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REVIEW PAPER

PHYTOCHROME-INTERACTING FACTORS: a promising tool to improve crop productivity

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Abstract

Light is a key determinant for plant growth, development, and ultimately yield. Phytochromes, red/far-red photoreceptors, play an important role in plant architecture, stress tolerance, and productivity. In the model plant Arabidopsis, it has been shown that PHYTOCHROME-INTERACTING FACTORS (PIFs; bHLH transcription factors) act as central hubs in the integration of external stimuli to regulate plant development. Recent studies have unveiled the importance of PIFs in crops. They are involved in the modulation of plant architecture and productivity through the regulation of cell division and elongation in response to different environmental cues. These studies show that different PIFs have overlapping but also distinct functions in the regulation of plant growth. Therefore, understanding the molecular mechanisms by which PIFs regulate plant development is crucial to improve crop productivity under both optimal and adverse environmental conditions. In this review, we discuss current knowledge of PIFs acting as integrators of light and other signals in different crops, with particular focus on the role of PIFs in responding to different environmental conditions and how this can be used to improve crop productivity.

Keywords: Cold, drought, grain size, heat, light signaling, phytochrome, PIF, plant architecture, plant breeding, plant yield, salinity.

Introduction

Plants adjust their growth and development to an ever-changing environment. As plants are autotrophic organisms, light is fundamental for photosynthesis but is also an important cue to fine-tune development in response to the environment. The perception of and response to light period, intensity, and quality impact plant fitness. Phytochromes are the red/far-red (R/FR) light photoreceptors, which are activated by R light and inactivated by FR light or dark. Plant phytochromes are particularly important as light sensors in (i) entrainment of the circadian clock to the day–night light fluctuations (recently reviewed by Sanchez *et al.*, 2020); (ii) response to light intensity, particularly important during seedling establishment (Xie *et al.*, 2007; Trupkin *et al.*, 2014); and (iii) response to variation in light quality, often a response to nearby plants causing an

enrichment in FR light (Trupkin *et al.*, 2014). The latter, called shade avoidance syndrome, enables plants to elongate away from competitors to reach sunlight. Light quality also affects plant responses to adverse conditions, such as biotic and abiotic stress (as reviewed by Courbier and Pierik, 2019). Therefore, phytochromes have been used as tools to develop crops with improved agronomic traits (Gururani *et al.*, 2015). However, due to their wide spectrum of action, clear breeding advantages after modulating the expression of either phytochrome A (phyA) or phytochrome B (phyB) have been elusive. For instance, *OsphyB* mutant of rice exhibits increased cold tolerance (He *et al.*, 2016), but a reduced 1000-grain weight (Sun *et al.*, 2020), while overexpression of the constitutively active OsphyB reduces tiller number and seed yield in rice (Hu *et al.*, 2020). The *AtphyB* mutant of Arabidopsis showed a higher survival rate after heat shock (Song *et al.*, 2017); nevertheless, it has a decreased germination rate (Heschel *et al.*, 2007). These studies suggest that a detailed mechanistic understanding of the phytochrome signaling cascade may be key to improve crop yield while overcoming the undesired effects of the phytochrome-modified plants (Hu *et al.*, 2020). Phytochromes have several interacting partners, but the PHYTOCHROME-INTERACTING FACTOR (PIF) family is the most known due to their central role as integrators of light signaling, environmental cues, and internal signals (e.g. hormones) to regulate plant growth and development. The function of PIFs has been widely studied and their role as integrators of light, temperature, and photoperiod was recently reviewed for Arabidopsis (Balcerowicz, 2020; Sanchez *et al.*, 2020). However, new findings in crop plant species have highlighted the potential of PIFs to improve crop resistance and/or yield. In this review, we discuss the role and importance of PIFs as integrators of external stimuli in rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and maize (*Zea mays*) crops, highlighting their potential as biotechnology tools to improve productivity under favorable and adverse environmental conditions.

PIFs in Arabidopsis, rice, tomato, and maize

PIFs belong to the basic helix–loop–helix (bHLH) transcription factor (TF) family. bHLH is one of the largest TF families in plants, comprising 167 members in Arabidopsis, 178 in rice, 152 in tomato, and 208 in maize (Carretero-Paulet *et al.*, 2010; Wang *et al.*, 2015; Zhang *et al.*, 2018). An important feature that distinguishes PIFs from the other bHLHs is the presence of an additional protein domain, the active phytochrome-binding motif.

PIFs' basic helix–loop–helix domain

The PIF protein bHLH structure comprises the basic (b) domain, fundamental for DNA interaction, and the HLH, fundamental for protein interactions namely, homo- and heterodimer formation. Therefore, the bHLH amino acid sequence of each TF allows the separation of bHLH TFs into subfamilies, which dictates their DNA binding sites and protein dimers, and consequently hints to their biological function. In Arabidopsis, rice, and tomato, PIFs belong to the bHLH subfamily VII (a+b) comprising 15, 14, and 11 members, respectively. Members of this show a very similar bHLH domain, but the bHLH proteins can be further divided. The subfamily VIIa members have an additional protein domain, the active phytochrome-binding motif, characteristic of PIFs, while the subfamily VIIb members do not have that domain (Heim *et al.*, 2003). In maize, PIFs are among the 15 bHLHs that compose the subfamily XIV (Pires and Dolan, 2010; Wang *et al.*, 2015; Catarino *et al.*, 2016; Zhang *et al.*, 2018). Phylogenetic analysis of the bHLH domain from these subfamilies from the four species (Fig. 1) resolved the presence of PIF subfamilies previously reported (Catarino *et al.*, 2016).

PIFs' active phytochrome-binding domain

We have searched for the active phytochrome B binding (APB) and active phytochrome A binding (APA) motifs among the bHLHs that belong to the PIF family in Arabidopsis, rice, tomato, and maize. Among the Arabidopsis bHLHs that compose the subfamily VII (a+b), 10 have the APB domain (ELxxxxG) (Fig. 2). The interaction with phyB is proven for eight, which are described as PIFs (PIF1 to PIF8) (Khanna *et al.*, 2004; Oh et al., 2004, 2020; Leivar *et al.*, 2008; Choi *et al.*, 2014; Luo *et al.*, 2014), including the Phytochrome-Interacting Factor3 Like 1 (PIL1) recently renamed PIF2 (Lee and Choi, 2017). The remaining two (At4g28800 and At4g28815), have not yet been tested for phyB interactions, although they contain the APB domain (Fig. 2). Additionally, Arabidopsis has another two bHLHs from the subfamily VII (a+b) that contain a variation of the APB (Fig. 2). At4g28790 has the substitution of a serine for the glycine (ELxxxxS), and it has been confirmed that it does not interact with phyB (Khanna *et al.*, 2004), showing the importance of this glycine for PIF–phyB interaction. At4g28811 has the APB sequence with smaller spacing between the leucine and the glycine (ELxxxG), but it is not known how this affects the interaction with phyB as this was not tested. These observations were also reported by Leivar and Quail (2011), but as far as we know the question remains and the exact number of Arabidopsis bHLHs that interact with AtphyB is unknown. Regarding the APA domain (NFxxFxR), it is present in the AtphyA-interacting PIFs, AtPIF1 and AtPIF3 (Ni *et al.*, 1998; Oh *et al.*, 2004). This is consistent with the assumption that APA and APB motifs are necessary for binding to phyA and phyB, respectively (Fig. 1).

In rice, among the members of the bHLH subfamily VII (a+b), seven contain the APB domain (Figs 1, 2). Six were reported as PHYTOCHROME-INTERACTING FACTOR 3 LIKE (OsPIL11 to OsPIL16), due to their homology to

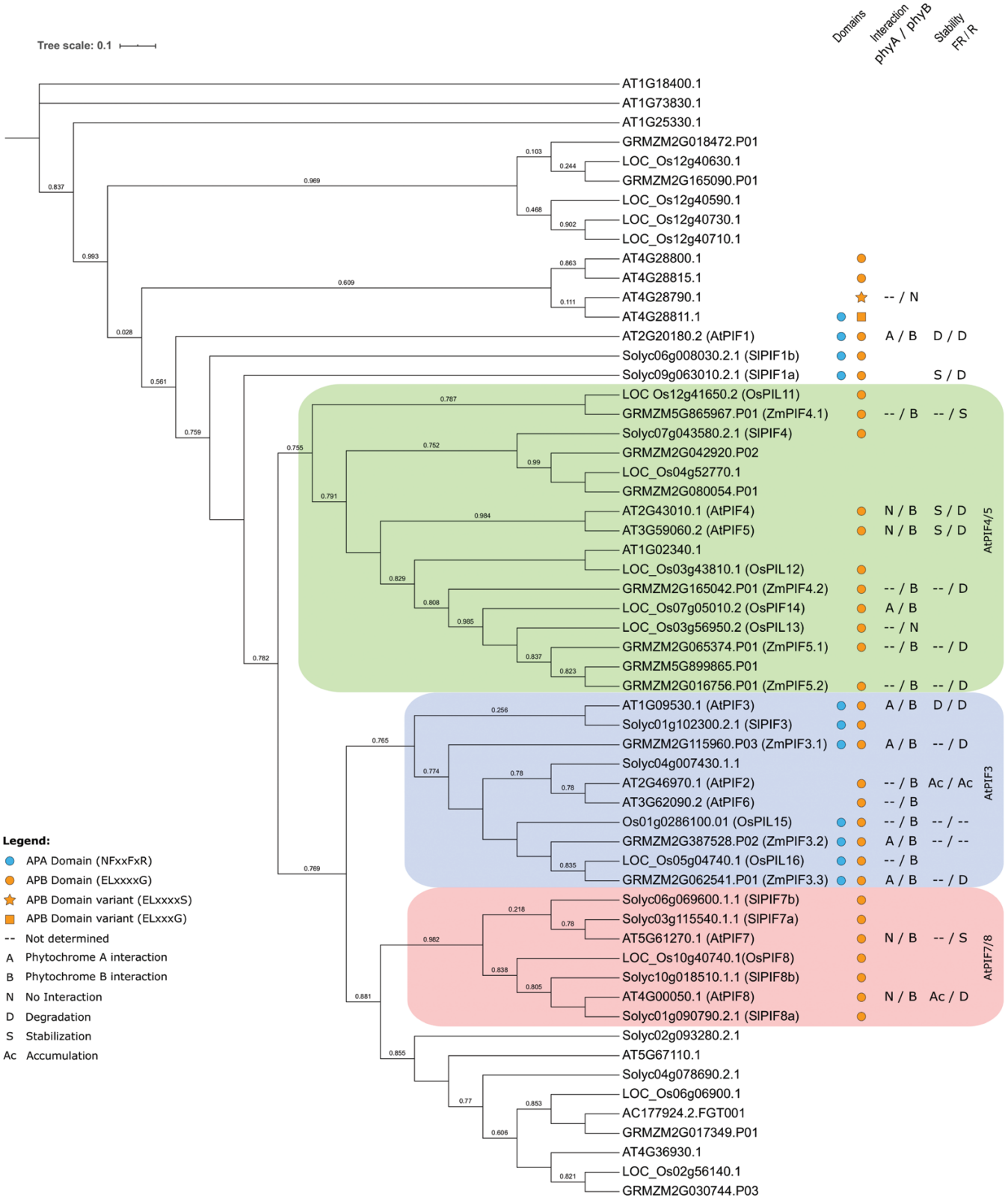

Fig. 1. Phylogenetic analysis of bHLH subfamily that includes Arabidopsis, rice, tomato, and maize PIFs. Protein sequences were aligned using MAFFT (Katoh and Standley, 2013). The bHLH region was manually selected in BioEdit (https://bioedit.software.informer.com/) and used to generate the phylogenetic tree. The maximum likelihood analysis was carried using the program PhyML v3.0 (Guindon and Gascuel, 2003) using the Jones, Taylor,

and Thornton (JTT) amino acid substitution model and an estimated gamma distribution. Branch was tested using the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-like aLRT). The phylogenetic tree was visualized using MEGA X (Kumar *et al.*, 2018). For this phylogenetic analysis, three Arabidopsis bHLH proteins of the subfamily XII (closest to subfamily VII (a+b)) were added as outgroup (Catarino *et al.*, 2016). The AtPIF3 clade is represented in blue, the AtPIF4/5 clade in green, and the AtPIF7/8 clade in pink. Information about the APA and/or APB domain sequence, interaction with phyA and/or phyB, and stability of the protein under far-red (FR) and/or red (R) is represented in three columns after each gene. The APA and APB domains are represented in blue and orange, respectively. Circles represents the canonical domain, and stars or squares represent a variation of the APB domain. The domain sequence is shown in the key at the bottom left. Interaction with phytochromes is represented in the form phyA/phyB. On the left is represented the information regarding the interaction with phyA, while on the right is information regarding the interaction with phyB. A and B represent interaction with phyA or phyB, respectively; N represents no interaction, and '--' represents interaction not determined. The stability of PIFs under light (far-red and red) is indicated as FR/R, where D represents degradation, AC represents accumulation of the protein, S indicates that the protein is stable, while '--' indicates that the effect of light on PIFs' stability is not determined. The information for ZmPIFs and SlPIFs stability was obtained in Arabidopsis and *N. benthamiana*, respectively.

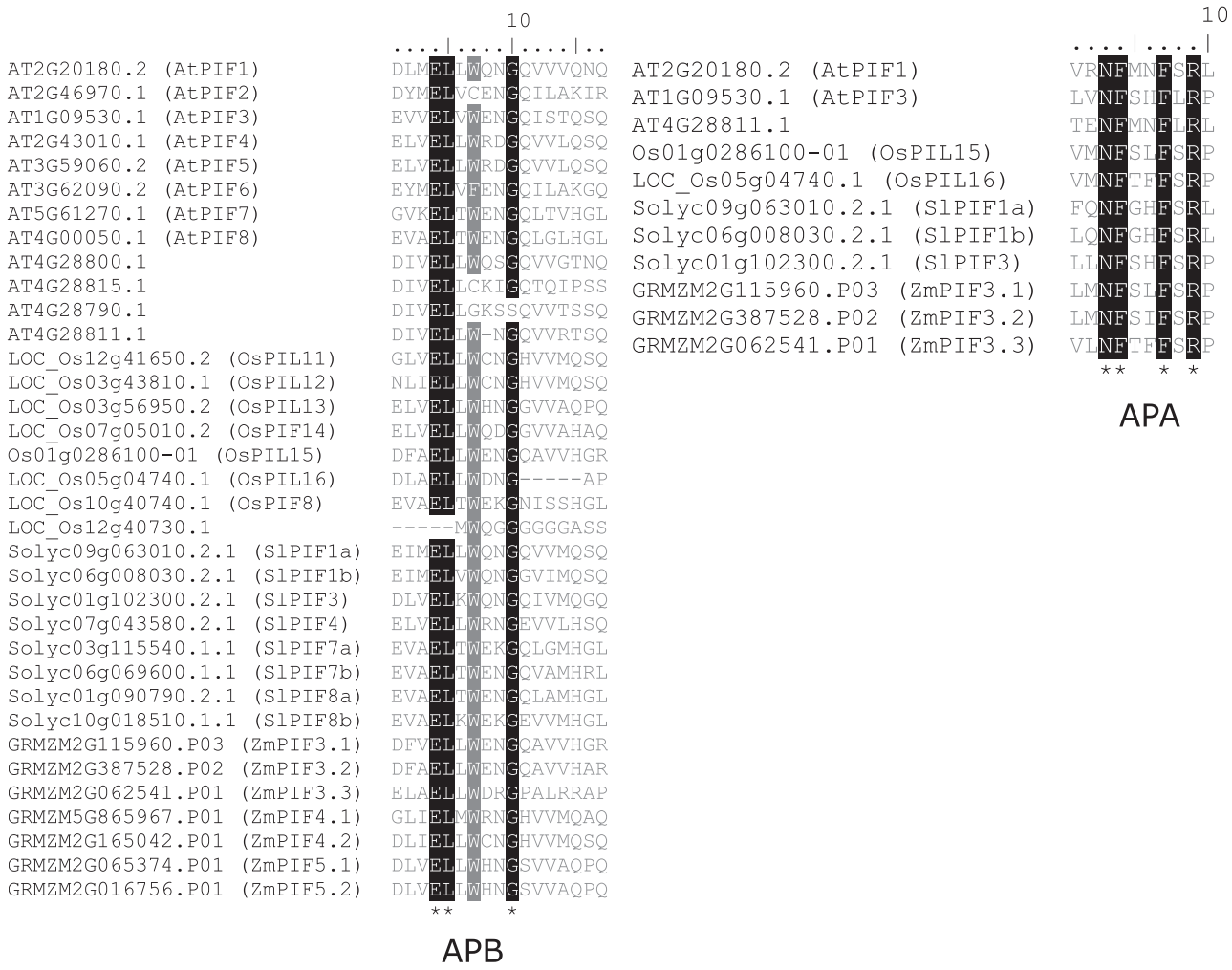

Fig. 2. APB and APA domain of PIF family in Arabidopsis, rice, tomato, and maize. Amino acid sequence alignment of APB (left) and APA (right) domain of PIF family. * represents conserved amino acids. The second most conserved amino acid has an aromatic group (W, tryptophan or F, phenylalanine) and is highlighted in gray.

Arabidopsis PIF3 (Nakamura *et al.*, 2007). The interaction between these OsPILs and OsphyB was confirmed for OsPIL14 (renamed OsPIF14), OsPIL15, and OsPIL16 (Cordeiro *et al.*, 2016; He *et al.*, 2016; Xie *et al.*, 2019), while OsPIL13, also known as OsPIL1, does not interact with OsphyB (Todaka *et al.*, 2012). It was suggested that this lack of binding was due to the absence of a glutamine (Q) after the APB domain (ELxxxxGQ) in OsPIL13 (Todaka *et al.*, 2012), a residue present after the APB domain in all AtPIFs. However, Q is not conserved in the OsPIF14 and OsPIL16 sequence and both interact with OsphyB (Fig. 2). This evidence suggests that the APB sequence-flanking region might be species-dependent,

highlighting the complexity of this interaction. Therefore, the PIF–phytochrome interaction needs to be tested on a case by case basis. The seventh rice PIF was recently reported and named OsPIF8 (Oh *et al.*, 2020). Among rice PILs, only OsPIL15 and OsPIL16 contain the APA domain (Fig. 1), but their interaction with OsphyA has not yet been tested. So far, only OsPIF14 was shown to weakly interact with OsphyA despite lacking the canonical APA domain (Cordeiro *et al.*, 2016).

In tomato, eight bHLHs containing the APB domain were identified and named as PIFs (SlPIF1a, 1b, 3, 4, 7a, 7b, 8a, and 8b) (Wang *et al.*, 2020). Among these, SlPIF1a, SlPIF1b, and SlPIF3 also contain the APA domain, suggesting that they might be regulated by SlphyA, similarly to their homologs AtPIF1 and AtPIF3, respectively (Figs 1, 2). However, the putative interactions between SlPIFs and SlphyA or SlphyB have not yet been tested.

In maize, among the 15 bHLHs identified as members of the subfamily XIV, seven contain the APB domain. These seven bHLHs were reported as PIFs (ZmPIF3.1, 3.2, 3.3, 4.1, 4.2, 5.1, and 5.2) (Zhang *et al.*, 2018) and all interact with both ZmphyB1 and ZmphyB2 in plant cells (Wu *et al.*, 2019). ZmPIF3.1, 3.2, and 3.3, which have the APA domain, were shown to interact with ZmphyA1 in yeast cells (Gao *et al.*, 2019).

Overall, these results suggest that within the four species analysed, the number of PIFs is similar and, based on their bHLH amino acid sequence, three main clades can be pointed to: the clade of AtPIF3 homologs, which have both APA and APB domains, thus interacting with phyA and phyB, and the clades of AtPIF4/5 and AtPIF7/8 homologs that are predominantly regulated by phyB (Fig. 1). Maize does not contain AtPIF7/8 homologs, indicating that the presence of these clades is species specific, which might be important to fine-tune light-dependent plant development.

PIFs as integrators of light cues

High solar radiation favors high-density planting (Yang *et al.*, 2019), which has been used to improve crop productivity. However, when density is too high, the tallest plants filter the light and smaller plants receive a decreased R/FR ratio. This alteration in light quality triggers the shade avoidance response, altering the source–sink balance and forcing plants to invest in elongation growth rather than productivity. Even in greenhouses, where plants grow under more controlled conditions, to avoid the negative effects of temperature changes, the materials used to build greenhouses filter sunlight and decrease crop production as compared with plants grown under direct sunlight (Chen *et al.*, 2019). To overcome this issue, LED lights have been used to supplement sunlight and thus improve crop productivity (Chen *et al.*, 2019). These observations clearly show the importance of the light signaling cascade, and phytochromes have been reported as important regulators of plant growth and productivity (Gururani *et al.*, 2015; Cao *et al.*, 2018a; Wies *et al.*, 2019; Sun *et al.*, 2020; Wies and Maddonni, 2020). However, due to the pleiotropic functions of phytochromes, the modulation of their expression *in planta* can lead to undesired effects. Given that PIFs' function is much more specific (compared with phytochromes), they have a much higher potential to improve crop productivity under optimal or adverse conditions. In this section, we will summarize the importance of light quality and photoperiod for the regulation of PIFs' transcript and protein levels in different plants.

The importance of light quality for PIF stability and activity

PIF stability and activity are dependent on the conformation of the R/FR photoreceptors, phytochromes. Upon activation by light, phytochromes interact with PIFs, through the APA and APB domains, and promote PIF degradation and/or inhibit their DNA interaction. Therefore, dark, FR, and R light, and PIF protein sequence, namely the presence of APA and APB domains, determine PIF stability and activity. The Arabidopsis PIF degradation mechanisms were recently reviewed (Legris *et al.*, 2019), but the molecular mechanisms regulating the degradation of PIFs in rice, tomato, and maize are largely unknown. Here, we summarize the latest findings for PIF stability and activity in these plants.

AtPIF1 and AtPIF3 are the only PIFs from Arabidopsis that contain the APA domain and are degraded after FR light exposure by the 26S proteasome via AtphyA alone (Park *et al.*, 2004; Oh *et al.*, 2006). All the other AtPIFs are stable under FR, except AtPIF8 (Fig. 1), which accumulates under FR due to the inhibition of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) by AtphyA (Oh *et al.*, 2020). These results indicate that phyA modulates PIF stability both directly and indirectly to regulate FR-mediated development in Arabidopsis. Information about the effect of FR on rice, tomato, and maize PIF stability is scarce. Two PIFs containing the APA domain, OsPIL15 and SlPIF1a (Figs 1, 2) have been reported to mediate the FR response. Rice seedlings overexpressing OsPIL15 show short above-ground development, a phenotype that reverts after FR exposure, suggesting that similarly to AtPIF1 and AtPIF3, OsPIL15 is degraded via OsphyA (Zhou *et al.*, 2014). To test this hypothesis it will be fundamental to analyse the interaction with OsphyA as well as OsPIL15 FR stability. The study of SlPIF1a stability in tobacco cells suggests that SlPIF1a is stable under FR (Llorente *et al.*, 2016), though these results must be confirmed in tomato cells.

R induces quick degradation of most AtPIFs via phytochromes and the 26S proteasome. There are, however, two exceptions, AtPIF2 and AtPIF7, which under R accumulate or maintain protein levels, respectively (Park *et al.*, 2004; Khanna *et al.*, 2004; Oh *et al.*, 2006, 2020; Al-Sady *et al.*, 2006;

Leivar *et al.*, 2008; Choi *et al.*, 2014; Luo *et al.*, 2014). Contrary to other AtPIFs, AtPIF2 APB domain does not contain an aromatic amino acid (Fig. 2, highlighted in gray). It would be interesting to understand if that change could regulate the stability of AtPIF2. Phytochromes regulate PIF activity by two complementary mechanisms, phosphorylation-mediated degradation (Al-Sady *et al.*, 2006; Shen *et al.*, 2007; Park *et al.*, 2018) and sequestration (Huq and Quail, 2002; Park *et al.*, 2018). As for the latter, phytochromes inhibit the interaction of PIFs with DNA and this is suggested to be a fast mechanism to regulate PIF activity under flickering light (Park *et al.*, 2018). In rice, very little is known about PIF regulation by R or white light. It was reported that R reverts the phenotype of etiolated *OsPIL15*-Ox seedlings (Zhou *et al.*, 2014). Since OsPIL15 has both APA and APB domains and is degraded under white light by OsphyB (Xie *et al.*, 2019), OsPIL15 R-induced degradation is the most likely scenario, but sequestration of OsPIL15 cannot be excluded. The stability of tomato and maize PIFs was analysed in *Nicotiana benthamiana* and Arabidopsis, respectively. Under R, SlPIF1a (Llorente *et al.*, 2016), ZmPIF3.1, ZmPIF3.3, ZmPIF4.2, ZmPIF5.1, and ZmPIF5.2 are degraded, while ZmPIF4.1 seems to be stable (Wu *et al.*, 2019). However, to validate the results, these functional studies must be performed within the respective species.

Diurnal regulation of PIFs

Diurnal gene expression is key for plant development, allowing plants to be prepared for the constant changes in the environment through the day, such as the next light or dark period. Different light spectra, associated with different times of day, such as early morning or late afternoon, can result in the activation of different circadian components since it was shown that different wavelengths modify phytochrome and cryptochrome light-sensor activity, influencing the length of the diurnal expression period of genes controlled by the circadian clock (Millar *et al.*, 1995; Somers *et al.*, 1998). Therefore, the direct regulation of PIFs by phytochromes places them in a pivotal position to integrate light signals (phototransduction) in the regulation of diurnal cycles. It was suggested that a ternary complex comprising AtPIF3, AtphyA, and AtphyB can bind *in vitro* to G-box motifs in the promoter of *CIRCADIAN CLOCK ASSOCIATED* (*AtCCA1*) and *LATE ELONGATED HYPOCOTYL* (*AtLHY*), important morning-expressed circadian clock components (Martínez-García *et al.*, 2000). Despite the importance of AtPIF3 in the regulation of circadian genes being later questioned when it was observed that *Atpif3* mutant plants maintain a robust expression phase and period of *CCA1* and *LHY* (Viczián *et al.*, 2005), studying the quadruple mutant *Atpif1pif3pif4pif5* demonstrated that AtPIFs bind to the promoter of morning circadian genes, regulating their expression (Shor *et al.*, 2017). Furthermore, this study found that AtPIFs are important to communicate sucrose signals to the oscillator, suggesting a PIF-dependent entrainment of the circadian clock by metabolic signals. Conversely, photoperiod and light intensity also contribute to the regulation of expression of *PIFs* (Flis *et al.*, 2016; Moraes *et al.*, 2019). It was shown that the expression of *AtPIF4* and *AtPIF5* is regulated by core circadian gene products, such as TIMING OF CAB EXPRESSION 1 (AtTOC1) (Yamashino *et al.*, 2003), PSEUDO-RESPONSE REGULATOR 5 (AtPRR5), and AtPRR7 (Nakamichi *et al.*, 2012; Liu *et al.*, 2013), and the evening complex (Nusinow *et al.*, 2011), all components of the internal oscillator active at dusk. Such fine regulation is thought to constrain the activity of PIFs in inducing plant growth to later in the night, where cell elongation is metabolically more favorable. Moreover, clock components such as AtTOC1 interact with both AtPIF3 and AtPIF4 to control temperature-mediated cell elongation at the end of the night (Soy *et al.*, 2016; Zhu *et al.*, 2016). In addition to transcriptional regulation, day length regulates the accumulation of PIF proteins as a mechanism of seasonal adaptation (Lee and Thomashow, 2012).

Transcriptional regulation of *PIFs* in other species is still highly elusive, yet the diurnal expression of *PIFs* in rice, tomato, and maize has been reported. Nakamura *et al.* (2007) studied the expression of some rice *PIFs* and found that in short-day conditions *OsPIL13*, *OsPIL15*, and *OsPIF14* expression is diurnally regulated, peaking at the beginning of the day, end of the day, and beginning of the night, respectively. Moreover, these authors showed that only *OsPIL13* is circadian-regulated by observing sustained rhythmic expression in a free-running system. We further analysed expression of *OsPIFs* by using an in-house-generated RNA-seq database of rice seedlings grown in a controlled environment under short-day (10 h light–14 h dark) and long-day (14 h light–10 h dark) photoperiods (Fig. 3A). We obtained similar results to the previously reported *OsPIL13* and *OsPIF14* mRNA expression levels (Nakamura *et al.*, 2007) and observed that *OsPIL11*, *OsPIL12*, and *OsPIF8* show a similar regulation to *OsPIL13*. On the other hand, *OsPIL15* and *OsPIL16* showed two expression peaks, one during the day and the other at light–dark transition in long days. While the expression of *OsPIL15* is similar in long and short days, *OsPIL16* shows only one expression peak, during light in short days. In both photoperiods, *OsPIF14* expression increased in the first hours of dark and was downregulated during light. This observation is in agreement with a previous report (Nakamura *et al.*, 2007). Altogether, these results indicate that *OsPIL11*, *OsPIL12*, and *OsPIL15* expression is not influenced by the day-length while *OsPIL13*, *OsPIL16*, *OsPIF8*, and *OsPIF14* expression seems to be dependent on the start of the night, where the first three are repressed and the last is induced. Since *OsPIF14* is the only rice *PIF* induced at dusk under both photoperiodic conditions, and given its preferable interaction with the active form of OsphyB (Cordeiro *et al.*, 2016), it is likely to play an important role under dark conditions. It has been shown that OsPIF14 strongly binds to G-box promoter sequences *in vitro* (Cordeiro *et al.*, 2016). Therefore, it would be interesting to determine whether OsPIF14 interacts

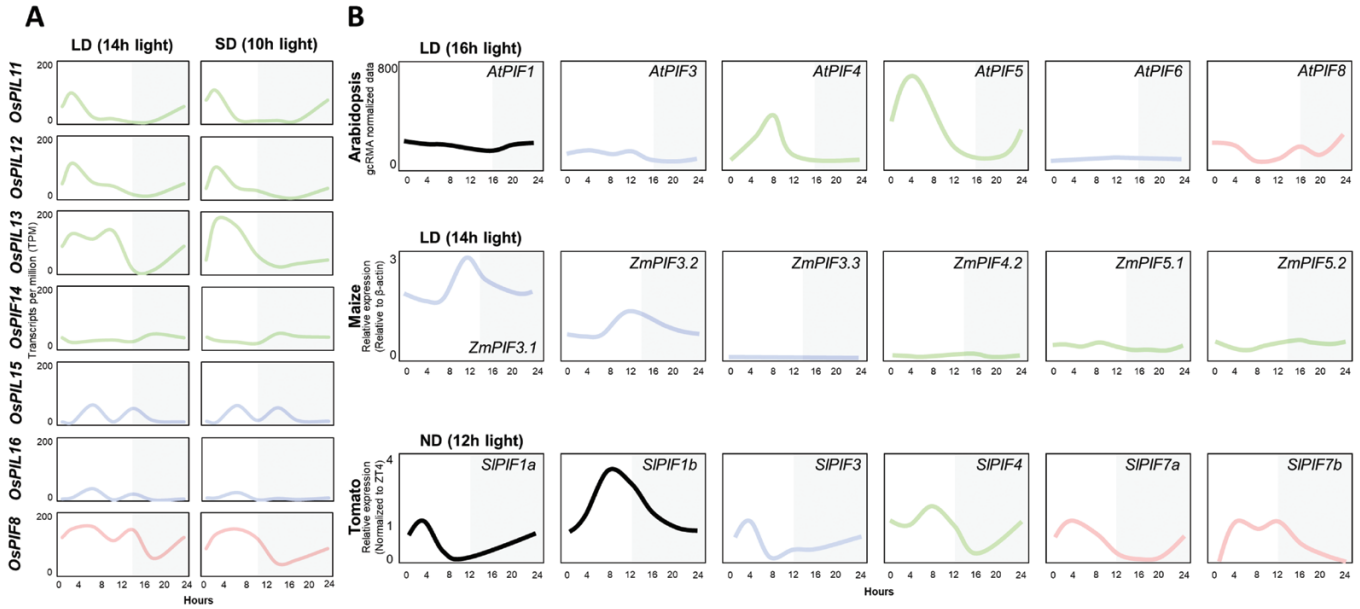

Fig. 3. Diurnal expression of *PIF* genes in different plants. (A) Expression of rice *OsPILs* in long days (LD; 14 h light–10 h dark) and short days (SD; 10 h light–14 h dark). Twenty one day old rice seedlings cv. Nipponbare grown in ½ Murashige and Skoog solid medium at constant 28 °C and 300 µmol photons m^{-2} s^{-1}. Pools of four to five plants were used for each time point and the experiment was repeated to obtain three biological replicates. Gene expression levels, in transcripts per million (TPM), were obtained by RNA-seq; data from Andrade *et al.* (2022). (B) Expression level of *PIFs* from Arabidopsis, maize, and tomato. Arabidopsis *PIF* expression (microarray data normalized using gcRMA) was obtained from DIURNAL database (http://diurnal.mocklerlab.org) by matching PIF IDs with 'longday' sub-database (16 h of light; Michael *et al.*, 2008). Maize *PIF* expression (RT-qPCR relative expression normalized to β-*actin*) was obtained from Gao *et al.* (2019) and since it is not directly specified, the photoperiod is an approximation inferred according to the length of the day during the summer in Nanton, Jiangsu, China (where 05.00 h corresponds to ZT0 and ~14 h of light was considered). Tomato *SlPIF* expression (RT-qPCR relative expression normalized to the mean of *TIP41* and *EXPRESSED*) in a neutral day (ND; 12 h light–12 h dark) was obtained from Rosado *et al.* (2016). Relative RNA levels are represented for all species in a way that allows comparison of gene expression between *PIF*s from the same species, except for tomato, which was plotted as normalized to ZT4 (Rosado *et al.*, 2016). Thus, the transcript levels among the different *SlPIFs* cannot be compared. For further information regarding the expression of *SlPIF* genes at ZT4 see Rosado *et al.* (2016). The color of the lines representing the transcript level is according to the clades from Fig. 1: black represents *AtPIF1* clade, blue *AtPIF3* clade, green *AtPIF4/5* clade, and pink *AtPIF7/8* clade. The gray shading represents the dark period of the 24 h cycle.

with the G-box promoter sequences that are present in the rice single ortholog to *AtCCA1* and *AtLHY* and to investigate if this interaction is circadian or metabolically regulated. Moreover, *in vitro* assays demonstrated that OsPIL11, OsPIL12, and OsPIL13 interact with OsTOC1 (Nakamura *et al.*, 2007) and that both OsPIL13 and OsPIL15 co-localize with OsTOC1 *in vivo* (Zhao *et al.*, 2011). These interactions between PIFs and circadian clock elements hint at a potential regulatory mechanism for plants responding to alterations in the photoperiod. A mechanism by which light-responsive PIF proteins modulate the expression of clock genes and interact with clock proteins to fine-tune the circadian clock to light conditions is advantageous in keeping a precise and robust internal clock, and responding to photoperiodic changes. Nonetheless, more research is required to elucidate the direct regulation of circadian clock gene expression by OsPIFs.

In tomato, it has been shown that transcripts of most *SlPIFs* are induced during the night period and peak after dawn, whereas *SlPIF1b* and *SlPIF7b* show a down-regulation during the night and a sharp expression increase in the first hours of light (Fig. 3B). *SlPIF1a*, *SlPIF3*, *SlPIF7a*, and *SlPIF7b* peak at ZT4, while *SlPIF1b* and *SlPIF4* peak at ZT8 in a 12/12 h photoperiod (Rosado *et al.*, 2016). *SlPIF8a* and *SlPIF8b* were not analysed since the authors did not consider them to be members of the canonical PIF family. It has been reported that SlphyA and SlphyB are negative regulators of *SlPIF* transcripts. RNAi lines for *SlphyA* or *SlphyB* at different fruit development stages showed induced expression of *SlPIF1a*, *SlPIF1b*, and *SlPIF4*, whereas *SlPIF3* was down-regulated (Ernesto Bianchetti *et al.*, 2018). This observation explains the induction of *SlPIF1a* and *SlPIF3* during the night. However, it is still to be determined how the opposite regulation of *SlPIF1a* and *SlPIF3* by Slphys results in a similar diurnal expression pattern of expression and why *SlPIF1b* accumulates sharply during the day. A possible explanation is that tomato fruit tissue shows a different regulation of *SlPIFs* by Slphys to induce ripening, and therefore new studies must be conducted using *Slphy* mutant lines to confirm their influence on the expression of *SlPIFs*. Furthermore, other regulators are likely to be unveiled, and hence it would be interesting to identify transcriptional activators present at dawn that may counteract the inhibition effect of phytochromes.

Currently, only a single report has been published demonstrating the diurnal expression of ZmPIFs (Gao *et al.*, 2019). In this study, field-grown maize plants showed that *ZmPIF3.1* and *ZmPIF3.2* are the most expressed and are diurnally regulated, with a peak in mRNA accumulation at dusk, although the precise length of the day is not discussed since plants were field grown. The diurnal regulation for all the other *ZmPIFs* (*ZmPIF3.3*, *ZmPIF4.2*, *ZmPIF5.1*, and *ZmPIF5.2*) was more difficult to identify, yet the authors suggest that the first three are more expressed during the day and ZmPIF5.2 shows a peak of expression at dusk. However, the diurnal expression of *ZmPIFs* must be validated under controlled conditions.

PIFs from the plant species discussed here show higher expression during the day as compared with the dark period, except for *OsPIF14*, which shows higher expression after dusk. It is also worth noting that the gene expression pattern of most *PIFs* from the four species is under diurnal rhythm regulation. However, *PIF* transcriptional regulation differs within and among species (Fig. 3). It would be interesting to investigate if the divergence in *PIFs* diurnal regulation can be compensated by a robust post-translational regulation. This is observed for AtPIF3, which despite not showing a strong diurnal gene expression pattern is rapidly degraded by light and therefore is only active at night (Park *et al.*, 2004). Further research is needed to elucidate not only diurnal gene expression patterns in different conditions but also PIF protein levels and activity during diurnal cycles. Continuous research in the diurnal regulation of *PIFs* in crop species is of particular importance to start pinpointing their influence in regulating light-dependent growth and development, such as daily and seasonal growth and the activation of flowering.

PIFs as integrators of multiple abiotic stimuli

Food security is currently threatened by the rapid change in the world's climate, where episodes of extreme temperatures, water shortages, and salinization of the soil are becoming more frequent. Therefore, it is of paramount importance to attain a better understanding of the molecular mechanisms used by different crops to cope with such adverse environmental conditions. Phytochromes and PIFs are emerging as important modulators of abiotic responses in crops. In this section, we summarize the role of PIFs in the regulation of abiotic responses and discuss their potential to improve crop production under adverse environmental conditions.

Temperature responses

Plant responses to temperature and light are closely related. Since light and temperature vary through the day in an associated manner, plants have evolved sensors that accumulate both functions (thermo- and photo-sensors). This dual function has been attributed to phytochromes, namely phyB (Jung *et al.*, 2016; Legris *et al.*, 2016), which regulates cold and heat responses in Arabidopsis (Song *et al.*, 2017; Jiang *et al.*, 2020) and cold in rice (He *et al.*, 2016). The PIF-mediated crosstalk between light and temperature has been widely studied in Arabidopsis (topics reviewed by Wigge, 2013; Leivar and Monte, 2014; Paik *et al.*, 2017; Balcerowicz, 2020).

The C-REPEAT-BINDING FACTOR/DEHYDRATION-RESPONSIVE ELEMENT-BINDING 1 (CBF/DREB1) transcription factors are key regulators of low temperature response in plants (Ito *et al.*, 2006). PIFs have been described as regulators of cold tolerance through regulation of *CBF/DREB1* genes, a mechanism known to be conserved at least in Arabidopsis, rice, and tomato (Fig. 4). In Arabidopsis, PIF3, PIF4, and PIF7 repress *CBF/DREB1* genes (Kidokoro *et al.*, 2009; Lee and Thomashow, 2012; Jiang *et al.*, 2017) and regulate cold response by different mechanisms. AtPIF4 and AtPIF7 are associated with seasonal temperature variations. During the cold season, AtPIF4 and AtPIF7 stability decreases, which releases the repression of *CBF/DREB1* to promote cold tolerance (Kidokoro *et al.*, 2009; Lee and Thomashow, 2012). More recently, it was demonstrated that not only the transcriptional regulation of CBF/DREB1s by AtPIFs is important, but the interaction of both is an important mechanism to control PIF stability and fine-tune freezing tolerance (Jiang *et al.*, 2020; Xu and Deng, 2020). In brief, cold promotes the interaction of CBF1 with AtPIF3, preventing the light-dependent co-degradation of AtPIF3 and AtphyB, leading to the stabilization of AtPIF3 and AtphyB under cold. In that way, AtphyB induces the degradation of three CBF1 non-binding PIFs, AtPIF1, AtPIF4, and AtPIF5, known as negative regulators of cold tolerance (Jiang *et al.*, 2020). The upregulation of *AtPIF1* and *AtPIF4* by low temperatures (Jeong and Choi, 2013) suggests a mechanism to balance growth and cold tolerance (Fig. 4).

In rice, OsPIF14 has been shown to be the linker between light and cold responses. OsPIF14 interacts with OsphyB and represses the expression of *OsDREB1B*, while cold induces the alternative splicing of *OsPIF14* (Cordeiro *et al.*, 2016). The alternative splice form, *OsPIF14β*, which is estimated to be non-functional, accumulates over time and its level is inversely proportional to temperature (Cordeiro *et al.*, 2016). This seems to be a mechanism to release *OsDREB1B* from repression under cold, but the role of OsPIF14 in cold tolerance *in planta* is still elusive. In rice, other *OsPIFs* are regulated by low temperatures. *OsPIL13* is repressed by cold (Jeong and Choi, 2013), while *OsPIL15* and *OsPIL16* are induced, but only the overexpression of OsPIL16 leads to cold tolerance via up-regulation of *OsDREB1* gene expression (He *et al.*, 2016). Moreover, *OsphyB* mutant also shows increased cold tolerance, associated with the constitutive up-regulation of *OsPIL16*, thus highlighting the importance of OsPIL16 as an integrator of light and cold signaling (He *et al.*, 2016) (Fig. 4).

In maize, cold down-regulates *ZmPIF3.1* and up-regulates *ZmPIF5.1* gene expression after 24 h of treatment. The

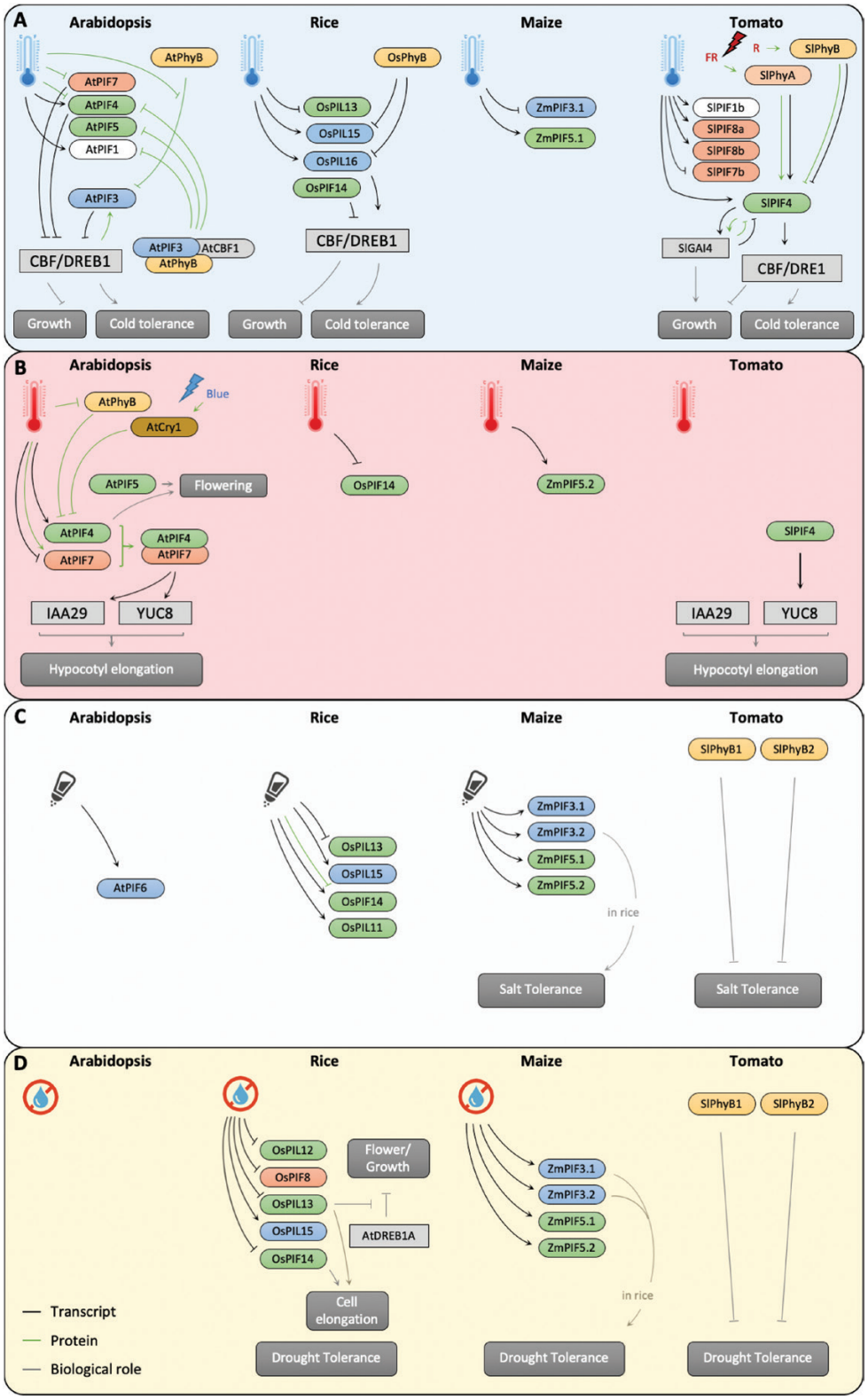

Fig. 4. The pivotal role of PIFs at the convergence of multiple pathways. Illustration of PIFs from Arabidopsis, rice, maize, and tomato involved in plant response to cold (A), heat (B), salinity (C), and drought (D). The illustration displays PIFs' upstream regulators and downstream targets. Line color represents the regulation level, namely at the transcript level (black), protein level (green), or the biological role (gray). The regulation can be positive/activation (pointed arrow) or negative/repression (blunt arrow). For each abiotic stress key genes (black letters, gray background) and key responses (white letters, gray background) are represented. Each PIF is represented with a color code that represents the associated clade from Fig. 1.

expression of other *ZmPIFs* seems not to be regulated by cold (Gao *et al.*, 2019). However, their stability, mechanisms of action, and role of ZmPIFs in maize response to cold are still to be elucidated.

In tomato, it is known that FR enhances cold tolerance via SlphyA (Wang *et al.*, 2016). Therefore, supplementation with FR during plant growth is being used to improve cold tolerance of tomato fruits during transportation at low temperatures. Among *SlPIF*s, *SlPIF4* is the most induced by cold, and FR enhances its cold-induced gene expression and protein accumulation via SlphyA (Wang *et al.*, 2020). Concomitantly, *SlPIF4*-Ox plants show increased cold tolerance, while *Slpif4*-KO mutant shows higher sensitivity (Wang *et al.*, 2020). On the other hand, R impairs *SlPIF4* mediated cold induction via SlphyB (Wang *et al.*, 2020). Thus, in opposition to AtphyB and OsphyB, SlphyB negatively regulates cold tolerance (Wang *et al.*, 2016). These results show the antagonistic effect of FR and R in the regulation of SlPIF4 expression and protein stability and suggest a different role of phyB in the species described. A molecular mechanism was proposed in which SlPIF4 regulates the CBF/DREB1 and the gibberellin (GA) pathway to confer cold tolerance (Fig. 4A). Under cold, a low R/FR ratio induces *SlPIF4* expression and SlPIF4 accumulation, which in turn induces *SlCBF1*, *2*, and *3*, and *GA-INSENSITIVE 4* (*SlGAI4*). The synthesis of SlGAI4 represses *SlPIF4* expression and protein accumulation by a negative-feedback loop to keep the balance between growth and cold tolerance (Wang *et al.*, 2020). In Arabidopsis and rice, PIFs are regulated by GA by interacting with DELLA proteins (Li *et al.*, 2016; Mo *et al.*, 2020), although the importance of such regulation in cold tolerance has not been elucidated in either species. In addition to *SlPIF4*, also *SlPIF1b*, *SlPIF8a*, and *SlPIF8b* are induced by low temperature, while *SlPIF7* is repressed (Wang *et al.*, 2020). However, further studies must be conducted to unveil if these PIFs play a role in tomato cold tolerance. Figure 4A shows that CBF–PIF interaction represents a feedback-loop mechanism used by different plants to fine-tune their response to cold.

In Arabidopsis, it has been shown that phytochromes and cryptochromes are key players in the light regulation of plant development at higher temperatures and their pathways converge on the regulation of AtPIF4 (Ma *et al.*, 2016; Song *et al.*, 2017; Qiu *et al.*, 2019). In brief, the inactivation of phyB by high temperature leads to the accumulation of AtPIF4, thus promoting cell elongation and flowering (Thines *et al.*, 2014; Qiu *et al.*, 2019). Nevertheless, AtPIF4 transcriptional activity is repressed by its interaction with cryptochrome 1, in a blue light-dependent manner, leading to inhibition of the high temperature-mediated hypocotyl elongation (Ma *et al.*, 2016). Although AtPIF4 seems to be a central hub regulating thermomorphogenesis, other PIFs, such as AtPIF1, AtPIF5, and AtPIF7, also play important roles (Thines *et al.*, 2014; Qiu *et al.*, 2019; Chung *et al.*, 2020). AtPIF4 and AtPIF5 induce flowering (Thines *et al.*, 2014), while AtPIF4 and AtPIF7 regulate hypocotyl elongation (Qiu *et al.*, 2019). *AtPIF7* transcript level is down-regulated by higher temperatures (Fiorucci *et al.*, 2020), but the abundance of the encoded protein increases (Chung *et al.*, 2020), thus promoting the formation of heterodimers with AtPIF4, a warm-temperature-induced PIF (Koini *et al.*, 2009; Stavang *et al.*, 2009; Sun *et al.*, 2012; Fiorucci *et al.*, 2020). This heterodimer induces hypocotyl elongation through direct activation of the auxin biosynthetic and responsive genes, such as *YUCCA8*/*YUC8*, *SAUR*, and *IAA29* (Franklin *et al.*, 2011; Sun *et al.*, 2012; Chung *et al.*, 2020; Fiorucci *et al.*, 2020) (Fig. 4B). The work in Arabidopsis shows the importance of PIFs for thermomorphogenesis and suggests that crop PIFs might be key to improving tolerance to higher temperatures. In fact, SlPIF4 promotes thermomorphogenesis in tomato by inducing *SlYUC8A* and *SlYUC8* at high temperature, showing that activation of auxin biosynthesis pathway might be a conserved mechanism through which PIFs modulate temperature-dependent development in plants (Hayes, 2019; Rosado *et al.*, 2019). SlPIF4 has been suggested to be a major integrator of light and temperature in tomato, regulating responses to both low and high temperature (Fig. 4A, B). In other crops, PIFs are also regulated by high temperature, but their role in high-temperature responses is not studied yet. For instance, *ZmPIF5.2* is induced (Gao *et al.*, 2019), while *OsPIF14* is repressed at higher temperatures (Jeong and Choi, 2013). All the other *ZmPIFs* and *OsPIFs* are not significantly modulated by high temperature at the transcriptional level (Todaka *et al.*, 2012; Jeong and Choi, 2013; Gao *et al.*, 2019). It would be interesting to understand if PIF protein stability is regulated by higher temperatures, as happens for AtPIF7. Altogether, we postulate that PIFs have a high potential to improve crop responses to adverse temperatures despite our still limited understanding of how PIFs modulate temperature responses in crops.

Drought and high salinity

Phytochromes and PIFs mediate plant responses to drought and salinity. However, the molecular mechanisms underlying these responses are still poorly understood. In tomato, *SlphyB1* and *SlphyB2* mutants show increased growth during drought and salt stress, suggesting that SlphyB1 and SlphyB2 act as negative regulators of drought and salt tolerance (Gavassi *et al.*, 2017). In agreement, tomato plants subjected to low R:FR ratio showed improved salt tolerance, mediated by SlphyB1 (Cao *et al.*, 2018b). In rice, OsPIL13 and OsPIF14 act as positive growth inducers under drought and salt stress, respectively. Under drought, *OsPIL13* is down-regulated and *OsPIL13-OX* rice lines show increased internode elongation associated with higher transcript level of cell wall organization and cell elongation genes (Todaka *et al.*, 2012; Jeong and Choi, 2013; Wei and Chen, 2018). Under high salinity, *OsPIF14* is up-regulated but the encoded protein is degraded (Mo *et al.*, 2020). This is consistent with the observed growth arrest under stress as well as with the observation that *OsPIF14-OX* rice lines show

enhanced shoot and root growth, through direct regulation of cell elongation-related genes (Mo *et al.*, 2020).

The importance of PIFs regulating plant drought and salt tolerance has also been studied in heterologous systems. When *OsPIL13* is overexpressed in Arabidopsis together with *AtDREB1A*, it recovers the dwarfism and late flowering observed in *AtDREB1A-OX* plants, maintaining the survival rate under drought (Kudo *et al.*, 2017). Rice overexpressing *ZmPIF3.1* and *ZmPIF3.2* shows improved drought tolerance, by inducing stomatal closure, while rice overexpressing *ZmPIF3.2* shows improved salt stress tolerance (Gao *et al.*, 2015, 2018a, b).

In addition to the crop PIFs referred to above, others shown to be regulated at the transcriptional level by drought and salinity, can play important roles modulating the responses to these stresses. For instance, drought up-regulates *OsPIL15*, *ZmPIF3.1*, *ZmPIF3.2*, *ZmPIF5.1*, and *ZmPIF5.2* and down-regulates *OsPIF8*, *OsPIL12*, and *OsPIF14* (Jeong and Choi, 2013; Cordeiro *et al.*, 2016; Wei and Chen, 2018; Gao *et al.*, 2019). High salinity up-regulates *AtPIF6*, *OsPIL11*, *OsPIL15*, *ZmPIF3.1*, *ZmPIF3.2*, *ZmPIF5.1*, and *ZmPIF5.2* but down-regulates *OsPIL13* (Jeong and Choi, 2013; Gao *et al.*, 2019).

Several links have already been found between PIFs and drought and salt stress response (Fig. 4C, D). However, a number of PIFs need to be tested and functionally validated in the corresponding species. For instance, it would be interesting to investigate (i) if the four ZmPIFs referred to above modulate drought and salt responses in maize; (ii) which and how SlPIFs mediate the increased drought and salt tolerance of *SlphyB1* and *SlphyB2* mutants; and (iii) the function of the different PIFs regulated by drought and salinity mediating the response to these stresses. The knowledge raised from the functional characterization of rice, maize, and tomato PIFs, among others, will contribute to designing better and more efficient strategies for crop improvement, ultimately helping breeding programs to produce varieties more suitable for climate changes.

The potential of PIFs as a biotechnological tool to improve grain size/plant productivity

Grain size and number are two major components of crop seed yield, and are dependent on the number and size of inflorescences and tillers. Therefore, an effort has been made to characterize the molecular mechanisms underlying these very important traits. Grain size and number are complex traits regulated by many players including, among others, phytohormones and transcription factors, as recently reviewed by Fan and Li (2019). Although the review by Fan and Li focuses on rice, it only very briefly describes the role of OsPIFs in the regulation of grain size and number. In this section, we will highlight the importance of light and phytochrome pathways, focusing on the role of PIFs on grain/fruit size and productivity in economically important crops such as rice, tomato, and maize.

PIFs are important regulators of plant growth and architecture

The role of Arabidopsis PIFs as regulators of plant growth is well documented (Leivar and Monte, 2014; Chaiwanon *et al.*, 2016). In brief, AtPIF1, 3, 4, 5, 7, and 8 promote (Kim *et al.*, 2003; Fujimori *et al.*, 2004; Oh et al., 2004, 2020; Khanna *et al.*, 2007; Leivar *et al.*, 2008; Franklin *et al.*, 2011), while AtPIF2 and 6 inhibit hypocotyl elongation (Penfield *et al.*, 2010; Luo *et al.*, 2014). AtPIFs regulate cell elongation by controlling the expression of genes involved in hormone biosynthesis/metabolism (ABA, auxins, ethylene, jasmonic acid, and cytokinins) and signaling (auxin, brassinosteroids, gibberellins). In crops such as rice, tomato, and maize, PIFs also regulate vegetative growth. OsPIL13 was shown to promote internode elongation and plant height by regulation of cell wall-related genes involved in cell elongation such as expansins (Todaka *et al.*, 2012), while OsPIF14 acts as an inducer of root growth mediated by gibberellins (Mo *et al.*, 2020). When overexpressed in the ZH11 rice cultivar all rice PIFs induced mesocotyl elongation under dark (Mo *et al.*, 2020). However, when overexpressed in the Nipponbare rice cultivar, etiolated *OsPIL15*-Ox seedlings showed inhibition of aboveground development (Zhou *et al.*, 2014), suggesting that OsPIL15 function might be dependent on cultivar. Under field conditions, *OsPIL15* CRISPR, RNAi, and Ox (*OsPIL15* under a strong endosperm-specific promoter, *Gt13a*) plants showed the same tiller number, while the CRISPR line showed decreased height compared with wild-type (WT) (Ji *et al.*, 2019). In parallel, it was reported that under long days at 25 °C, a different *OsPIL15*-Ox line (under the CaMV 35s promoter) showed lower tiller number and tiller angle (Xie *et al.*, 2019). The differences observed in tiller number might be related to the different promoters used, but the effect of photoperiod and/or temperature cannot be discarded since *OsPIL15* is regulated by both stimuli. In addition, OsPIL16 seems to be a negative regulator of plant height (He *et al.*, 2016).

In dark-grown maize seedlings, the triple (*Zmpif3.1Zmpif3.2Zmpif3.3*), double (*Zmpif4.1Zmpif4.2*), and single (*Zmpif5.1*) knockout mutants showed a shorter mesocotyl, clearly showing the function of ZmPIFs in maize development. In addition, *ZmPIF4.1*-Ox in Arabidopsis displayed a strong constitutive shade phenotype, including long petioles, reduced leaf number, and early flowering (Wu *et al.*, 2019). Moreover, when *ZmPIF3.1* and *ZmPIF3.2* were overexpressed in rice, both transgenic lines showed an increased tiller and panicle number, under control conditions (Gao *et al.*, 2018a, b). The same lines showed a significantly wider tiller angle compared with WT, which is the same phenotype as *OsPIL15* fused with a repressor domain (*OsPIL15*-RD) (Xie *et al.*, 2019). These observations suggest that ZmPIF3.1 and ZmPIF3.2 act

antagonistically to OsPIL15 in the regulation of tiller angle. However, the role of ZmPIF3.1 and ZmPIF3.2 must be studied in maize.

In tomato, *SlPIF4-RNAi* plants were smaller than WT at the seedling stage, and differences were accentuated during the life cycle, most likely caused by the reduction of auxin levels. *SlPIF4* silencing caused 15% reduction in vegetative weight and 23% reduction in fruit weight, accounting for a total reduction of 21% in plant aerial mass (Rosado *et al.*, 2019). Altogether, in the analysed species, PIFs have a key and conserved role in the regulation of plant growth and architecture by integrating internal and external stimuli.

PIFs play a role in determining grain size and productivity

Cell elongation, division, and differentiation are key for grain size and number. In rice, lemma and palea size plays a determinant role in grain size by limiting the storage capacity of grain and consequent grain growth. OsPIL11, OsPIL13, OsPIL15, and OsPIL16 control grain size by regulating cell expansion and/or cell division, but the information about the molecular mechanisms is scarce. The best characterized are OsPIL15 and OsPIL16, which have the same role on seed length but a distinct mechanism of action.

OsPIL16 is a negative regulator of grain length and the effect on grain size is proportional to the *OsPIL16* transcript level. *OsPIL16*-RNAi lines with the lowest level of *OsPIL16* expression showed the longest grains (Heang and Sassa, 2012a). However, only when the overexpression of *OsPIL16* reaches 70–80-fold increase is a decrease of 7–6% in grain length observed (Heang and Sassa, 2012a). The low effect on grain size observed in the overexpressing lines might be related to the post-translational regulation of OsPIL16 that prevents the continuous accumulation of OsPIL16 protein. OsPIL16 regulates grain size by decreasing lemma and palea inner epidermal cell length, but not width. However, the mechanisms and network of action by which OsPIL16 controls cell length and grain size are largely unknown. Two atypical non-DNA-binding bHLHs, the POSITIVE REGULATOR OF GRAIN LENGTH 1 (PGL1) and PGL2, were identified as interacting with OsPIL16, which is also known as ANTAGONIST OF PGL1 (APG) (Heang and Sassa, 2012b, c). Atypical bHLH proteins can interact with bHLH TFs, blocking their transcriptional activity. Thus, the formation of the heterodimer OsPIL16–PGL1 and/or OsPIL16–PGL2 may impair OsPIL16 function, a hypothesis supported by the longer grain size observed in *PGL1*-Ox, *PGL2*-Ox, and *OsPIL16*-RNAi lines. In addition to OsPIL16, OsPIL15 is also a negative regulator of grain size (Heang and Sassa, 2012b). However, OsPIL15 does not interact with PGL1, suggesting a different molecular mechanism underlying grain size control. Grain length and width are higher in *OsPIL15*-KO and *OsPIL15*-RNAi lines and reduced in *OsPIL15*-Ox as compared with WT (Ji *et al.*, 2019). The weight of 1000 grains was increased in *OsPIL15*-KO and -RNAi, leading to an increased yield of 13.07% to 16.59% and 14.33% to 22.08%, respectively. *OsPIL15*-Ox lines showed lower 1000-grain weight and yield compared with WT (Ji *et al.*, 2019). Contrary to OsPIL16, OsPIL15 regulates grain size by controlling cell division. The number of parenchyma cells of the spikelet hull of KO lines is higher than WT, and no significant differences were found in the cell area. More cells were observed in the lemma along the longitudinal axis in *OsPIL15*-KO lines compared with WT, suggesting that OsPIL15 regulates grain size by controlling cell number instead of cell expansion (Ji *et al.*, 2019). Recently two complementary mechanisms of action by which OsPIL15 regulates grain size were proposed (Fig. 5). (i) OsPIL15 induces the expression of a purine permease gene, *OsPUP7*, by binding to the N-box (CACGCG) present on *OsPUP7* promoter. Consequently, the cytokinin transport is altered and cell division is inhibited. This OsPIL15–OsPUP7 pathway only explains in part the increased grain size observed in *OsPIL15*-KO lines, since *Ospup7* mutants show longer but not wider grains, and thus OsPIL15 must regulate other genes that control grain width (Ji *et al.*, 2019). (ii) OsPIL15 induces *OsMIR530* expression by directly binding to three G-box (CACGTG) elements in the *OsMIR530* promoter. Thus, the accumulation of mature OsmiR530 represses *PLUS3 domain-containing* (*OsPL3*) expression, inhibiting grain length and regulating cell division and expansion in spikelet hulls (Sun *et al.*, 2020). This regulatory cascade is supported by the decreased grain length and width and consequent lower 1000-grain weight observed in

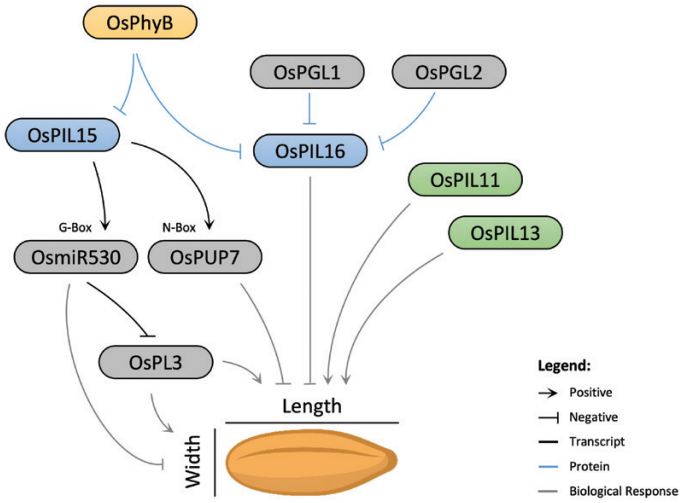

Fig. 5. Role of PIFs regulating rice grain size. Illustration of rice PIFs involved in the regulation of grain length and width. The illustration displays known PIFs' upstream regulators and downstream targets. Line color represents the regulation level, namely at transcript level (black), protein level (blue), or the biological response (gray). G-box represents the sequence CACGTG, while N-box represents the sequence CACGCG. The regulation can be positive/activation (pointed arrow) or negative/repression (blunt arrow). Each PIF is represented with a color code that represents the associated clade from Fig. 1.

OsPIL15-Ox, *OsmiR530*-Ox, and *OsPL3*-KO lines (Ji *et al.*, 2019; Sun *et al.*, 2020). In addition, the inflorescence number is altered in these lines. *OsPIL15*-Ox shows a reduced tiller number (Xie *et al.*, 2019), while *OsmiR530*-Ox and *OsPL3*-KO show reduced panicle branches (Ji *et al.*, 2019; Sun *et al.*, 2020). Although *OsPL3* is the major target of OsmiR530 concerning grain size, it does not fully explain the yield loss in *OsmiR530*-Ox, suggesting the presence of other players in this signaling cascade (Sun *et al.*, 2020). Additional OsPIL15 direct target genes and OsPIL15 interactors still need to be identified to fully characterize and understand the network by which OsPIL15 regulates grain size and plant architecture.

In contrast with the negative effect of OsPIL16 and OsPIL15 on grain development, OsPIL11 and OsPIL13 are positive regulators of grain length (Fig. 5). *OsPIL11*-Ox and *OsPIL13*-Ox lines showed increased grain length, while *OsPIL11*-KO lines had shorter grains compared with WT (Todaka *et al.*, 2012; Yang *et al.*, 2018). In addition, *OsPIL13* T-DNA mutant shows lower grain per panicle and reduced 500-grain weight (Sakuraba *et al.*, 2017). It is known that OsPIL13 induces cell expansion by regulating the expression of expansins in rice (Todaka *et al.*, 2012), but it is not known how OsPIL11 and OsPIL13 regulate grain size and productivity. Further studies are needed to unveil the molecular mechanisms underlying OsPIL-mediated rice grain development (Fig. 5).

The role of PIFs in grain/fruit size and yield in other economically important crops, such as tomato and maize, is poorly understood. Rice plants overexpressing *ZmPIF3.1* showed an increased grain yield due to an increased tiller and panicle number and increased 1000-grain weight, under control conditions. When overexpressing *ZmPIF3.2*, rice plants also showed an increased number of tillers and panicles, but it was not transduced in higher yield due to the lower filling rate (Gao et al., 2018a, b). These results are contrary to what is observed for their rice homologs, OsPIL15 and OsPIL16, which are repressors of grain size and show lower 1000-grain weight and yield when overexpressed (Heang and Sassa, 2012a; Ji *et al.*, 2019). Overall, these observations show that ZmPIF3.1 and ZmPIF3.2 have the capability to regulate grain yield and are interesting breeding candidates; nevertheless, their role and molecular mechanism must be better characterized in maize plants.

In tomato, SlPIF1a, SlPIF3, and SlPIF4 were characterized as playing a role in fruit quality and productivity. SlPIF1a inhibits the accumulation of carotenoids by repressing the expression of the gene encoding a carotenoid synthesis enzyme, PHYTOENE SYNTHASE 1 (SlPSY1). This is a direct regulation in which SlPIF1a binds to the PBE-box (CACATG) present in the *SlPSY1* promoter, thus repressing *SlPSY1* expression (Llorente *et al.*, 2016). SlPIF3 was shown to decrease tocopherol content during fruit ripening, by repressing the expression of the gene encoding GERANYLGERANYL DIPHOSPHATE REDUCTASE (GGDR), one of the first enzymes in tocopherol biosynthesis (Gramegna *et al.*, 2019). SlPIF4 was suggested to be a key player in determining fruit quality and yield as *SlPIF4*-silenced lines not only showed lower flower production and consequent lower fruit production but also fruits with 23% less weight compared with WT (Rosado *et al.*, 2019). However, due to the upregulation of key genes, such as *SlPSY1*, *GERANYLGERANYL DIPHOSHATE SYNTHASE 2* (*SlGGPS2*), and *RIPENING INHIBITOR* (*SlRIN*), *SlPIF4*-silenced fruits showed increased carotenoid content and faster ripening compared with WT. This phenotype was suggested to reflect the reduction in auxin levels and a change in the source–sink relationship, which is most probably caused by a lowered leaf area of the *SlPIF4*-silenced line and leads to an altered carbon partitioning (Rosado *et al.*, 2019). Again, the molecular mechanisms underlying the role of these tomato PIFs regulating fruit development are poorly understood and require more investigation.

Conclusion and perspective

Increased demand for food through population growth and rising living standards will continue to increase demand for crop productivity, while climate change and reduced arable land contribute to decreases in yield. The overexpression of stress-responsive genes has been used successfully to increase stress tolerance, but often negative effects are observed on plant development. Hence, new alternatives are needed and PIFs have emerged as key players that can be used to increase crop productivity and stress tolerance. The role of PIFs as central integrators of internal and external stimuli to regulate plant development is well established in the model plant Arabidopsis. In crops, an increasing number of studies point towards PIFs as important regulators of agronomic traits and integrators of external environmental conditions to modulate plant development and yield. As regulators of plant growth, PIFs control plant size, architecture, grain number and size, and fruit quality in an environment-dependent manner, showing a vast potential for improving crop productivity. However, the molecular mechanisms underlying PIF-mediated stress responses in crops are poorly understood. A comprehensive understanding of these molecular mechanisms will allow modulating PIF function to improve crop productivity under optimal or adverse environmental conditions, which may help safeguard food security during climate change. New breeding technologies such as CRISPR, which have proved very successful in rice and tomato (Wang *et al.*, 2019), will be useful to generate single and multiple *PIF* mutants in different plant species. This will allow the advance of this field of research, while cheaper mRNA sequencing may allow for the widespread generation of time-course data in different growth conditions, such as different photoperiods, developmental stages, temperature, and growth conditions. Ultimately, this effort will contribute to a better and more comprehensive understanding of the molecular mechanisms underlying PIF-mediated environmental responses.

Overall, this knowledge will be a crucial step towards crop productivity improvement. It will allow generating crops more resistant to either adverse environmental conditions or high crop density, without yield loss. Such advances would allow for expansion of the cultivated area and increased crop yield.

Author contributions

All authors contributed to conceiving the manuscript. AC wrote the manuscript and coordinated the writing/revision process and made most of the figures. LA wrote the manuscript, analysed the RNA-seq non-published data mentioned in the manuscript, and made the respective figure. CM and GL wrote the manuscript. PW and NS raised funds and revised the manuscript.

Conflict of interest

The authors declare that the review manuscript was written in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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Data availability

The RNA-seq data used to make Fig. 3A are openly available in the Gene Expression Omnibus at https://www.ncbi.nlm.nih.gov/geo with the accession number GSE181836.

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