses and reflecting optics objectives. One drop of each solution was set on top of a sapphire slice that is transparent at the range of 220nm to 3.5μm. A visible near-Infrared spectrometer was used to register light transmittance. Right panel: The optical transmittance spectra of flavonols (Quercetin 3-β-D-glucoside and Kaempferol-3-glucoside) and anthocyanins (Malvidin chloride, Malvidin-3-galactoside chloride, and Delphinidin 3-O-β- D-glucoside chloride). *X*-axis indicates the light wavelengths, classified in UV-C (200-280 nm), UV-B (280-315 nm), UV-A (315-400 nm), visible (400-700 nm) and infrared (IR; 780- 900 nm) light. *Y*-axis shows the percentage of light transmitted through the tested samples. (B) The relative mRNA levels of light-related genes (high-light responsive, UV-B responsive, and light/UV stress protection) at different ripening stages in red and white skin sections of variegated berries of cv. 'Béquignol Noir'. Gene expression data from three biological replicates are shown (averages shown as horizontal lines). Asterisks indicate significant differences ($p<0.05$) between tissues based on one-way ANOVA followed by Tukey’s post hoc test (performed independently for each developmental stage). Full gene names are as follow: *HPR1* (hydroxypyruvate reductase), *ACD1* (accelerated cell death 1), *SEN1* (dark inducible 1), *PHOT2* (phototropin-2), *sigE* (RNA polymerase sigma subunit SigE), *BBX22* (zinc finger B-box transcription factor, AtBBX30 homologue), *FOT6-4* (6-4 photolyase), *FOT1* (cyclobutane pyrimidine dimer photolyase), SEP2 (stress enhanced protein 2), *UVR1* (UV-B receptor 1), *SOD* (superoxide dismutase [Cu-Zn]), and *LPP1* (lipid phosphate phosphatase 1).

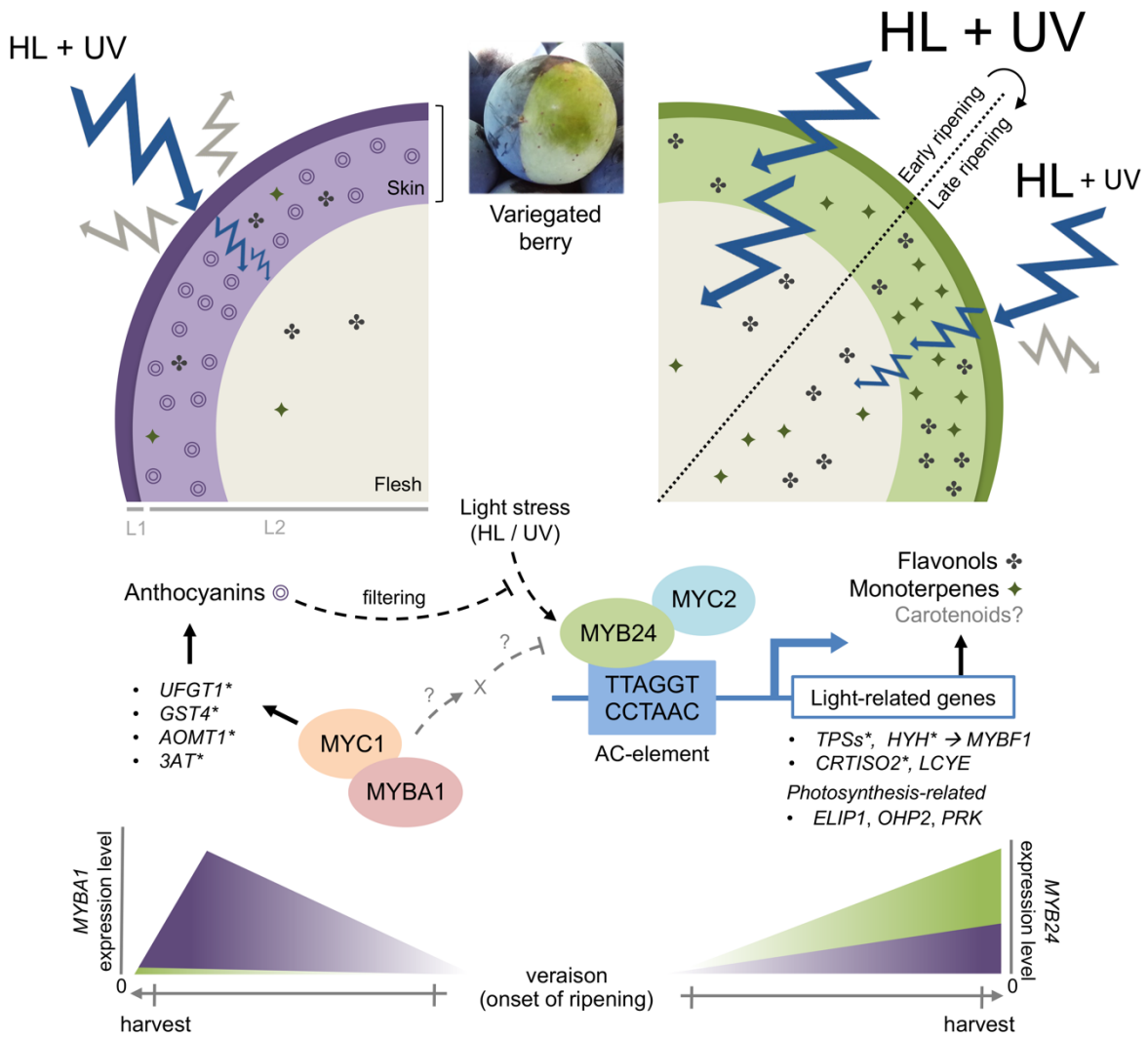

Figure 8. A proposed model for the response of white skin sections of variegated berries coping with high-intensity light and UV radiation stress. In red-skinned sections, the *MYBA1* regulator is expressed, thus activating the production of anthocyanin pigments that prevent damage by filtering excessive radiation. Due to the lack of these intrinsic sunscreens, the white-skinned sections of variegated berries experience high-intensity light and UV stress, resulting in *MYB24* induction. MYB24 regulates light-related processes, including monoterpene and flavonol biosynthesis, by binding to the promoter regions of terpene synthases (*TPSs*) and regulator genes (*HYH*), respectively. Carotenoid pathway genes are also regulated. The known antioxidant properties of the accumulated monoterpenes and flavonols confer berries a gradual oxidative protection that is maximum at harvest. Alternative specialized metabolic pathways are present in these skins to protect cells from the harmful effects of excessive light stress.