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Comparisons of controlled environment and vineyard experiments in Sauvignon blanc grapes reveal similar UV-B signal transduction pathways for flavonol biosynthesis

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ABSTRACT

UV-B radiation is an environmental challenge affecting a number of metabolic functions in plants. Plants protect themselves from this potentially damaging radiation through synthesising UV-absorbing compounds such as flavonoids. This study aims to investigate the effect of UV-B on flavonoid biosynthesis in Sauvignon blanc grapes. In particular, a comparison has been made between controlled environment (CE) and vineyard trials to better understand molecular mechanisms of low/high fluence UV-B responses and how the results relate to each other in the context of flavonoid biosynthesis. Following exposure to supplemental UV-B in the CE, both flavonols and gene expression exhibited UV-B induced response. Flavonols, particularly quercetin/kaempferol 3-*O*-glycosides were increased at distinct stages of berry development. All genes measured showed a significant developmental regulation. *VvFLS4*, *VvCHS1*, *VvMYB12*, *VvHY5* and PR (*VvTL1* and *VvChi4A/4B*) increased due to UV-B in the CE experiments. However, PR were not responsive to the natural UV-B fluence in vineyard but were significantly induced at later stages of development. Overall, despite very different conditions in the CE and vineyard the majority of UV-B induced responses are similar. Only PR activities in the CE cabinets reflect a higher fluence stress response that is not reflected in the natural lower UV-B fluence environment.

1. Introduction

UV-B radiation (280–320 nm) has been implicated in a wide variety of plant responses from changes in phenology to modification of gene expression [1,2]. While earlier studies frequently focus on UV-B as a damaging environmental stress, UV-B is now more likely to be considered as an important signal at the ecosystem level that regulates plant metabolism, growth, development and morphology [3,4]. This view is supported by the recent discovery of a specific UV-B photoreceptor, UV RESISTANCE LOCUS 8 (UVR8) and the corresponding signal transduction pathway [5–9]. Much of the data on UV-B responses in plants has been obtained from controlled environment (CE) experiments that have historically been criticised for a lack of environmental authenticity. In particular, the inability to provide sufficiently high photosynthetically active radiation (PAR) can therefore contribute to an overestimation of UV-B effects in CEs, caused by a relatively low PAR/UV-B ratio [10]. Controlled environmental conditions include CE cabinets, greenhouses or glasshouses and growth chambers. Compared to field conditions, the main benefit of conducting experiments in the CEs is the relative ease of providing supplemental radiation with UV-B lamps or by reducing UV with filters to potentially evaluate the effects of UV-B on plants. CEs can also provide the ability to study and mitigate additional effects caused by other parameters including temperature, humidity and biotic factors such as insect damage [11]. Over the last few decades, dozens of studies regarding UV-B effects have been carried out on a wide group of crop species, including bean, maize, rice, pea, soybean and wheat [11]. However, the research is limited utilising CE conditions to study the UV-B responses of the important commercial crop, grapevine [12], of which the biochemical composition and flavour characteristics are significantly impacted by increased UV-B exposure in the vineyard [13]. Furthermore, most experiments on the molecular biology associated with UVR8 have been on model plants, such as Arabidopsis, with few studies carried out on commercial crops, such as grapevine.

Plants are able to mitigate the harmful effects of high UV-B radiation by synthesising flavonoids, a class of UV-absorbing compounds

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located mainly in the epidermis which act as internal filters [14] and reactive oxygen species (ROS) scavengers [15]. In the vineyard, flavonoid accumulation in grape berries can be affected by changes in the light conditions, specifically exposure to UV-B [16]. This is of particular relevance, especially when canopy leaves are removed as a viticultural measure to reduce humidity and increase light coverage on the grape bunches, with the aim to mitigate the disease burden [17,18]. An increase of UV-B exposure can significantly influence the levels of flavonoids in the harvest berries [19] and potentially, modify the biochemical structure of the wine [1,15].

The induction of the signal transduction pathway for flavonoid biosynthesis is one of the most extensively characterized and widespread plant responses to UV-B radiation [20–22]. The UV-B induction of flavonoid biosynthesis is achieved through a complex regulation of genes and transcription factors involved in the flavonoid biosynthetic pathway [23]. A transcriptional activation complex, consisting of R2R3-MYB transcription factor (R2R3-MYB), a basic helix-loop-helix domain protein (MYC/bHLH) and WD40 repeat (WDR) proteins (MYB-MYC/bHLH-WDR) has been reported to play an important role in the regulation of the flavonoid biosynthetic pathway, especially the flavonol synthase (FLS) genes that are involved in the last step of the flavonol biosynthesis [24–27]. Recent studies have shown that the effects of UV-B are mediated by both a non-specific response initiated by high fluence UV-B, and specific signalling pathways that mediate a photomorphogenic response to low fluence UV-B [28]. The photoreceptor UVR8 is involved in perceiving UV-B wavelengths at low fluence and facilitates signalling using the transcription factor CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) and a bZIP transcription factor ELONGATED HYPOCOTYL 5 (HY5) [29,30]. In *Arabidopsis thaliana,* chalcone synthase (CHS) can be utilised by the low fluence UV-B transduction pathway to influence flavonoid biosynthesis [28,31]. In contrast, high fluence wavelengths are frequently associated with a general stress response in plants [31] and have been shown to induce a wide range of genes and enzymes overlapping with many other known signal transduction mechanisms, involving ROS, mitogen-activated protein kinase (MAPK) signalling cascades jasmonic acid, salicylic acid and pathogen-related (PR) proteins [1,32].

This research assumes that the CE experiments, as a verification and complementary to vineyard environments, can provide similar UV-B responses which are reliable to be used to interpret UV-B responses on flavonoid biosynthesis in grapevines. In this study, we examined the effects of UV-B on flavonoid biosynthesis in *Vitis vinifera* L. Sauvignon blanc berries through a comparison of vineyard treatments (two successive seasons) and four CE experiments using grape cuttings (with different developmental stages of berries). We have focused our study to recent understanding of UVR8 and the high/low UV-B fluence response signal transduction pathways as it relates to flavonoid biosynthesis. To our knowledge, this is the first comparison of CE and vineyard research investigating flavonoid biosynthesis specifically in grapevine and contributes significant understanding to UV-B induced plant responses in context of many years of debate relating to natural environments and artificial experimental systems [33].

2. Materials and methods

To investigate the UV-B effects on flavonoid biosynthesis in Sauvignon blanc grapes throughout berry development, potted grapevines *Vitis vinifera* cv. Sauvignon blanc at distinct developmental stages were transferred to a controlled cabinet with supplemental UV-B radiation. After different periods of UV-B exposure, berries were collected and analysed for flavonol composition and gene activity. Both the total UV absorbance and flavonoid composition were determined. Genes of the flavonoid biosynthetic pathway (*VvFLS1-5* and *VvCHS1-3*) and the transcription factors that are known to regulate this pathway were also analysed. These were two repeat WD40 proteins (*VvWDR1–2*), R2R3/MYB transcription factors (*VvMYB12/MYBF1*) and a helix-loop-helix domain protein (*VvMYCA1/bHLH*). To study UV-B signalling from light interception leading to flavonol biosynthesis, genes that are considered to be related to fluence responses to UV-B were analysed. These included, genes associated with UV-B wavelengths at low fluence, namely *VvUVR8* and its reaction partners *VvCOP1* and *VvHY5* [31]. The expression of five PR protein genes (*VvTL1-3* and *VvChi4A/4B*) were investigated as genes reported to be influenced by UV-B wavelengths at high fluence. In addition to the CE experiments, a screen system has been developed to create different UV environments in the research vineyard at Lincoln University [13]. Berries were collected from the vineyard at different stages throughout development, flavonol levels and the activity of the same candidate genes were analysed in berries with different UV environments.

2.1. CE experiments

The fruit-bearing cuttings model system for grapevine (potted vines developed from rooted cuttings) used in this study was according to Mullins and Rajasekaran [34]. Grapevine cuttings were collected from the mature canes of Sauvignon blanc grapevines from Lincoln University research vineyard (Canterbury, New Zealand) during 2011 winter. Cuttings were grown in a greenhouse until required developmental stages (Fig. A1a). Potted fruit-bearing cuttings were moved to a CE cabinet (1.37 m × 2.45 m, two separate spaces divided by a UV-excluding polycarbonate screen in the middle; Fig. A1b) at least two weeks before the treatments to adjust the micro-environments (22 °C day /18 °C night; 70–80% humidity; PAR 300 μmol/m^2/s, 12 h/day; UV-B radiation 300 mW/m^2, 9:00–17:00/day). PAR (Osram warm-white fluorescent tubes, New Zealand Lighting System Ltd., New Zealand) was measured by LI-COR LI-188B Quantum Radiometer (LI-COR Biosciences–Biotechnology, USA). UV-B radiation (UVB-313 UV fluorescent tubes with Acrylic filter to minimize UV-C radiation, Q-Lab Company, USA) was measured by a UVB Biometer model 501 radiometer (Solar Light Company, USA).

Experiments were performed on potted vines at the developmental stages of °Brix 3.5–4.0, 5.7–8.5, 11.2–12.5 and 13k16. For each experiment, 30 potted vines were treated with pure PAR (300 μmol/m^2 s), while the other 30 vines were exposed to PAR + UV-B radiation (UV-B 300 mW/m^2). Sampling time points were selected from 1, 2, 3, 4 to 5 or 7 d of PAR or PAR + UV-B exposure. At each time point, 3 vines with PAR treatment and 3 vines with PAR + UV-B treatment were moved out of the cabinet, berries were collected and frozen in liquid nitrogen immediately, then stored in −80 °C for further analysis.

Flavonol analysis was carried out on all four stages of development and gene expression carried out on developmental stages of 3.5–4.0 °Brix (considered pre-veraison) and 13–16 °Brix (as equivalent to harvest, the potted vine grapes being unable to ripen further).

2.2. Vineyard treatments

The experimental design of vineyard trials and characteristics of the screening materials has been described previously with minor modifications [13] (Fig. A1c,d). The vineyard trials were based on four treatments, including a full canopy (full canopy, FC) and three leaf removed treatments: one with no screening (LR), one screened with UV-transmitting material (ACRYLIC) and one screened with UV-B excluding material (PETG). Berry development and vineyard environmental parameters were monitored as previously described [13], including the total soluble solids (°Brix) of berries throughout development, PAR levels, temperature variation among treatments and UV-B level (UV-B 127.5–147.5 mW/m^2) on the grape bunches during the growth season (Table A1).

Sample regimes for the vineyard trials have been detailed previously with minor modifications [13]. In 2010 and 2011, samples were collected at 3.7, 5.6, 14.5, 18.3° Brix and 4.6, 4.9, 13, 17.3 °Brix developmental stages respectively. At each sampling, 16 berries were