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Auxin-mediated plant architectural changes in response to shade and high temperature

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Abstract

The remarkable plasticity of their architecture allows plants to adjust growth to the environment and to overcome adverse conditions. Two examples of environmental stresses that drastically affect shoot development are imminent shade and high temperature. Plants in crowded environments and plants in elevated ambient temperature display very similar phenotypic adaptations of elongated hypocotyls in seedlings and elevated and elongated leaves at later developmental stages. The comparable growth responses to shade and high temperature are partly regulated through shared signalling pathways, of which the phytohormone auxin and the phytochrome interacting factors (PIFs) are important components. During both shade- and temperature-induced elongation growth auxin biosynthesis and signalling are upregulated in a PIF-dependent manner. In this review we will discuss recent progress in our understanding of how auxin mediates architectural adaptations to shade and high temperature.

Abbreviations

bHLH, basic Helix-Loop-Helix; IAA, Indole-3-Acetic Acid; NPA, 1-Naphthylphthalamic acid; phy, phytochrome; PIF, Phytochrome Interacting Factor; PIN, Pin-formed; SAUR, Small Auxin Up RNA; TAA1, Tryptophan Aminotransferase of Arabidopsis; YUC, YUCCA, flavin-containing monooxygenase

Introduction

The term auxin literally means ‘to grow’ and its role in cell division and elongation is well established. It is thus not surprising that auxin has emerged as an important regulator of adaptive growth responses to environmental stresses. Two such environmental stresses are vegetative shading and high temperature, which induce strikingly similar changes in shoot architecture. In Arabidopsis, they rapidly lead to elongated hypocotyls and petioles in seedlings and to hyponastic leaves with elongated petioles and reduced lamina size in adult plants (Gray et al. 1998; Casal 2012; Crawford et al. 2012, Fig.1). In the long term, both vegetational shade and increased ambient temperature lead to early flowering as an ultimate escape response. It has become clear that the comparable growth responses to shade and increased temperature partly share signalling components, with a prominent role for auxin. However, as no role for auxin has been established in the regulation of flowering we have excluded the accelerated flowering response from this review.

The phenotypical adaptations to shade and increased temperature serve to bring the photosynthetic organs away from the stress. In the case of shade, neighbouring vegetation poses a threat to a plant's light capture. When the presence of proximate neighbours is perceived, growth is rapidly diverted to elongation of hypocotyls, stems or internodes, thus bringing the photosynthetic organs higher up in the vegetation to avoid becoming overgrown (Casal 2012, Fig.1). These growth adaptations are costly, as a shade avoidance phenotype in the absence of dense vegetation leads to decreased biomass and reproduction (Casal and Smith 1989; Casal et al. 1994; Schmitt et al. 1995). However, in the presence of surrounding vegetation the shade avoidance response allows plants to compete with their neighbours and secure light capture. Consequently, plants that fail to respond to their neighbours have reduced fitness in dense stands and will eventually be out-competed (Ballare et al. 1988; Schmitt et al. 1995; Pierik et al. 2003; Keuskamp et al. 2010). The significance of the shade avoidance response is furthermore underlined by the fact that it is prioritised over defence, as shade-avoiding plants are more susceptible to pathogens and herbivorous insects (Moreno et al. 2009; Cerrudo et al. 2012; de Wit et al. 2013).

The similar changes in plant architecture upon increased ambient temperature are thought to prevent high temperature damage to the leaves. Plants pre-grown at 28°C were cooler than plants pre-grown at 22°C when moved to 28°C, despite a lower number of stomata in high temperature-grown plants (Crawford et al. 2012). It was therefore suggested that the less compact shoot architecture and leaf hyponasty of Arabidopsis plants in high temperature facilitates transpiration and thus cools the leaves (Clum 1926; Radin et al. 1994; Crawford et al. 2012; Bridge et al. 2013).

Shade and temperature sensing

Proximate vegetation may be perceived through volatiles and physical touch (Kegge and Pierik 2010; de Wit et al. 2012), but is mainly sensed by changes in the intensity and spectral composition of the light (reviewed in Vandenbussche et al. 2005; Casal 2012). Red (R) and blue (B) light are depleted from the spectrum as they are absorbed for photosynthesis, while far-red (FR) light is reflected by plants. The primary signal through which plants perceive neighbours is the ratio of red to far-red (R:FR) light, which decreases even before actual shading occurs (Morgan and Smith 1978; Morgan et al. 1980; Ballaré et al. 1990). Most laboratory studies use a decrease in R:FR to induce the shade avoidance response by supplementing the background light with FR. Therefore we will focus on the plant response to this early neighbour detection signal without a decrease in blue light or light intensity in this review.

The R:FR is perceived through a set of phytochrome (phy) photoreceptors, which exist in two photoconvertible conformation states: the active, FR-absorbing form (Pfr) and the inactive, R-absorbing form (Pr). When the R:FR decreases, the phytochrome photoequilibrium shifts to the inactive form and thus acts as a sensor for light qualitative changes (Holmes and Smith 1975; Smith and Holmes 1977). Among the different phytochromes (phy A-E in Arabidopsis), phyB is the main phytochrome triggering the shade avoidance response in Arabidopsis (Franklin et al. 2003). Phytochromes are present in all plant tissues but not all tissues are equally responsive to a low R:FR signal (Sharrock 2008). In *Sinapis alba* (white mustard) both local FR irradiation of the stem and the primary leaves increased internode elongation, but the induction was faster when the stems were irradiated (Morgan et

al. 1980). In a FR-responsive Arabidopsis reporter line GUS expression was strongly induced in the hypocotyl both when the whole seedlings or only the cotyledons were treated with FR, whereas this was not the case when only the lower part of the hypocotyl was irradiated (Tanaka et al. 2002). Similarly, petiole elongation of adult Arabidopsis leaves was enhanced when the leaf lamina were locally treated with FR, but not when the petioles were irradiated (Kozuka et al. 2010). It thus appears that in Arabidopsis the cotyledons and at a later stage leaf lamina are the primary perception site of the low R:FR neighbour detection signal (Bou-Torrent et al. 2008).

A rise in ambient temperature could be sensed at different levels and it is likely that different temperature sensing mechanisms operate simultaneously. Temperature may be perceived through increased fluidity of the plasma membrane and associated activation of ion channels and lipid signalling, through changes in protein structure or through ROS accumulation (as reviewed in Mittler et al. 2012). In this review we focus on the temperature response to a relatively mild increase in ambient temperature from around 20°C to temperatures still below 30°C. Unlike the classical heat stress response that is usually studied at temperature shifts to above 40°C, this milder increase in temperature leads to a moderate increase of typical heat stress markers (Saidi et al. 2005; Balasubramanian et al. 2006). A temperature-sensing mechanism that has been proposed in the context of mildly elevated ambient temperature (27°C) depends on chromatin accessibility through temperature-mediated occupancy of the H2A.Z histone in nucleosomes, which wraps DNA tightly. It was shown that at high temperature H2A.Z nucleosome occupancy declined at the promoters of temperature-responsive genes, thereby rendering the chromatin more accessible for transcriptional regulators (Kumar and Wigge 2010).

Where temperature is perceived in the plant is not known. In a relatively small plant as Arabidopsis that lacks thick structures temperature changes might be perceived in all tissues. In apple trees it has been shown that increased root temperature can induce bud break (Greer et al. 2006), indicating that in bigger species local temperature perception can evoke a systemic signal that leads to a response in distal tissues.

Although perception of shade and increased temperature is different, both stress signals employ the auxin pathway to redirect growth drastically to adapt to adverse conditions. The great majority of studies concerning the role of auxin in these responses have been done with young seedlings of Arabidopsis, using hypocotyl elongation as a convenient read-out of the growth response. Inhibition of different steps of the auxin pathway through mutations or pharmacological treatments affects both shade- and temperature-induced hypocotyl elongation (Gray et al. 1998; Steindler et al. 1999; Tao et al. 2008). In the next section we will discuss the known signalling steps that link auxin to the shade avoidance- and high temperature growth responses. For a succinct summary of the auxin pathway (biosynthesis, transport and response) we refer to Box 1 and references therein.

The auxin pathway in the shade- and high temperature response

Localisation of auxin action depends both on increased biosynthesis and polar auxin transport (Morelli and Ruberti 2000; Benjamins and Scheres 2008). All parts of Arabidopsis seedlings are able to produce auxin, with the highest synthesis capacity and auxin concentrations in young dividing leaves (Ljung et al. 2001). Inhibition of

auxin transport prevents induction of the auxin reporter *DR5::GUS* in the hypocotyls but not in the cotyledons of low R:FR-treated seedlings (Tao et al. 2008). This suggests that shade induces auxin production mainly in the cotyledons and that auxin is then transported to the hypocotyl to promote elongation growth (Fig. 2). In increased temperature, induction of the auxin biosynthesis genes *YUC8* and *YUC9* was shown to be stronger in cotyledons than in hypocotyls (Stavang et al. 2009), which may indicate that also during high temperature stress the cotyledons are the main auxin source. Interestingly, for shade avoidance the site of signal perception coincides with the site of stress-induced auxin production, which may also be the case in response to increased temperature.

Auxin production

Auxin accumulates in seedlings within one hour in response to a decreased R:FR ratio or a transfer from 22 to 28°C (Tao et al. 2008; Franklin et al. 2011; Li et al. 2012; Sun et al. 2012; Hornitschek et al. 2012). Increased auxin levels were also found in hypocotyls after two days of low R:FR treatment (Keuskamp et al. 2010). However, in leaves of adult plants treated with end-of-day FR, a treatment that mimics the shade avoidance response, no increase in auxin concentration was found (Kozuka et al. 2010).

Auxin accumulation in seedlings in shade or increased temperature is due to an increase in auxin production. The predominant biosynthesis pathway in both responses is the TAA1-YUC -dependent route (Tao et al. 2008, Fig. 2). Other pathways may be involved additionally, as appears to be the case for the CYP79B2 pathway in elevated temperature (Franklin et al. 2011). In accordance with a central

role for auxin, the *sav3/taa1* mutant that does not accumulate TAA1 protein is strongly impaired in hypocotyl elongation in response to shade and increased temperature (Tao et al. 2008, Fig. 1). Adult *sav3/taa1* plants show a strong reduction in shade-induced leaf hyponasty and petiole elongation and have a less pronounced reduction in leaf area in simulated shade (Tao et al. 2008; Moreno et al. 2009, Fig. 1), indicating that TAA1 is also important for shade avoidance responses at later developmental stages. Despite its obvious relevance for the response to shade and high temperature, TAA1 is not the rate-limiting step in auxin production since its over-expression does not phenocopy plants with increased auxin content (Tao et al. 2008). Furthermore, *TAA1* expression seems to be regulated in certain conditions in response to higher temperatures, while its expression decreased in response to two hours of low R:FR (Tao et al. 2008; Franklin et al. 2011; Nomoto et al. 2012). Although TAA1 may be regulated at the level of protein activity, it rather seems that auxin production in response to shade and increased temperature is boosted through induced expression of *YUCCA* (*YUC*) genes. Consequently, *YUC1* overexpression can rescue the short hypocotyl of the *sav3/taa1* mutant under shade conditions (Won et al. 2011). There are eleven *YUC* genes, which catalyse auxin biosynthesis downstream of TAA1 (Zhao et al. 2001; Won et al. 2011). Several *YUCs* are strongly and rapidly induced in response to shade and increased temperature (Tao et al. 2008; Stavang et al. 2009; Sun et al. 2012; Brandt et al. 2012), but their exact contribution to auxin production in these responses is still unknown. *YUC8* expression is upregulated in high temperature and the *yuc8* mutant has reduced hypocotyl elongation compared to wild type when transferred to 29°C (Sun et al. 2012). In response to low R:FR *YUC2, YUC5, YUC8* and *YUC9* are strongly induced (Tao et al. 2008; Won et al. 2011; Li et al. 2012; Brandt et al. 2012). However, shade-

induced hypocotyl elongation was only slightly reduced in the quintuple *yuc35789* mutant. This mild phenotype may indicate that the YUCs act redundantly as this mutant can still express *YUC2* in response to shade (Li et al. 2012). The *yuc1yuc4* double mutant on the other hand has a *sav3/taa1*-resembling phenotype in shade (Won et al. 2011).

Auxin transport

The main source of newly synthesised auxin is assumed to be in the cotyledons/lamina and shoot apical meristem while it may be required in distal plant parts to affect growth. Therefore, auxin transport is an important component of the auxin pathway. Blocking auxin transport using the inhibitor of polar auxin transport 1-naphthylphthalamic acid (NPA) inhibits hypocotyl elongation in response to shade (Steindler et al. 1999; Tao et al. 2008; Keuskamp et al. 2010). Correspondingly, reduced hypocotyl elongation in low R:FR was also observed in a *pin3* mutant that is affected in polar auxin transport (Keuskamp et al. 2010). In response to low R:FR PIN3 protein was furthermore shown to relocate from a basal to a lateral location in the endodermis (Keuskamp et al. 2010). This supports a previously proposed model in which the root-ward auxin flux is redirected to a more lateral auxin distribution to regulate growth in the hypocotyl (Morelli and Ruberti 2000). Consistently, the auxin-responsive reporter line IAA19::GUS showed a more lateral expression pattern in petioles of low R:FR-treated plants (Pierik et al. 2009). NPA treatment also eliminates high temperature-induced hypocotyl elongation, indicating that auxin transport is also required for the response to increased temperature (Gray et al. 1998; Stavang et al. 2009).

Auxin perception and signalling

Auxin is perceived by nuclear receptors of the TIR/AFB family and targets the transcriptional inhibitors called IAA proteins for degradation through the 26S proteasome (Box 1). Inactivation of the receptors by mutations or chemical treatments with competitive inhibitors strongly reduces hypocotyl elongation in response to shade and increased temperature (Gray et al. 1998; Keuskamp et al. 2010). This reduced response is also observed in plants expressing a dominant-negative mutation of IAA protein, in which inhibition of auxin-mediated responses is correlated with a stabilised IAA (Gray et al. 1998; Sun et al. 2012)). This suggests that turnover of IAAs plays a role in auxin-dependent growth regulation.

Auxin-regulated responses

IAA degradation and ARF activation (Box 1) leads to the induction of auxin-responsive genes. Transcriptomic analysis of the shade and high temperature responses revealed that auxin-responsive genes are strongly enriched among the induced genes (Devlin et al. 2003; Sessa et al. 2005; Tao et al. 2008; Stavang et al. 2009; Kozuka et al. 2010; Li et al. 2012; Hornitschek et al. 2012). There are several known mechanisms through which this auxin-driven gene induction affects stress-related growth.

One way is through activation of different components of its own pathway. Upregulation of *PIN3* and *PIN7* is likely to reinforce auxin transport towards sites of growth besides previously mentioned PIN relocalisation (Devlin et al. 2003;

Keuskamp et al. 2010). Expression of several small auxin up RNAs (*SAURs)* is strongly induced in response to both shade and increased temperature. A few hours of shade was shown to induce *SAUR19, SAUR21, SAUR23* and *SAUR24* in the basal part of hypocotyls (Spartz et al. 2012). *SAUR19, SAUR23* and *SAUR24* are also induced in the hypocotyl elongation zone after a few days of elevated temperature (Franklin et al. 2011). The corresponding proteins were recently proposed to be positive regulators of hypocotyl elongation possibly through modulation of auxin transport (Spartz et al. 2012; Chae et al. 2012). Plants expressing a miRNA targeting the *SAUR61SAUR68* subfamily showed only slightly reduced hypocotyl length, while plants expressing GUS or GFP fusions to *SAUR63* had longer hypocotyls and increased basipetal auxin transport (Chae et al. 2012). Similarly, expression of a GFP fusion with *SAUR19* increased leaf size and cell and hypocotyl length (Spartz et al. 2012), suggesting that a GUS/GFP fusion leads to SAUR stabilisation and a subsequent gain-of-function phenotype. The absence of strong phenotype in *saur* loss-of-function mutants could be due to redundancy of the different family members (Chae et al. 2012). In parallel to genes that promote elongation, genes participating in a negative feedback are also activated. This is the case for the induction of the *IAAs*, which may have a role in preventing exaggerated responses rather than promoting growth.

Auxin also controls other hormonal pathways to coordinate growth. Exogenous auxin treatment activates several genes (*GA20OX1, GA20OX2, GA2OX8, GA3OX1*) in the gibberellin (GA) biosynthesis pathway, which is required for hypocotyl growth and enhanced elongation in response to both shade and increased temperature (Djakovic-Petrovic et al. 2007; Chapman et al. 2012). Binding of GA to its receptor

leads to degradation of the growth-inhibiting DELLA proteins (Fu et al. 2004; Ueguchi-Tanaka et al. 2005). The DELLA protein RGA was found to bind to the transcriptional activators phytochrome interacting factor (PIF) 3 and 4 (see below) and to thereby prevent their transcriptional activity (Feng et al. 2008; de Lucas et al. 2008). Activation of the GA pathway by auxin leads to degradation of RGA both in shade and high temperature (Djakovic-Petrovic et al. 2007; Stavang et al. 2009), thus releasing the PIFs to promote growth. Another hormonal pathway affected by auxin is the cytokinin pathway. Shade induces the expression of the cytokinin oxidase-coding gene (*CKX6*) (Carabelli et al. 2007), which likely triggers cytokinin degradation. This leads to inhibition of leaf primordium growth through reduced cell division, a phenotype that is not observed in the *tir1* mutant (Carabelli et al., 2007). A similar hormonal interaction may cause the reduced leaf blade expansion in shade.

Independently of gene expression, auxin rapidly activates proton pumps (H^+-ATPase), which leads to acidification of the apoplast (Takahashi et al. 2012). An acidic pH in the apoplast increases the activity of cell wall-degrading enzymes such as expansins and xyloglucan endotransglucosylase/hydrosylases (XTHs) that is necessary to loosen the cell walls and to allow for elongation. Apart from its effect on cell wall loosening through apoplast acidification, auxin treatment also induces the expression of several *EXPANSIN* and *XTH* genes (Chapman et al. 2012). Accordingly, expression of several *XTH* genes as well as xyloglucan degrading activity is upregulated in shade-treated petioles of adult plants (Sasidharan et al. 2010). Increased expression and activity of a certain XTH may not make it essential for growth (Kaewthai et al. 2013) and redundancy among the many XTHs may exist.

Nevertheless, *xth15* and *xth17* single mutants show reduced petiole elongation in response to shade (Sasidharan et al. 2010).

Shade and temperature control of the auxin pathway

Besides auxin temperature and shade responses both depend on members of the phytochrome interacting factors (PIFs), bHLH transcription factors that recently appeared as central regulators of growth adaptation to the environment (Leivar and Quail 2011; Proveniers and Van Zanten 2013). PIFs were originally described as proteins interacting with the active form of the phytochromes, but it has now become clear that they play a more general role in growth responses. Plants overexpressing *PIF4* or *PIF5* phenocopy shade-grown plants with elongated petioles and hypocotyls while the corresponding mutants present a reduced response to low R:FR (Lorrain et al. 2008). Interestingly, different PIFs are involved in different responses. Adaptation to increased temperature depends exclusively on PIF4, as a *pif4* mutant shows neither hypocotyl/petiole elongation nor leaf hyponasty at 29°C (Koini et al. 2009, Fig. 1). In response to low R:FR PIF7 seems to be the predominant regulator, as *pif7* seedlings show strongly reduced hypocotyl elongation in response to low R:FR (Li et al. 2012, Fig. 1). PIF4 and PIF5 are also required for a full response to shade particularly under low light with PIF4 acting predominantly on hypocotyl elongation and PIF5 on gene expression (Lorrain et al. 2008; Keller et al. 2011; Leivar et al. 2012). Other members of the PIF family also moderately contribute to the shade avoidance response such as PIF1 and PIF3 (Leivar et al. 2012).

How the PIFs are regulated by environmental signals is better understood for shade than for high temperature. As proteins interacting with phytochromes PIFs are the perfect sensors to integrate changes in the R:FR ratio. Interaction with the phytochromes mediates PIF phosphorylation and/or degradation, while inactivation of the phytochromes in low R:FR releases their repression and allows the PIFs to act as transcriptional regulators (Lorrain et al. 2008; Li et al. 2012). At elevated temperatures, increased *PIF4* expression in the cotyledons has been reported (Koini et al. 2009; Stavang et al. 2009; Nomoto et al. 2012) especially during the dark period (Nomoto et al. 2012), which leads to increased PIF4 levels (Yamashino 2013). Whether the rise in PIF4 levels in response to temperature elevation is exclusively a transcriptional response or also includes post-transcriptional regulation is still debated (Stavang et al. 2009; Kumar et al. 2012; Foreman et al. 2011). Shade and temperature also control PIF accessibility to DNA through dimerization that inhibits DNA binding (see before with DELLA and below with HFR1) and possibly through changes in DNA structure. The temperature-mediated eviction of H2A.Z from nucleosomes could facilitate PIF4 binding to promoters of growth-promoting genes, as is the case for PIF4 binding to *FLOWERING LOCUS T* in temperature-induced flowering (Kumar et al. 2012).

The impaired response of *pif* mutants to shade or increased temperature correlates with impaired auxin production in response to these signals (Franklin et al. 2011; Li et al. 2012; Sun et al. 2012; Hornitschek et al. 2012). The expression of auxin-responsive genes is also affected in *pif* mutants (Nozue et al. 2011; Li et al. 2012; Leivar et al. 2012; Hornitschek et al. 2012). For instance, shade- and temperature-induced expression of *IAA29* is strongly reduced in the *pif4* mutant (Hornitschek et al.

2009; Koini et al. 2009; Nozue et al. 2011; Franklin et al. 2011; Sun et al. 2012; Hornitschek et al. 2012). Furthermore, the *pif7* transcriptome after one hour in low R:FR is similar to the one of *sav3/taa1* (Li et al. 2012), which suggests that auxin is the driving force for hypocotyl elongation in low R:FR. Chromatin immunoprecipitation experiments have shown that *TAA1* and several *YUCs* are direct target genes of PIF4, PIF5 and PIF7 (Li et al. 2012; Sun et al. 2012; Hornitschek et al. 2012). In response to 29°C, PIF4 also targets another auxin biosynthesis pathway through *CYB79B2* (Franklin et al. 2011). PIF-mediated auxin production may therefore explain how these transcription factors promote growth in response to changes in temperature or light. Manipulating the auxin pathway can rescue temperature and shade responses when PIF activity is disturbed. Hypocotyls of *pif4* mutants do elongate in response to increased temperature when *SAUR19* is over-expressed and application of the auxin analogue picloram can restore the response to low R:FR in the *pif7* mutant (Franklin et al. 2011; Li et al. 2012). Conversely, the long hypocotyl of a *PIF4* overexpressing line is reduced when *YUC8* is mutated or the auxin signalling pathway is inhibited by a dominant-negative version of IAA3 (Sun et al. 2012).

Auxin production is not the only step in the auxin pathway controlled by the PIFs. PIF4 and PIF5 can also target genes affected in auxin signalling (such as *IAA19, IAA29*), auxin transport (such as *PIN3*) and auxin inactivation (such as *GH3.3*) (Hornitschek et al. 2012; Oh et al. 2012). Correspondingly, the *pif4pif5* double mutant is affected in auxin sensitivity (Nozue et al. 2011; Hornitschek et al. 2012). PIF4, PIF5 and PIF7 are however not required for the induction of auxin-responsive genes in all conditions, since auxin treatment still induces a robust expression of some of these marker genes in *pif* mutants (Li et al. 2012; Chapman et al. 2012; Hornitschek

et al. 2012). This is paralleled by data from Chapman et al. (2012) showing that early hypocotyl responses to picloram treatments are indistinguishable from wild type in the *pif4pif5* double mutant.

Shade induces an extensive transcriptional network comprising both positive and negative regulators of elongation growth that also affect auxin signalling (Sessa et al. 2005). Among these genes is a set of atypical basic helix-loop-helix (bHLH) transcription factors such as *HFR1, PAR1* and *PAR2* that inhibit the shade avoidance response (Sessa et al. 2005; Roig-Villanova et al. 2007). These bHLHs lack the typical E and G-Box DNA-binding domain, but act as transcriptional co-regulators by inhibiting DNA-binding of PIF4 and PIF5 through the formation of heterodimers with the PIFs (Hornitschek et al. 2009; Galstyan et al. 2011; Hao et al. 2012). HFR1 and PAR1 can also form heterodimers with the HLHs KDR and PRE1, respectively, which interferes with PIF heterodimerization and thus counteracts the inhibiting action of HFR1 and PAR1 on the PIFs (Hao et al. 2012; Hong et al. 2013). This reveals a complex competitive network of HLH/bHLH transcription factors that may be employed to tightly control the growth responses. Plants overexpressing PAR1 have an impaired shade-avoidance response and a reduced response to elevated temperature (Hao et al. 2012), suggesting that a similar network of repressors and enhancers might be at play during high temperature signalling.

Another group of genes rapidly induced by shade is the homeodomain-leucine zipper (HD-ZIP) class II transcription factors such as *ATHB2* and *ATHB4*, which can form an intricate regulatory network (Steindler et al. 1999; Ciarbelli et al. 2008; Sorin et al. 2009). More recently, the HD-ZIPIII REVOLUTA (REV) was shown to induce the expression of *ATHB2* and *ATHB4* in shade and to be required for shade-induced

hypocotyl elongation (Brandt et al. 2012). Moreover, it was shown that REV can regulate the expression of *YUC5* and thus seems to be directly linked to auxin. REV could indeed induce auxin levels and its overexpression could partially restore low R:FR-induced hypocotyl elongation in the *sav3/taa1* mutant (Brandt et al. 2012).

Integrating light and temperature signals

Light and temperature provide important information about the environment, time of day and season that together coordinate plant growth and developmental stage transition. Furthermore, light and temperature are both important input signals for the circadian clock, as both day/night and temperature cycles can be used to entrain the clock. Therefore, it is perhaps not so surprising that both signals feed into the same growth pathway. Moreover, the plant's response to one of the signals can be affected by the other.

A clear example of such interaction between shade and temperature was shown in *Abutilon theophrasti.* Plants grown at 26/20°C (d/n) showed longer hypocotyl elongation in response to simulated shade than plants grown at 18/16°C (Weinig 2013), indicating that shade and high temperature can have an additive effect on growth. Similarly, high temperature-induced hyponasty was enhanced in low light intensity as compared to higher light intensity (Vasseur et al. 2011). Interestingly, plants grown at 16°C show a reversed response to low R:FR with no petiole elongation and increased leaf expansion and biomass (Patel et al. 2013), suggesting that temperature has a major impact on the eventual growth output of the shade avoidance signalling pathway.

Conversely, light also affects plant responses to changes in temperature. Many temperature-induced responses (flowering, bud break, thermotolerance) are known to integrate signal input over a longer period, in which light-derived information on for instance day length may also play a role. It was shown that low R:FR can induce expression of the CBF regulon that is involved in freezing acclimation (Franklin and Whitelam 2007). As the R:FR also decreases during twilight, plants may use the information on shorter day length and longer twilight periods to prepare for subsequent temperature stress. Interestingly, PIF4 and PIF7 were found to be negative regulators of the CBF-component DRE-Binding (DREB)1, indicating that shade and temperature signalling pathways may directly interact (Kidokoro et al. 2009; Lee and Thomashow 2012). Importantly, the shade induction of the CBF regulon and PIF repression of *DREB1* expression are regulated by the circadian clock and day length. This might be a way for plants to distinguish between seasonal fluctuations and unusual stress situations.

Similar to the response to cold, light responses are also gated by the circadian clock. For shade, it has been shown that plants are most responsive in terms of hypocotyl elongation to a short period (2h) of low R:FR when applied towards the end of the photoperiod (Salter et al. 2003; Sellaro et al. 2012). PIF4 and PIF5 are crucial for rhythmic growth promotion and their circadian expression is regulated both by light and temperature (Nozue et al. 2007; Nomoto et al. 2012). In diurnal cycles, the evening complex ELF3-ELF4-LUX regulates the rhythmic expression of *PIF4* and *PIF5* (Nusinow et al. 2011). The coincidence of high transcript levels induced by the evening complex and PIF protein accumulation in the dark leads to growth promotion at the end of the night during normal growth (Nozue et al. 2007). Although during

shade avoidance mechanisms exist to regulate PIF abundance (phyB inactivation, DELLA degradation) this circadian PIF abundance may cause the gating of responsiveness to the stress signal. However, the *pif4pif5* mutant as well as mutants in the evening complex retained the gated shade response (Sellaro et al. 2012). This indicates that there is not a direct link between diurnal PIF4/PIF5 levels and gating for the shade avoidance response. One possibility is that clock gating of shade avoidance is due to PIF7, which appears to be the predominant PIF in shade avoidance. Auxin levels, signalling and responsiveness show oscillations that coincide with hypocotyl growth rhythms (Covington and Harmer 2007; Michael et al. 2008; Rawat et al. 2009; Nozue et al. 2011). The gated shade avoidance response to afternoon shade was also found to coincide with stronger responsiveness to exogenous auxin, but unlike the shade response this gated auxin responsiveness was affected in clock mutants (Sellaro et al. 2012). Although perhaps not induced by the same stimulus, it thus seems that circadian oscillations of the auxin pathway create a window of opportunity to allow for growth-related responses.

Altogether, auxin appears to be an important signal integrator that determines plant architecture in a complex environment. Although a number of important elements linking environmental sensing to auxin-mediated growth responses have been identified we still have a rather poor understanding of how perceived signals are integrated at the level of the whole organism. Besides strong evidence for induced auxin production there are indications that auxin sensitivity is also increased in response to stress. Whether sites of auxin production and auxin action overlap or are spatially separated, and how changes in sensitivity are regulated remains elusive. Currently available molecular tools will provide exciting new insight into spatio-

temporal aspects of signal transduction and tissue-specific responses and further our understanding of auxin-mediated stress responses.

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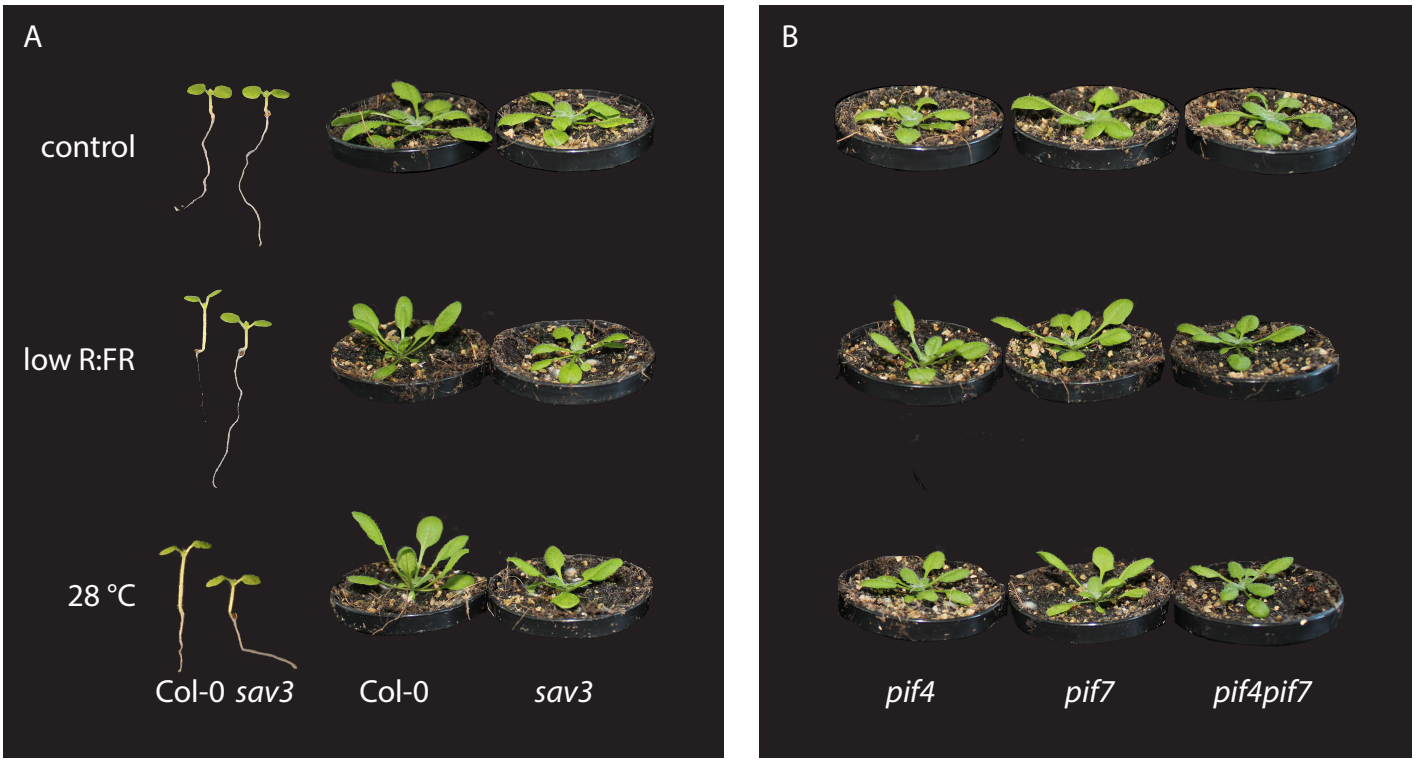
Figure legends

Figure 1. Phenotypes of Col-0 and the auxin biosynthesis mutant *sav3/taa1* seedlings and adult plants (A) and of various adult *pif* mutant plants (B) in control conditions, low R:FR and increased temperature. PIF7 is the predominant PIF regulating the shade response, whereas the response to elevated temperature is mainly regulated by PIF4.

Figure 2. Model of shade- and high temperature-induced auxin signalling in an Arabidopsis seedling. Both low R:FR and high temperature lead to a PIF-induced increase of auxin production through the TAA1-YUC pathway (see Box 1) in the cotyledons. Inactivation of PhyB in low R:FR leads to stabilisation of PIF4 and PIF5 and to dephosphorylation of PIF7. The relieved PhyB suppression of the PIFs allows them to bind to their targets, among which are the *YUCCA* auxin biosynthesis genes. During perception of elevated ambient temperature PIF4 mediates auxin biosynthesis through the TAA1-YUC pathway and the CYP79B2 pathway. Auxin is transported from the cotyledons to the hypocotyl, where PIF4 and PIF5 possibly play a role in enhanced auxin sensitivity. Auxin is laterally distributed by PIN proteins, eventually leading to enhanced hypocotyl elongation. Red colour: regulation in high temperature, blue colour: regulation in low R:FR, purple colour: regulation in both high temperature and low R:FR.

BOX1

Auxin is mainly synthesized from its precursor L-Trp through different pathways in the cytosol (reviewed by Ljung 2013). Of these, the pathway depending on the aminotransferase TAA1 for conversion to IPyA and on the YUCCA enzymes for subsequent conversion to IAA has recently emerged as a major pathway in Arabidopsis (Stepanova et al. 2008; Tao et al. 2008; Mashiguchi et al. 2011; Won et al. 2011). Its negative charge prevents auxin diffusion out of the cell, therefore it needs to be transported through the cell membrane via the PIN and ABCB families of efflux carrier proteins (reviewed by Zazímalová et al. 2010). In the low pH of the apoplastic environment auxin becomes protonated and in this less polar form it can enter cells through diffusion. Auxin can furthermore be transported into the cell through the AUX1/LAX family of influx carriers (reviewed by Swarup and Péret 2012). Auxin is perceived in the nucleus, where it binds to the F-box TIR/AFB family of auxin receptors. This stimulates degradation of the Aux/IAA repressor proteins, which relieves their repression of the ARF transcriptional regulators (reviewed by Benjamins and Scheres 2008). ABP1 is believed to be another, membrane-bound, auxin receptor. It appears to play an important role in cell expansion during leaf growth (reviewed in Perrot-Rechenmann 2010), and may therefore be involved in the auxin-dependent growth responses to shade and high temperature.


A
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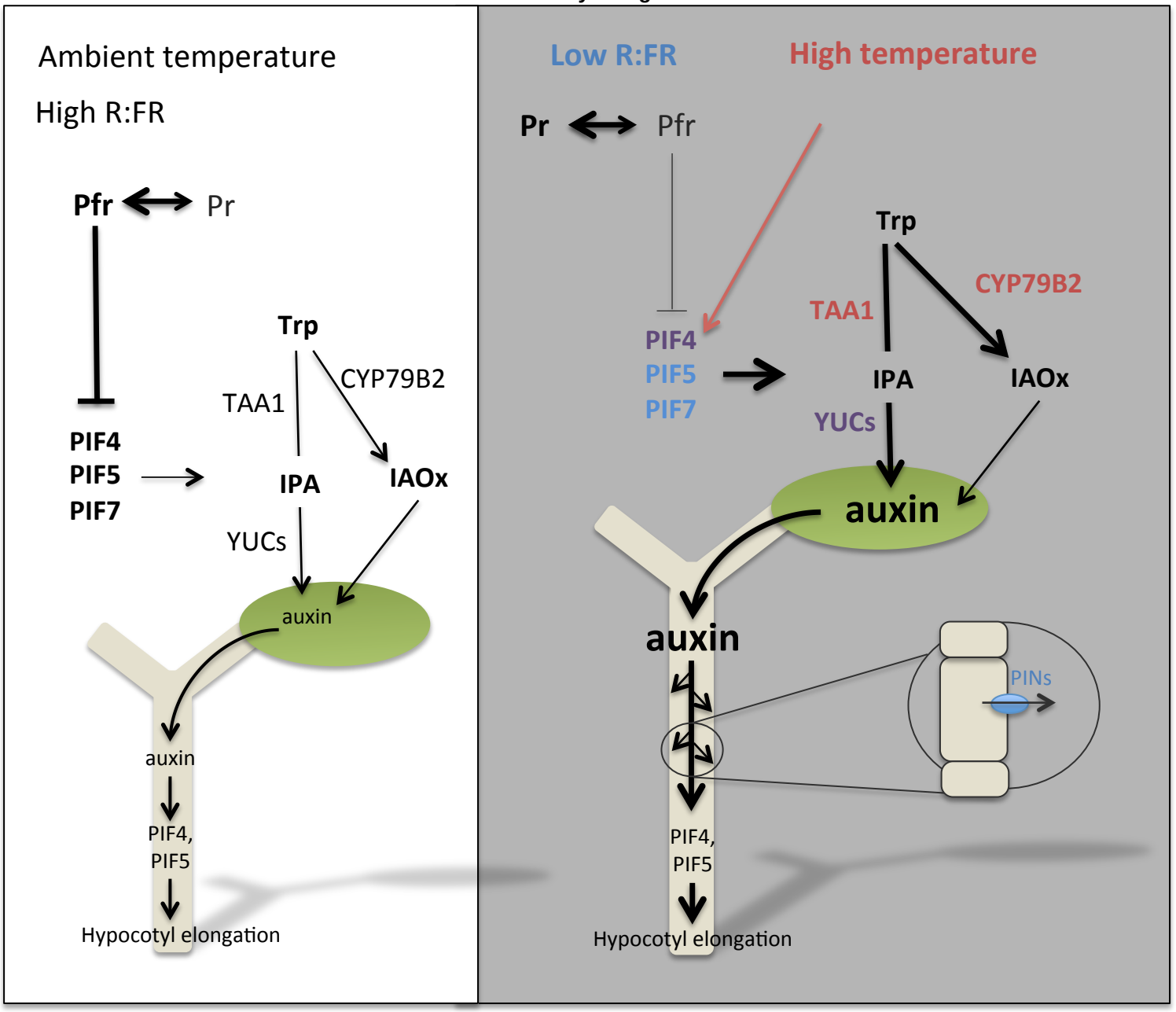
Ambient temperature
High R:FR
Pfr
Pr
PIF4
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Trp
TAA1
CYP79B2
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auxin
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PIF4,
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Hypocotyl elongation
Low R:FR
High temperature
Pr
Pfr
PIF4
PIF5
PIF7
Trp
TAA1
CYP79B2
IPA
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PIF4,
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Hypocotyl elongation