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Short title: Light, Oxygen and Bud Burst

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Title: Roles for Light, Energy and Oxygen in the Fate of Quiescent Axillary Buds.

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One sentence summary:
The decision of a quiescent axillary bud to commit to regrowth is governed by both metabolic and signalling functions, driven by light, energy and oxygen availability.

Key words: bud burst, hypoxia, reactive oxygen species, transcriptomics, grapevine, meristem, quiescence.

Abbreviations:
BRC1, BRANCHED1; COP1, CONSTITUTIVE PHOTOMORPHOGENIC1; CRY, CRYPTOCHROMES; CK, Cytokinin; DIN6, DARK-INDUCED6; HY5, ELONGATED HYPOCOTYL5; GUN4, GENOMES UNCOUPLED4; ERF-VII, Group VII ETHYLENE RESPONSE FACTORS; ROP2, GTPase RHO-LIKE PROTEIN; HRU1, HYPOXIA RESPONSIVE UNIVERSAL STRESS PROTEIN1; LHC, LIGHT HARVESTING COMPLEX; PhANGs, PHOTOSYNTHESIS-ASSOCIATED NUCLEAR GENES; PHY, PHYTOCHROME; PPD5, PsbP DOMAIN PROTEIN 5; RAM, root apical meristem; SAM, shoot apical meristem; SnRK1, SUCROSE NON-FERMENTING1-RELATED KINASE1; SUS, SUCROSE SYNTHASE; TOR, TARGET OF RAPAMYCIN; T6P, trehalose-6-phosphate; TPP, T6P PHOSPHATASE; TPS, T6P SYNTHASE.

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ABSTRACT

The hierarchy of events governing the resumption of growth of a quiescent axillary bud are poorly understood. During quiescence, a homeostasis exists in phytohormone and source/ sink regulation, which represses the metabolic and mitotic progression of the bud. Environmental change and shoot development can alter the homeostasis, leading to a binary state change and the commitment to growth. Within this context, light and oxygen availability, respiration and photosynthesis can serve both metabolic and signalling functions. However, the question of substrate versus signal has proven challenging to resolve; in the case of sugars, there are disparities in the data from apical and axillary buds in juvenile shoots, while in post-dormant perennial buds, light has only a facultative role in the decision, but signalling may still be essential for bud fate. We briefly update the roles and hierarchies of light- energy- and oxygen-dependent functions in axillary bud outgrowth of annual shoots, before focusing discussion on the role of chloroplast-to-nucleus retrograde signalling genes such as *GENOMES UNCOUPLED 4* (*GUN4*) and *ELONGATED HYPOCOTYL 5* (*HY5*) in bud burst responses to light, examining available transcriptome data from post-dormant grapevine buds (*Vitis vinifera* L.). We discuss the evidence implicating cryptochromes (CRY) in the activation of *HY5* expression in grapevine, leading to chloroplast biogenesis in the buds, and that this occurs via a biogenic, rather than an adaptive developmental process. The cytokinin (CK) signalling pathways and the light-regulated expression of chloroplast processes, especially those involved in carbon and oxygen metabolism, may also play an important role in bud burst.

INTRODUCTION

The mechanisms that control apical dominance in juvenile or annual shoots are well characterised. Removing the apex can result in axillary bud outgrowth, as can changes in light intensity and quality. Here, axillary bud outgrowth is regulated by signals arising from the apex, which contain several light quality and quantity sensing pigments. Of these, phytochromes are perhaps the best characterised. Phytochromes (PHY) sense red and far-red light while cryptochromes (CRY) and phototropins are involved in the perception of blue light. These photoreceptors regulate the expression of different transcription factors in order to coordinate light-dependent photomorphogenesis. Some plant species require light for axillary bud outgrowth (annual shoots) but in others the requirement for light is facultative (Leduc et al., 2014). In addition, the buds of many perennial plants can resume growth following a period of dormancy. In this case, apical suppression may temporarily break down, and the axillary bud may be considered more independent, at least until a new homeostasis is established along the shoot. Moreover, there is no evidence post-dormant perennial buds require light, although increased intensity can accelerate bud burst in a range of species (Maynard et al., 1990; Rageau et al., 1998; Sogaard et al., 2008; Caffarra and Donnelly, 2011).

Light-dependent influences on meristem activity involve at least two distinct but possibly cross-regulatory processes: direct regulation of gene expression via photoreceptors, and an indirect process involving the generation of energy through photosynthesis and respiration. A potential third pathway is the signalling of tissue oxygen status, which has been shown to be a primary cue for developmental transitions in plants, including photomorphogenesis (Considine et al., 2017). In this update, we consider the respective roles of light, energy and oxygen, as primary cues for axillary bud outgrowth, with a particular focus on the signalling pathways that trigger the resumption of growth following quiescence. We provide a concise overview of (i) the physiology of axillary meristems and buds, focusing on genotypic differences in bud requirements for light and energy to trigger outgrowth, and; (ii) the importance of transcriptional regulation of plastid functions in the resumption of growth in quiescent grapevine (*Vitis vinifera* L.) buds following dormancy.

LIGHT AND ENERGY DEPENDENCY OF AXILLARY BUD OUTGROWTH, AND A PUTATIVE ROLE FOR OXYGEN-DEPENDENT SIGNALLING

Vascular plants display indeterminate growth and a branched root and shoot structure, which is enabled by the spatial distribution and activation of meristems (Sussex and Kirk, 2001). Most

terrestrial species exhibit axillary branching, rather than the more ancestral dichotomous branching. Axillary buds are classed as sylleptic or proleptic, and both types may be quiescent for sustained periods of time, being able to resume growth immediately upon perception of appropriate developmental, metabolic or environmental cues. Additionally, proleptic buds of some species possess the ability to exhibit true dormancy, which is a developmental and internally repressed condition that requires environmental entrainment to enable a transition to quiescence (Considine and Considine, 2016). Dormant buds are metabolically isolated from the shoot by physiological barriers such as the deposition of callose. In this situation, apical dominance in its strictest sense may not apply, at least until dormancy is relieved. In the following discussion, we will focus on quiescence and the role of light in the processes promoting axillary bud outgrowth, particularly in intact juvenile or annual shoots.

The dominance behavior of the apical meristem, which enforces and maintains axillary bud quiescence, is enforced by mobile signals such as sucrose and phytohormones, particularly auxin. The role of apically-derived auxin in maintaining axillary bud quiescence was established nearly a century ago (Thimann and Skoog, 1934; refer to Rameau et al., 2015 for a detailed review). However, auxin signalling intersects with other phytohormones such as strigolactones and cytokinins (CK) to regulate the outgrowth of axillary buds. Each phytohormone functions downstream of light signalling pathways initiated by photoreceptors (Leduc et al., 2014). Phytohormone signalling pathways are thought to converge at the level of the BRANCHED1 transcription factor (BRC1, and homologues), which is a central repressor of axillary bud outgrowth (Dun et al., 2012). However, auxin transport may be too slow to account for observed bud outgrowth kinetics, while sucrose availability may provide a more rapid regulatory trigger (Renton et al., 2012; Mason et al., 2014). The application of sucrose results in a dose-dependent activation of bud outgrowth, a process that apparently antagonises auxin- and strigolactone-mediated signalling, although sucrose effects were at least partly independent of these pathways (Barbier et al., 2015a).

Light and sucrose can act both as signals and sources of energy for bud growth. Sucrose functions both as a metabolic substrate and signal controlling development, notably via the TARGET OF RAPAMYCIN (TOR) kinase and SUCROSE NONFERMENTING1-RELATED KINASE1 (SnRK1). Several species such as Rosa sp. and pea require light for axillary bud outgrowth, while others have varying facultative requirements for light (Leduc et al., 2014). In axillary buds of Rosa sp., the expression of genes involved in sucrose hydrolysis and mobilisation is promoted by light, however sucrose cannot compensate for light in

activating bud outgrowth (Girault et al., 2008). Application of sucrose and non-metabolisable analogues such as palatinose promotes the rate of bud outgrowth in Rosa, *Arabidopsis thaliana* (arabidopsis) and pea when light is present (Rabot et al., 2012; Barbier et al., 2015b). These data suggest that photoreceptor-mediated signalling is a primary requirement for bud outgrowth, and that sucrose synthesis and metabolism via photosynthesis is an essential downstream component.

Several lines of evidence suggest that sucrose may function as a signal rather than energy substrate in augmenting bud outgrowth (Barbier et al., 2015a). The altered shoot branching phenotype of arabidopsis mutants deficient in trehalose-6-phosphate (T6P) cannot be explained by metabolic or energy functions because T6P only accumulates to low concentrations even in wild type plants (Chary et al., 2008). Over expression of *HEXOKINASE1* leads to increased bud outgrowth and expression of genes involved in abscisic acid-related processes, together with reduced expression of auxin-related genes (Kelly et al., 2012). Nevertheless, other studies have linked the effects of sucrose to metabolic requirements (Leduc et al., 2014; Otori et al., 2017). Further insights into the question of whether sucrose acts as a signal rather than a substrate come from studies of the shoot apical meristem (SAM). Auxin- and sucrose-mediated pathways independently promote the cell cycle by activating TOR kinase, which in turn directly activates key cell cycle regulators, as well as the stem cell identity protein WUSCHEL (Pfeiffer et al., 2016; Li et al., 2017). The fact that both auxin and sucrose are required is particularly interesting for two reasons: Firstly, the auxin response in the SAM is dependent on a small GTPase Roh-like protein (ROP2). This protein was shown to be activated by both the direct application of auxins and the light-induced auxins in shoot apices (Li et al., 2017). In addition, the application of auxin effectively substituted light to activate the TOR-dependent formation of true leaves, when sucrose was present (Li et al., 2017). The ROP2 was shown to directly interact with TOR kinase, promoting its kinase activity (Cai et al., 2017). ROP2 also functions in oxygen- and redox-dependent survival (Baxter-Burrell et al., 2002). The expression of ROP2 is promoted by a HYPOXIA RESPONSIVE UNIVERSAL STRESS PROTEIN1 (HRU1) that transduces the hypoxic cue via Group VII ETHYLENE RESPONSE FACTORs (ERF-VII), which are stabilised in hypoxic conditions (Gonzali et al., 2015). Hence, these data strongly suggest auxin and sucrose pathways converge with oxygen signalling upstream of TOR kinase (Considine, 2017). We will return to oxygen signalling below. Secondly, the sucrose effect on TOR and WUS is consistent with a metabolic function because

glucose and not palatinose is able to substitute for sucrose (Pfeiffer et al., 2016), which conflicts with reports on axillary buds (Rabot et al., 2012; Barbier et al., 2015b).

The above points demonstrate the incomplete nature of current understanding of how auxin and sucrose function together in axillary bud outgrowth. Interestingly, the addition of sucrose is sufficient to trigger the growth of the root apical meristem (RAM) but not the SAM. This finding may be explained by the relatively higher concentrations of auxin in the RAM compared to SAM, and also the light dependency of auxin synthesis in the SAM (Li et al., 2017). Increased auxin synthesis and transport from the axillary buds occurs during the transition to bud outgrowth, suggesting that photoreceptor-dependent auxin synthesis in the axillary bud meristems may be a primary trigger for bud outgrowth. However, strigolactone has also been suggested to be a signal output from photosynthesis. Increased axillary branching is evident in an arabidopsis mutant lacking the PsbP Domain Protein5 (PPD5), which is a key component of photosystem II (Roose et al., 2011). While PPD5 is essential for autotrophic metabolism and optimal oxygen-evolving activity, the *ppd5* mutants are able to sustain electron transport, and the phenotype can be rescued by the application of strigolactone, indicating that the phenotype is more likely to be due to hormone defects than energy deficits. Perhaps also relevant, axis initiation in tomato requires light signalling via phytochromes but not photosynthesis (Yoshida et al., 2011). Meristems cultured with sucrose in darkness, or in the presence of the carotenoid inhibitor norflurazon in the light, fail to initiate new leaf primordia. Nevertheless, axis initiation is a different process to organ development, i.e. the resumption of growth following quiescence.

LIGHT, OXYGEN AND CHLOROPLAST FUNCTIONS IN PERENNIAL BUD BURST; AN ILLUSTRATION WITH GRAPEVINE BUDS

In many perennial species, proleptic buds resume growth following a prolonged period of dormancy (Considine and Considine, 2016). The dormant bud becomes desiccated and metabolically isolated by callose deposition in the plasmodesmata (Rinne et al., 2011). In this state, the meristem tissues are enclosed, typically by lignified bracts and scales (Figure 1). Following dormancy, the bud resumes a quiescent but receptive state with a connected symplast. Studies of several woody species have shown that the internal tissues and leaf primordia of quiescent buds are largely etiolated and lack chlorophyll (Solymosi et al., 2012). The plastids in such buds however, exist in different developmental stages that are partly related to the nature of the tissues in which they reside (Solymosi et al., 2012). For example,

proplastid-like and etio-chloroplasts respectively were identified in the inner and outer leaf primordia of compactly closed common ash buds (Solymosi et al., 2012). After bud burst, the emerging leaves contain regular chloroplasts, although they are not fully developed (Solymosi et al., 2012). However, in horse chestnut, closed buds contain proplastids, and the leaf primordia of the opening buds contain etioplasts or etio-chloroplasts, but not chloroplasts (Solymosi et al., 2006). In tree-of-heaven buds, both inner and outer leaf primordia contain chloroplasts and etio-chloroplasts (Solymosi et al., 2012). Hence, outer leaf primordia do not always contain more developed plastids than the inner leaf primordia.

There is also evidence of regulated oxygenation during bud burst in grapevine. The post-dormant bud is hypoxic (<10 % saturation; Figure 1), and oxygen concentration gradually increases in a spatially regulated manner during the first week of bud burst, prior to leaf emergence (Meitha et al., 2015; 2017). Independent studies show the seed of several species, as well as fruits show spatially and developmentally regulated tissue oxygen status (Verboven et al., 2008; Borisjuk and Rolletschek, 2009; Cukrov et al., 2016). In grapevine buds, as in seeds, the outer scales were shown to be a barrier to oxygen diffusion, however this did not explain the elevated levels of oxygen in the primary bud after bud burst commenced, particularly where the oxygen minima was not at the core of the bud (Figure 1; Meitha et al., 2015). Although not yet demonstrated in buds, the low oxygen status (hypoxia) of seeds is reflected in the spatial patterns of metabolic control, particularly in relation to anaerobic glycolysis and energy status (Borisjuk and Rolletschek, 2009). It has since emerged that oxygen status (and nitric oxide) has a regulatory role in seed dormancy and germination, where the oxygen-dependent degradation of ERF-VII regulate the effective transition from anaerobic to aerobic metabolism and quiescence to growth (Holman et al., 2009; Gibbs et al., 2014). No such research has been applied directly to bud outgrowth, however it is notable that arabidopsis mutants impaired in the regulated degradation of the ERF-VII transcription factors show reduced apical dominance (Graciet et al., 2009).

Gene expression data of grapevine buds may provide some insight into the roles of light and oxygen in regulating bud burst. Post-dormant grapevine buds do not require light to burst, however dark-grown buds are impaired in chlorophyll synthesis and develop an etiolated phenotype (Meitha et al., 2017). We have contrasted the gene expression of buds, grown in single-node cuttings, during bud burst in the presence (DL) and absence of light (D) at 72 and 144 h (Supplemental Table S1; FC≥|2|, FDR P≤0.05), which preceded leaf emergence (Data available at NCBI BioProject PRJNA327467,

186 http://www.ncbi.nlm.nih.gov/bioproject/327467). A complementary study investigated the
187 developmental control of gene expression and primary metabolism (Meitha et al., 2017).
188 Interestingly, there were few changes in physiological status or global transcript profiles of
189 light- and dark grown buds over the term; a total of 436 genes were differentially expressed at
190 one or both time points, 47 genes consistently regulated at both (Supplemental Table S1). A
191 small subset of genes showed quite starkly differential expression in response to light, and these
192 will now be discussed in detail.

193 A key component of photomorphogenesis is ELONGATED HYPOCOTYL5 (HY5), a bZIP
194 transcription factor known to bind the promoters of light-inducible genes to activate their
195 expression (Chattopadhyay, 1998). This transcription factor is activated by different types of
196 light, through the action of the photoreceptors PHYA, PHYB, CRY1 or CRY2 (Eberhard,
197 2008), at least in part due to their negative regulation of CONSTITUTIVE
198 PHOTOMORPHOGENIC1 (COP1), which targets HY5 to the proteasome (Ang et al., 1998).
199 Although the function of HY5 in seedling photomorphogenesis in arabidopsis has been
200 reported, its expression and response to light in perennial buds had not been described. From
201 the transcriptome analysis of the grapevine buds (Supplemental Table S1), we observed that
202 the expression of genes coding for the HY5, or in its activators PHYA, PHYB, CRY1 and
203 CRY2 were not differentially regulated by the presence of light at 72 h of growth. However,
204 the expression of two *CRY* genes and *HY5* was increased at 144 h in the buds exposed to light
205 (Supplemental Table S1; Figure 2). In rose species and cultivars, blue light is sufficient to
206 promote bud outgrowth until flowering (Girault et al., 2008; Abidi et al., 2013). Together this
207 evidence suggests that in perennials buds, CRY photoreceptors are capable of stimulating bud
208 burst by promoting *HY5* expression. Known HY5 target genes encode proteins involved in the
209 chlorophyll biosynthesis, light harvesting and the Calvin cycle (Eberhard et al., 2008). The
210 expression of many genes involved in these processes was upregulated at 144 h in illuminated
211 buds compared to those kept in continuous darkness (Figure 2, Figure 3). Homologues of many
212 of the light-regulated genes in grapevine buds are also induced during photomorphogenesis in
213 arabidopsis (Ghassemian et al., 2006) and in rice (*Oryza sativa;* Kleffmann et al., 2007; Su et
214 al., 2007). The subset of light-regulated genes in these species includes those coding for
215 photosystem components such as PsaD, G, H, K, L & N, PsbS, LHCA1, A2, A4 & A6, LHCII
216 B2 & B3, as well as ATP synthase epsilon, Ferredoxin, Ferredoxin NADP-reductase, rubisco
217 subunits, and chlorophyll biosynthesis. Moreover, the expression of genes encoding two
218 ankyrin domain-containing proteins, which are involved in successful insertion of light

harvesting complex (LHC) components in the thylakoid membrane, was upregulated in grapevine buds at 144 h under illumination (Supplemental Table S1). Furthermore, the levels of transcripts encoding several enzymes of the Calvin cycle were also higher in illuminated buds at 144 h, as described in further detail below).

From the upregulated genes in DL condition at 144 h, a total of 48 genes contained the target G-Box sequence (CACGTG) of HY5 (Supplemental Table S2), including homologues of genes known to be regulated by HY5, as well as likely candidates in light- and energy-dependent functions. This includes genes coding for two T6P phosphatases, the malic enzyme, the CK-responsive GATA factor 1, cryptochrome and GUN4, among others (Figure 2, Supplemental Table S2). Some evidence has been provided that links CK signalling pathways with HY5 (Vandenbussche et al., 2007; Das et al., 2012). It may be that the CK-responsive GATA factor 1 is responsible for this crosstalk. In further studies, it would be interesting to evaluate whether HY5 can modulate the expression of these genes.

Early markers of light perception or prolonged darkness were differentially expressed according to the presence of light. For example, the expression of a homologue of *EARLY LIGHT-INDUCIBLE PROTEIN* was upregulated in the light (Figure 2, Supplemental Table S2). Conversely, the expression of a homologue of *DARK-INDUCED6* (*DIN6*, also known as *ASPARAGINE SYNTHETASE1*, *ASN1*) was progressively downregulated in the presence of light, relative to continuous darkness. The upregulation of *DIN6* is a hallmark of stresses such as extended darkness and hypoxia, which limit photosynthesis and/or respiration (Baena-González et al., 2007). The expression of *DIN6* is repressed by sucrose and glucose, and is specifically induced by the arabidopsis homologues of the catalytic subunits of SnRK1 (KIN10, KIN11), a conserved hub for starvation signalling (Baena-González et al., 2007).

These facets of the transcript profiles of developing grapevine buds demonstrate that a light-dependent photomorphogenesis becomes apparent at 144 h of exposure of the buds to environmental favorable conditions, but not earlier (i.e. 72 h). This finding suggests that at the beginning of bud burst other environmental cues, such as temperature, are required to promote skotomorphogenic development. Thereafter, growth in the light provides signals that induce photomorphogenic development.

Chloroplast to nucleus, and mitochondria to nucleus retrograde signals are very important for organelle development (Chan et al., 2016). Components that act as retrograde signals participate in biogenic and operational processes. Some genes that are involved in retrograde

signalling such as GENOMES UNCOUPLED4 (GUN4) and HY5 are differentially expressed in grapevine buds in response to light. The *gun* mutants are defective in tetrapyrrole metabolism, suggesting that this pathway is important in biogenic signalling. The expression of six genes involved in tetrapyrrole metabolism was changed in grapevine buds in response to light at 144 h. In particular, GUN4 participates in the biosynthesis of Mg-Protoporphyrin-IX, which in turn binds to a Heat Shock 90-type protein and interacts with HY5 to regulate the expression of photosynthesis-associated nuclear genes (PhANGs; Chan et al., 2016). The expression of Protoporphyrin-IX biosynthetic genes and HY5 was upregulated by light in grapevine buds at 144 h, suggesting that the retrograde activation of PhANGs occurs in illuminated buds. Hence, the plastids in the buds of perennials species may be undergoing a biogenic process rather than an operational adaptation to the environmental conditions at the early stages of bud burst.

Light adaptation also occurs through the induction of CK signalling pathways in plants. The expression of a gene coding for a histidine-containing phosphotransfer protein was upregulated by light at 72 h in grapevine buds (Figure 2, Supplemental Table S1). This protein plays a key role in propagating CK signal transduction (Hwang, 2002). The expression of the CK-responsive GATA factor 1 is known to respond to light and CK (Naito et al., 2007). It also plays a role in chloroplast development (Hudson et al., 2013). The expression of the CK-responsive GATA factor 1 was increased at 144 h DL in grapevine buds (Figure 2). This transcription factor represses gibberellic acids signalling downstream of PIF and DELLA regulators (Richter et al., 2010). The expression of genes coding for repressors of CK signalling, such as ARR1 type B and APRR7, was downregulated by light in grapevine buds. These findings suggest that the influence of light on grapevine buds involves CK signalling pathways. The expression of two other components (*ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER AHP1* and *HISTIDINE KINASE 1 AHK3*) involved in CK signalling were downregulated by light. Since there is considerable redundancy in the functions of the different AHP proteins (AHP1,2,3 and 5), which act as positive regulators of CK signalling to promote development, the significance of this observation is uncertain (Hutchison et al., 2006). Moreover, *AHK1* expression is related by stress signals through the mediation of a MAPK cascade, rather than by developmental process (Higuchi et al., 2004).

As described above, evidence now suggests that sucrose and light-dependent auxin signalling converge upon meristem activators in arabidopsis, promoting meristem growth. We found few primarily auxin-related functions in the grapevine data shown here (Figure 2, Supplemental

Table S1). Auxin has previously been shown to function in the removal of dormancy callose in grapevine buds, and to accumulate during bud swell, however direct application has apparently little effect (Aloni et al., 1991; Lavee and May, 1997, and references therein). A more recent, limited transcript analysis in developing grapevine buds (pre-dormant, paradormant) showed no relationship between genes selected as auxin- and sucrose-function markers, nor with auxin-function markers and the outgrowth potential (He et al., 2012). Nevertheless, none of these studies were designed to elaborate auxin or sucrose functions, and hence any relationships may be obscured.

SUGAR METABOLISM IS REGULATED BY LIGHT AT EARLY STAGES OF GRAPEVINE BUD BURST

Several transcripts encoding enzymes involved in starch, sucrose and hexose metabolism were strongly regulated by light in grapevine buds at 72 h in the light. These include homologues of *STARCH PHOSPHORYLASE*, *BETA-1,3-GLUCANASE* and two *SUCROSE SYNTHASE* (*SUS*) (Figure 2, Supplemental Table S1). The light-induced activation of expression of starch and sucrose hydrolytic genes was largely attenuated at 144 h, although *STARCH PHOSPHORYLASE* transcripts remained at higher levels at 144 h. Transcripts encoding a homologue of *CALLOSE SYNTHASE* were decreased in the buds in the light at 144 h. In Rosa sp. the light-dependent upregulation of *VACUOLAR INVERTASE* is considered to be important in promoting sugar degradation and bud burst (Girault et al., 2008; Henry et al., 2011). The finding that the expression of a *VACUOLAR INVERTASE*, *GIN2*, was not differentially regulated by light in grapevine, may partially explain the differences in the light requirements of bud burst in Rosa sp. and grapevine.

As illustrated in Figure 4, the expression of genes encoding plastid carbon metabolism enzymes in grapevine buds were clearly upregulated by light at 144 h. Moreover, transcripts encoding a homologue of the plastid-localised $NADP^+$-dependent *MALIC ENZYME* were increased in the light, suggesting a need for regulation of $NADP^+$/NADPH homeostasis and provision of reducing power for Calvin cycle activity (Wheeler et al., 2005). In contrast, the upregulated expression of genes encoding proteins involved in the catabolism of branched-chain amino acids in the plastid was increased in the dark, as were the levels of transcripts encoding a cytosolic PHOSPHOENOLPYRUVATE CARBOXYKINASE (Figure 4). These findings

suggest a requirement for alternative substrates to fuel the mitochondrial tricarboxylic acid pathway (Araújo et al., 2010; Avin-Wittenberg et al., 2015).

T6P is a primary sensor of cellular energy status. Transcripts encoding two *T6P PHOSPHATASE* homologues (*TPP*) were increased by light at 144 h, while *TPP* and a *T6P SYNTHASE* (*TPS*) mRNAs were decreased in abundance (and increased in the dark, Figure 2, Figure 4). These transcriptional differences suggest that reduced T6P levels or alternatively increased T6P turnover occurs in the buds in the light compared to the dark condition.

We then compared the grapevine bud differential gene expression at 144 h (Supplemental Table S1) against the public data of arabidopsis transcriptional perturbation database in Genevestigator (Hruz et al., 2008). We used the accession identifiers of the arabidopsis homologues of the grapevine DEGs and selected unique genes, leaving 317 DEGs (Supplemental Table S3a). The corresponding arabidopsis accession were entered using the Signature tool and compared to all available arabidopsis data using the Perturbations profile, with the Manhattan Distance algorithm (Affymetrix arabidopsis ATH1 Genome Array, all genetic backgrounds, 9552 samples). Some of the arabidopsis accessions submitted did not match a probe from the ATH1 microarray, leaving 306 probes (Supplemental Table S3b**)**. Nearly all of the top 50 most similar of 3020 Perturbation studies attended to post-germination photomorphogenesis. Each of the top five most similar were wild-type studies that investigated light signalling and contrasted light conditions against continuous darkness (Supplemental Table S3b). For example, the role of plastid biogenesis in mediating light-dependent signalling (GEO accession GSE24517; Ruckle et al., 2012) and the role of light-dependent translational regulation in photomorphogenesis (GEO accession GSE29657; Liu et al., 2012). Several of the studies involving mutant lines which had similar profiles to grapevine buds data (BioProject PRJNA327467) also related to light and carbon signalling. For example, a study of the role of the *COP1* (also known as *FUSCA1*) in coordinating light-dependent signalling (GEO accession GSE22983; Chang et al., 2011) and a study identifying *CARBON AND LIGHT INSENSITIVE* (*CLI186*) mutants (ArrayExpress accession E-MEXP-1112; Thum et al., 2008).

We then constrained our query of the Genevestigator data to developmental studies of germination or post-germination seedlings, which retrieved 136 perturbations (Supplemental Table S3c). The similarity of our data with comparisons from Narsai et al. (2011; GEO accession GSE30223) of germinating seed against dark-stratified seed, suggested the DL condition in our study was more developmentally advanced than the D condition. Also of

interest were comparisons of glucose-treated against control seedlings of wild-type or conditional mutants of the TOR protein kinase, indicating the DL condition was consistent with active metabolism of sugars (GEO accession GSE40245; Xiong et al., 2013).

In addition, the comparison to the core 600 putative targets of the arabidopsis KIN10 (Baena-González et al., 2007) corroborated the identification of components involved in the catabolism of branched-chain amino acids, and the regulation of *T6P SYNTHASE* expression under continuous darkness (repressed in DL/ D). Furthermore, this analysis supported conclusions regarding light-mediated regulation of *DORMANCY/AUXIN ASSOCIATED1* (*DRM1*), two genes coding for thioredoxins and two members of the NBS-LRR leucine-rich repeat superfamily, each implicated in sugar starvation responses (Baena-González et al., 2007).

Together, these data suggest that transcriptional changes induced by light in grapevine buds are similar to those observed in arabidopsis, evidencing a prominent role for chloroplast processes in carbon and oxygen (energy) metabolism during bud burst and the requirement for light to orchestrate chloroplast biogenesis. It also provides considerable evidence of the effect of light on sugar signalling. Alternative pathways for catabolism became evident under continuous darkness, suggesting catabolism of branched-chain amino acids to fuel the mitochondrial tricarboxylic acid cycle.

CONCLUDING REMARKS

The commitment to resume growth of post-dormant perennial buds is driven by developmental activators such as CK and auxins. While light can function as an upstream regulator of these phytohormones, light is only a facultative requirement for the decision in many species. he body of evidence discussed here demonstrates that light promotes/ enhances, rather than drives, photomorphogenic development, while other cues such as temperature promote the initial skotomorphogenic outgrowth. Sucrose, resulting from emerging photosynthesis may also participate in the light-independent activation process, acting as both a metabolite and signalling molecule. While the present discussion has focused on the importance of white light, blue light may also play a key role in bud burst. Accumulating evidence supports the function of CRY photoreceptors in blue light perception resulting in *HY5* expression, which in turn activates photomorphogenic gene expression, stimulating bud outgrowth. PHYA and PHYB may also fulfil roles in light perception as they do in arabidopsis seeds. The developmental stages of plastids of buds can vary between different perennials plants but also within different

379 tissues of the same bud. The developmental regulation of the hypoxic state also plays important
380 but largely undefined roles in bud burst. The role of hypoxia in regulating mitochondrial and
381 plastid numbers and composition at the early stages of bud burst is largely unexplored. Finally,
382 our analysis of the literature evidence highlights the conservation of light-induced signalling
383 cascades and associated transcriptional changes that drive the resumption of growth after a
384 period of quiescence in perennial buds and arabidopsis seeds. Several exciting questions
385 remain, particularly in regard to the role of light and oxygen in bud burst (see Outstanding
386 Questions). Increasingly, the tools required to investigate them, even in perennials are
387 becoming available.

388

ADVANCES BOX

- There is no evidence the developmental state, ultrastructure and photosynthetic capacity of plastids are directly related to the state of quiescence in perennial buds, suggesting independent pathways of regulation for the chloroplast development and the dormancy/quiescent state of the organ.
- Evidence in grapevine buds strongly suggests CRY photoreceptors participate in light perception causing *HY5* expression, which in turn triggers photomorphogenic gene expression in perennial buds.
- The developmental resumption of growth following quiescence in perennial buds is transcriptionally associated with the hypoxic responses of plants.
- A ROP2 GTPase has recently been identified as a pivotal regulator of TOR kinase in orchestrating meristem functions. Evidence suggests oxygen status may also regulate this pathway via hypoxia-dependent stabilisation of ERF-VII transcription factors.
- A conserved light-induced transcriptional signalling cascade accompany the resumption of growth in perennial buds and arabidopsis seeds.

OUTSTANDING QUESTIONS BOX

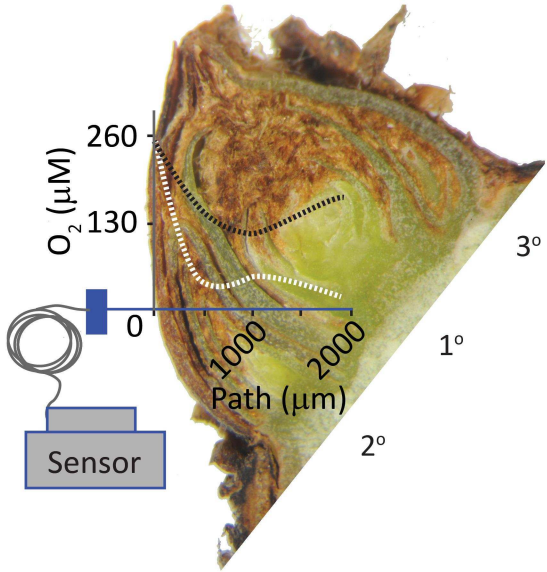
- Which molecular cues determine the developmental state and energetic capacity of plastids in dormant or quiescent perennial buds? Are these molecular cues under the regulation of master regulators of dormancy, or are they independent processes?
- Are PHYA and PHYB, via posttranslational modification, involved in the photomorphogenic process of perennial buds?
- Would gene silencing of *CRY* or *HY5* attenuate photomorphogenesis and preserve an etiolated state in bursting perennials buds?
- Does the development of a hypoxic state of transcriptional regulation play a functional role in organogenesis or is it merely consequential of the increase in respiration?
- Does oxygen status regulate TOR kinase activity *in vivo* in plants via hypoxia-dependent stabilisation of the ERF-VII transcription factors, which indirectly influence the ROP2?
- Does hypoxia play a role in regulating the mitochondrial and plastid numbers and composition at early stages of bud burst?

FIGURE LEGENDS

Figure 1. **Morphology, tissue oxygen status and light-affected growth of single-node cuttings of post-dormant grapevine buds**. A longitudinal section of a quiescent grapevine bud, showing three preformed shoots (1°, 2°, 3°), enclosed by layers of bracts, hairs and lignified scales. A stylised plot of the tissue oxygen concentration of a bud during quiescence (dotted white line) and bud burst (dotted black line), as determined by an oxygen microsensor is overlaid. The path of the probe, from external scales to the core of the primary meristem, is the x-axis (blue line), and 260 μM [O_2] approximates the air-saturated concentration in water at standard temperature and pressure (refer to Meitha et al., 2015).

Figure 2. Scatterplot showing the differential expression and functional category of grapevine genes specifically discussed here. Full data presented in Supplemental Table S1. Differential expression analysis was carried out from grapevine buds grown at 22 °C in the presence (DL) or absence (D) of light at 72 and 144 h following removal from 4 °C storage (FC ≥|2|, FDR $P \leq 0.05$). Letters from A to L summarise the functional categories. Size of dots represents the log10(Adjusted *P*-Value). Colour scale proportional to FC values; green (downregulated genes), grey (not differentially expressed) and purple (upregulated genes).

Figure 3. Differential expression of genes during grapevine bud burst coding for photosynthetic and chlorophyll metabolic functions at 144 h in the presence (DL) or absence (D) of light. Purple colour indicates upregulation at 144 h of DL respect to D. ALA, Aminolevulinic acid; CAO, CHLOROPHYLL A OXYGENASE; CHL, Mg-CHLOROPHYLLASE 1; CHLH, Mg-CHELATASE subunit; CRD, Mg-PROTOPORPHYRIN IX MONOMETHYLESTER CYCLASE; Cyt*b*6/F, CYTOCHROME *b*6-F COMPLEX IRON-SULFUR subunit (PETC); Fd, FERREDOXIN; FLU, FLUORESCENT IN BLUE LIGHT; FNR, Fd $NADP^+$ OXIDOREDUCTASE; GUN4, GENOMES UNCOUPLED4; HCF136, PSII STABILITY/ASSEMBLY FACTOR; HEMA, GLUTAMYL-TRNA REDUCTASE; LHC, LIGHT-HARVESTING COMPLEX; POR, NADPH-PROTOCHLOROPHYLLIDE OXIDOREDUCTASE; PSI, PHOTOSYSTEM I; PSII, PHOTOSYSTEM II; PsaD, PSI REACTION CENTRE (RC) subunit II, chloroplast precursor; PsaE B, PSI RC subunit IV B; PsaG, PSI RC subunit V; PsaH, PSI RC subunit VI; PsaK, PSI subunit X; PsaL, PSI subunit XI; PsaN, PSI RC subunit N; PsaO, PSI subunit O; PsbS, PSII 22 kDa protein; PsbW, PSII RC W; PsbX, PHOTOSYSTEM II subunit X; PsbY, PSII CORE COMPLEX PROTEIN (chloroplast precursor); psbZ, PSII core complex proteins;



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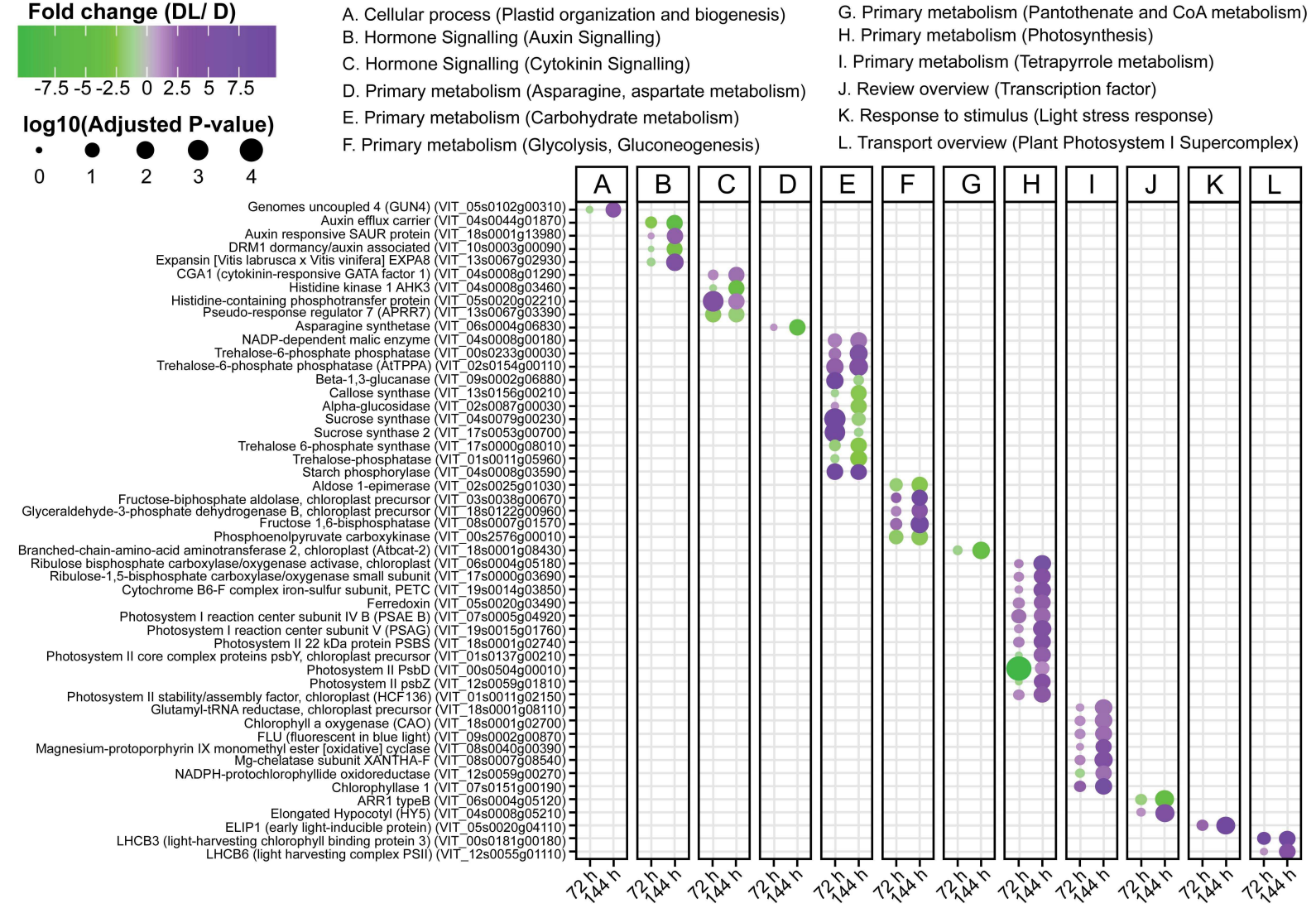

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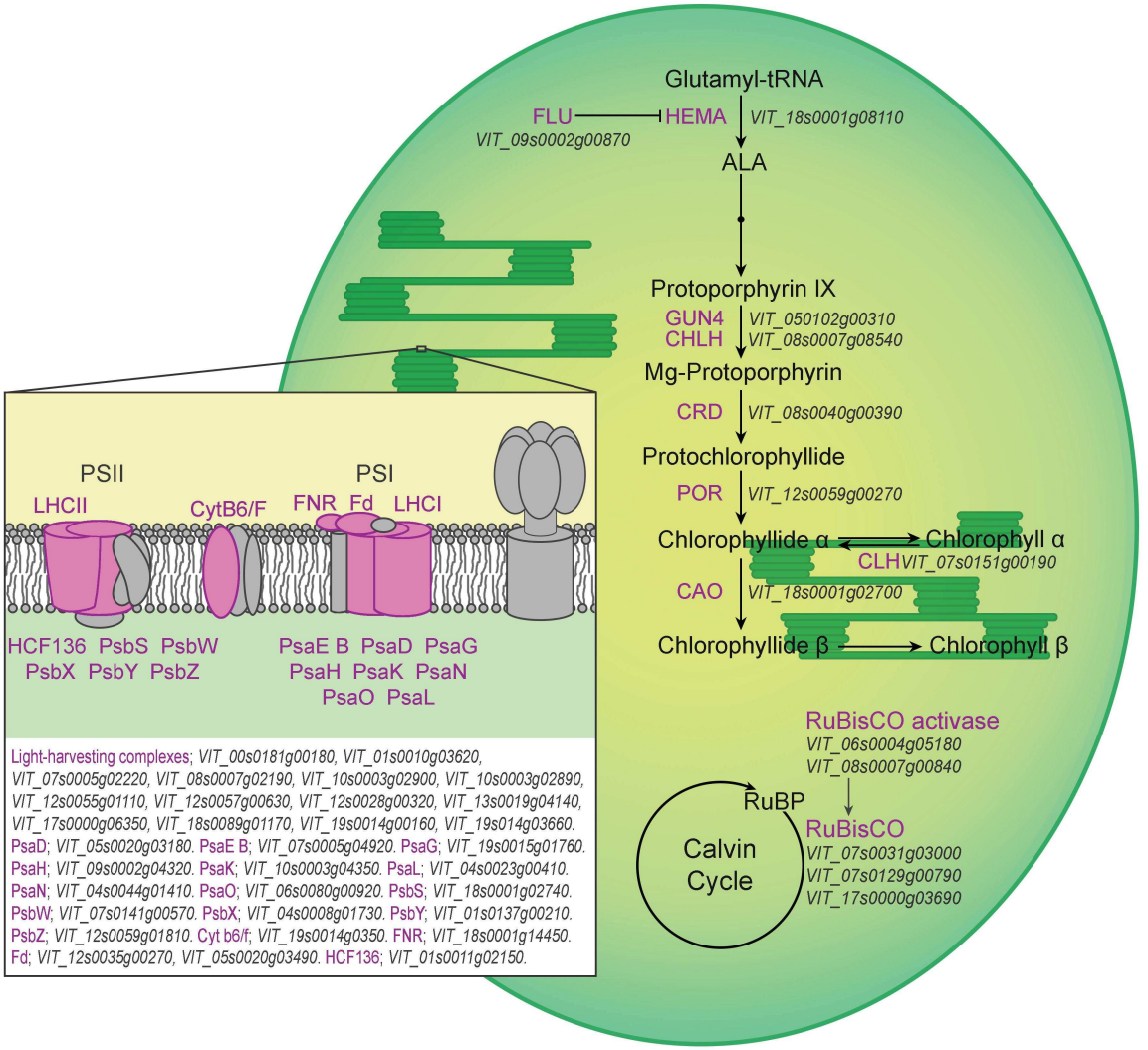

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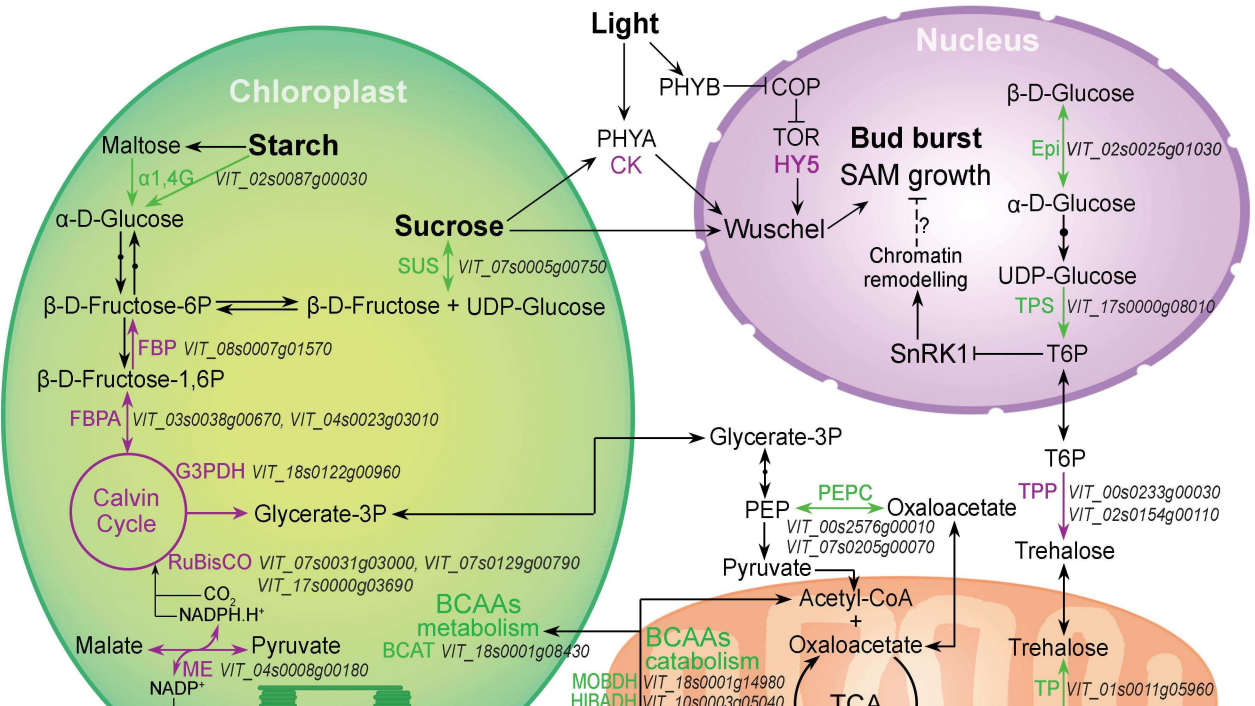
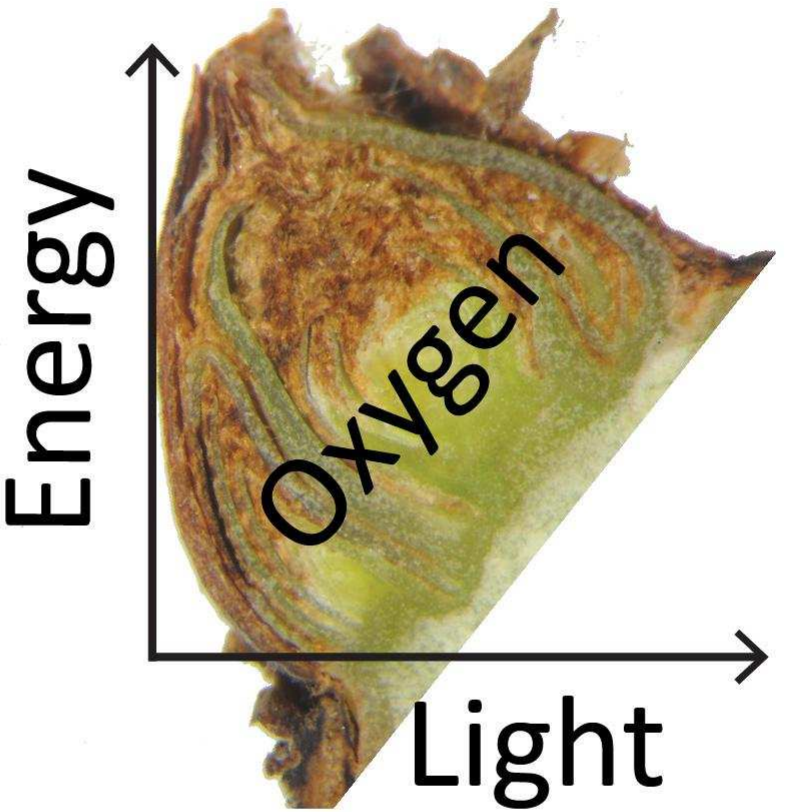


Figure 4. Differential expression of genes during grapevine bud burst coding for carbon- and energy-related functions at 144 h in the presence (DL) or absence (D) of light. Processes and reactions in purple and green reflect up- and downregulation respectively at 144 h in the DL/ D comparison. α1,4G, α-1,4-GLUCOSIDASE; BCAAs, branched-chain-amino acids; BCAT, BRANCHED-CHAIN-AMINO-ACID AMINOTRANSFERASE; CK, cytokinins; Epi, ALDOSE 1-EPIMERASE; FBP, FRUCTOSE 1,6-BISPHOSPHATASE; FBPA, FRUCTOSE-BISPHOSPHATE ALDOLASE; G3PDH, GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE B; ME, NADP-DEPENDENT MALIC ENZYME; PEPC, PHOSPHOENOLPYRUVATE CARBOXYKINASE; RuBisCO, RIBULOSE BISPHOSPHATE CARBOXYLASE; SUS, SUCROSE SYNTHASE; TP, TREHALOSE-PHOSPHATASE; TPP, TREHALOSE-6-PHOSPHATE PHOSPHATASE; TPS, TREHALOSE-6-PHOSPHATE SYNTHASE.


Energy
Oxygen
Light