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REVIEW PAPER

CDF transcription factors: plant regulators to deal with extreme environmental conditions

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Abstract

In terrestrial environments, water and nutrient availabilities and temperature conditions are highly variable, and especially in extreme environments limit survival, growth, and reproduction of plants. To sustain growth and maintain cell integrity under unfavourable environmental conditions, plants have developed a variety of biochemical and physiological mechanisms, orchestrated by a large set of stress-responsive genes and a complex network of transcription factors. Recently, cycling DOF factors (CDFs), a group of plant-specific transcription factors (TFs), were identified as components of the transcriptional regulatory networks involved in the control of abiotic stress responses. The majority of the members of this TF family are activated in response to a wide range of adverse environmental conditions in different plant species. CDFs regulate different aspects of plant growth and development such as photoperiodic flowering-time control and root and shoot growth. While most of the functional characterization of CDFs has been reported in Arabidopsis, recent data suggest that their diverse roles extend to other plant species. In this review, we integrate information related to structure and functions of CDFs in plants, with special emphasis on their role in plant responses to adverse environmental conditions.

Keywords: Abiotic stress, CDF, extreme environments, C/N metabolism, DOF, photosynthesis, transcription factors.

Introduction

Abiotic stresses such as salinity drought, extreme temperature, and low soil fertility are among the most important environmental conditions that constrain plant growth, development, and productivity. The gradual colonization of different terrestrial habitats, including a wide of range of extreme environments, during plant evolution has been possible thanks to the development of complex molecular and physiological mechanisms to modulate growth depending on availability of resources and environmental conditions (Xiong *et al.*, 2002; Shinozaki and Yamaguchi-Shinozaki, 2007; Ahuja *et al.*, 2010; Skirycz and Inzé, 2010; Osakabe *et al.*, 2011; Nishiyama *et al.*, 2012; Zhu, 2016).

Genome-wide expression analyses in multiple plant species have revealed a large set of genes that are regulated by different environmental stress conditions (reviewed in Todaka *et al.*, 2015; Ohama *et al.*, 2017; Zhu, 2016) and which encode proteins with functions in stress tolerance, such as chaperones implicated in *de novo* protein folding and the refolding

of misfolded proteins, osmoregulatory proteins, as well as antioxidant proteins and enzymes involved in the detoxification of reactive oxygen species (ROS) and xenobiotics (Stitt & Krapp, 1999; Xiong *et al.*, 2002; Yanagisawa, 2004; Foyer *et al.*, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007). Moreover, abiotic stresses promote changes in the expression of genes involved in carbon (C) metabolism, regulation of carbon/nitrogen (C/N) balance, and signalling transduction and the control of gene expression, such as protein phosphatases and kinases and a large group of transcription factors (TFs). Several of the TFs implicated in the regulation of gene expression and abiotic stress signalling have been identified so far, including different members of large gene families such as bHLH, HD-ZIP, WRKY, MYB, bZIP, DOF, and NAC (Shinozaki *et al.*, 2003; Osakabe *et al.*, 2014; Todaka *et al.* 2012; Zhu *et al.*, 2016). These data suggest the existence of complex transcriptional regulatory networks in which TFs are likely to physically interact to form complexes, as shown recently for several bZIP, bHLH, MYB, and DOF TFs (Zhang *et al.*, 1995; Kang and Singh, 2000; Washio, 2003; Diaz *et al.*, 2005; Skirycz *et al.*, 2008; Wei *et al.*, 2010).

Plant specific *D*NA binding with *O*ne *F*inger (DOF) proteins are a group of TFs characterized by a 50-amino-acid conserved DNA binding domain that is usually located in their N-terminal region and linked to a basic region (Yanagisawa, 1995). The conserved DOF domain is a particular zinc finger domain, with a specific C_2–C_2 finger structure, that binds specifically to *cis* DNA regulatory elements containing the common core 5′-T/AAAG-3′ motif present in the promoter regions of its target genes (Yanagisawa and Schmidt, 1999; Yanagisawa, 2002). In addition, recent studies reported that, although it was originally identified as a DNA binding domain, the DOF domain might display multiple functions including nuclear localization, interaction with other TFs, as well as cell-to-cell trafficking (Krebs *et al.*, 2010; Chen *et al.*, 2013).

DOF proteins have been reported to display a wide range of functions controlling many different aspects of plant growth and development, including shoot branching, vascular system development, flowering time, germination, and seed maturation (reviewed by Le Hir and Bellini, 2013; Noguero *et al.*, 2013; Yanagisawa, 2016). Moreover, new functions have been described for DOF TFs related to the control of the balance between carbon and nitrogen metabolism in maize (*ZmDOF1-2*), pine (*PpDOF5*), tomato (*SlCDF3*), and Arabidopsis (*AtCDF3*), through the control of the expression of key genes such as *pyruvate kinase* (*PK*), *phosphoenolpyruvate carboxylase* (*PEPC*) and *glutamine synthetase* (*GS*) (Yanagisawa and Sheen, 1998; Yanagisawa, 2004; Rueda-López *et al.*, 2008; Kurai *et al.*, 2011; Corrales *et al.*, 2017; Peña *et al.*, 2017; Renau-Morata *et al.*, 2017).

During the past few years, different detailed phylogenetic studies using the complete repertory of amino acid sequences of DOF proteins from different plant species (i.e. tomato, pepper, poplar, Arabidopsis, rice, barley, and *Brachypodium*), revealed four main families or groups of orthologous genes, named A–D (Lijavetzky *et al.*, 2003; Yang *et al.*, 2006; Hernando-Amado *et al.*, 2012; Sugiyama *et al.*, 2012; Corrales *et al.*, 2014; Wu *et al.*, 2016). Among the components of group D, a set of *DOF* genes known as *Cycling Dof factors* (*CDF*) were described, whose expression levels oscillate with a circadian rhythm (Imaizumi *et al.*, 2005; Fornara *et al.*, 2009; Corrales *et al.*, 2014). It is well established that CDFs are key factors in the photoperiodic pathway of flowering-time regulation in Arabidopsis (Imaizumi *et al.*, 2005; Fornara *et al.*, 2009) by controlling expression patterns of key regulators such as CONSTANS (CO) and FLOWERING LOCUS (FT). CDF protein stability is regulated in long days by a protein complex formed by the product of the clock gene GIGANTEA (GI) (Park *et al.*, 1999) and the blue-light absorbing protein FLAVIN BINDING KELCH REPEAT F-BOX PROTEIN 1 (FKF1), which bind to the CDF C-terminal region through specific conserved motifs comprising approximately 10–30 amino acid residues (Imaizumi *et al.*, 2005; Sawa *et al.*, 2007; Kloosterman *et al.*, 2013). Thus, a protein complex formed by GI and an F-box protein, FKF1, is required to degrade the CDF proteins in the long-day afternoon, releasing repression of CO and FT transcription (Imaizumi *et al.*, 2005; Sawa *et al.*, 2007). Additional functional protein analyses have provided new insights into the transcriptional control of CDF factors (Goralogia *et al.*, 2017), and a molecular mechanism by which CDFs repress the expression of *CO* and *FT* target genes has been proposed (Goralogia *et al.*, 2017). Arabidopsis CDF proteins contain in their N-terminal region a conserved domain required for the interaction with the TOPLESS (TPL) co-repressor protein (Liu and Karmarkar, 2008). This TPL interaction confers a repressive function on CDF1, since single mutations of the N-terminal TPL binding domain impair CDF1 protein to repress its *CO* and *FT* targets (Goralogia *et al.*, 2017). Consequently, CDF1 repression is exerted through the formation of a CDF–TPL transcriptional complex, which reduces the expression levels of *CO* and *FT* during a specific period of the day for seasonal flowering.

Lately, different reports showed that Arabidopsis, tomato, and rapeseed CDFs play additional functions in plant metabolism and abiotic stress responses (Corrales *et al.*, 2014, 2017; Fornara *et al.*, 2015; Xu and Dai, 2016; Renau-Morata *et al.*, 2017). Based on these observations, it is possible to speculate that CDFs are likely to regulate directly or indirectly processes associated to carbon and nitrogen assimilation, abiotic stress tolerance, and flowering time control. In the present review we summarize current knowledge about the CDF family emphasizing its role in the control of abiotic stress responses.

CDFs: structure and molecular characteristics

The domain structure of CDF TFs

CDFs display variable size ranging from 200 to 450 amino acid residues (Corrales *et al.*, 2014; Yanagisawa, 2016). Their protein sequences include a DOF DNA binding domain usually located close to the N-terminal region, a nuclear localization signal (NLS) (Fig. 1), and, typically, other domains involved in the control of transcriptional activities or protein stability (Imaizumi *et al.*, 2005; Sawa *et al.*, 2007; Kloosterman *et al.*,

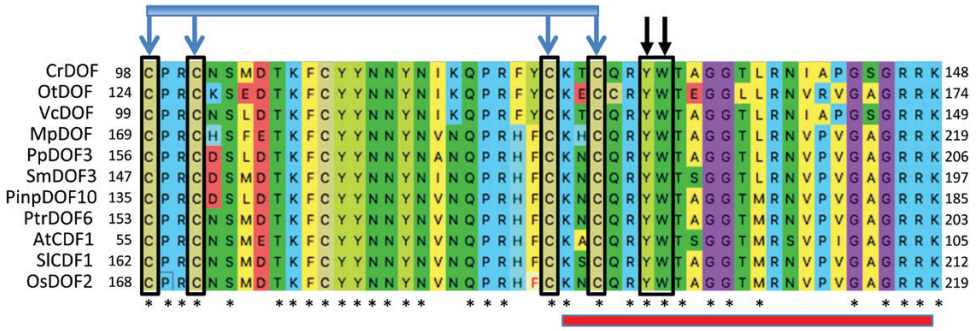

Fig. 1. Alignment of the amino acid sequences of DOF domains of CDF TFs from representative plant species. DOF domains were aligned of the CDFs selected from representative plant species: *Chlamydomonas reinhardtii* (*CrDOF*), *Ostreococcus tauri* (*OtDOF*), *Volvox carteri* (*VcDOF*), *Marchantia polymorpha* (*MpDOF*), *Physcomitrella patens* (*PpDOF3*), *Selaginella moellendorffii* (*SmDOF3*), *Pinus pinaster* (*PinpDOF10*), *Populus trichocarpa* (*PtrDOF6*), *Oryza sativa* (*OsDOF2*), Arabidopsis (*AtCDF1*), and *Solanum lycopersicum* (*SlCDF1*). The amino acid residues conserved in all proteins are marked by an asterisk. Cysteine residues that are likely involved in a C_2–C_2 Zn finger are connected by blue arrows. The red bar indicates a region located outside the Zn finger involved in DNA binding. The positions of aromatic amino acid residues conserved, involved in DNA binding, are indicated by black arrows.

2013). While the amino acid sequence of the DOF domain is highly conserved, the C-terminal regions are usually highly variable in both amino acid composition and length across the different members of the CDF family (Corrales *et al.*, 2014; Yanagisawa, 2016).

DOF domains

A DOF domain typically contains a zinc finger motif of about 30 amino acid residues, and a motif of about 20 amino acid residues located at its N-terminal region (Fig. 1, labelled in red). These amino acid segments have been proposed to be involved in the specificity and affinity of the DOF–DNA interaction (Yanagisawa, 2016). Specific mutations of two conserved amino acids, Tyr and Trp located outside the Zn finger, conserved across DOF TFs including CDF (Fig. 1), significantly reduced the sequence specific DNA binding capacity of these protein factors (Shimofurutani *et al.*, 1998; Umemura *et al.*, 2004). DOF DNA-binding activities have been analysed by different *in vitro* and *in vivo* approaches revealing that all the DOF TFs tested bind a similar 5′-AAAG-3′ DNA sequence or its complementary sequence, 5′-CTTT-3′ (reviewed in Yanagisawa, 2004, 2016), with the exception of AOBP, a pumpkin DOF protein, which recognizes a 5′-AGTA-3′ motif (Kisu *et al.*, 1998). Similarly, by using different *in vitro* assays, a similar sequence, 5′-(A/T)AAAG-3′, has been established as the recognition core for several CDFs, including Arabidopsis CDF1 and CDF3 (Imaizumi *et al.*, 2005; Corrales *et al.*, 2017), tomato SlCDF1–5 (Corrales *et al.*, 2014), and potato StCDF1 factor (Kloosterman *et al.*, 2013). However, these short putative CDF binding sites are quite often found in the regulatory regions of many genes and regions across the genome, and likely only a few would be true functional sites. As described for maize ZmDOF1, the specific position of the 5′-(A/T)AAAG-3′ motif in the genome defines the binding of ZmDOF1 to DNA *in vivo* (Cavalar *et al.*, 2003), suggesting that both the location and the interaction with other factors might determine the capacity of these TFs to bind to DNA and control transcription at precise sites in the genome. Only definitive chromatin immunoprecipitation followed by sequencing (ChIP-seq) and computational integration of these data with other functional genomic assays, such as RNA sequencing (RNA-seq) for gene expression levels and ATAC-/DNase-seq/FAIRE-seq for chromatin accessibility, will provide accurate functional binding sites depending on the different environmental stress conditions.

C-terminal motifs: interactions between CDFs and other proteins

A number of functionally significant interactions of CDFs have been reported with other proteins, several mediated by domains different from the conserved DOF domain (Imaizumi *et al.*, 2005; Sawa *et al.*, 2007; Kloosterman *et al.*, 2013). CDF proteins typically contain in their C-terminal region GI- (labelled in blue) and FKF1-binding (pale green) domains, which are specific for CDF TFs (Fig. 2). They both participate in the protein–protein interactions that control their post-translational regulation (Imaizumi *et al.*, 2005; Kloosterman *et al.*, 2013; Corrales *et al.*, 2014). Besides, CDFs contain an additional domain with the consensus sequence SPTLGKHSRDE of unknown function (Fig. 2; labelled in purple). Moreover, CDF proteins contain in their N-terminal region a non-EAR motif-like conserved domain (marked in yellow), which has been reported to be required for interaction with the co-repressor TPL protein (Goralogia *et al.*, 2017).

Molecular evolution of the CDF TF family

Different genome-wide searches highlight that CDF factors are widely spread throughout the plant kingdom (Corrales *et al.*, 2014, 2017; Yanagisawa, 2016) and their evolutionary relationships have been previously described (reviewed in Moreno-Risueno *et al.*, 2007; Shigyo *et al.*, 2007; Lucas-Reina *et al.*, 2015; Yanagisawa, 2016). The number of *CDF* genes identified in the available sequenced plant genomes is variable, and range from one in unicellular green algae like *Ostreococcus tauri* and *Chlamydomonas reinhardtii* and the colonial green alga *Volvox carteri*, to 15 in soybean (Table 1; Moreno-Risueno *et al.*, 2007).

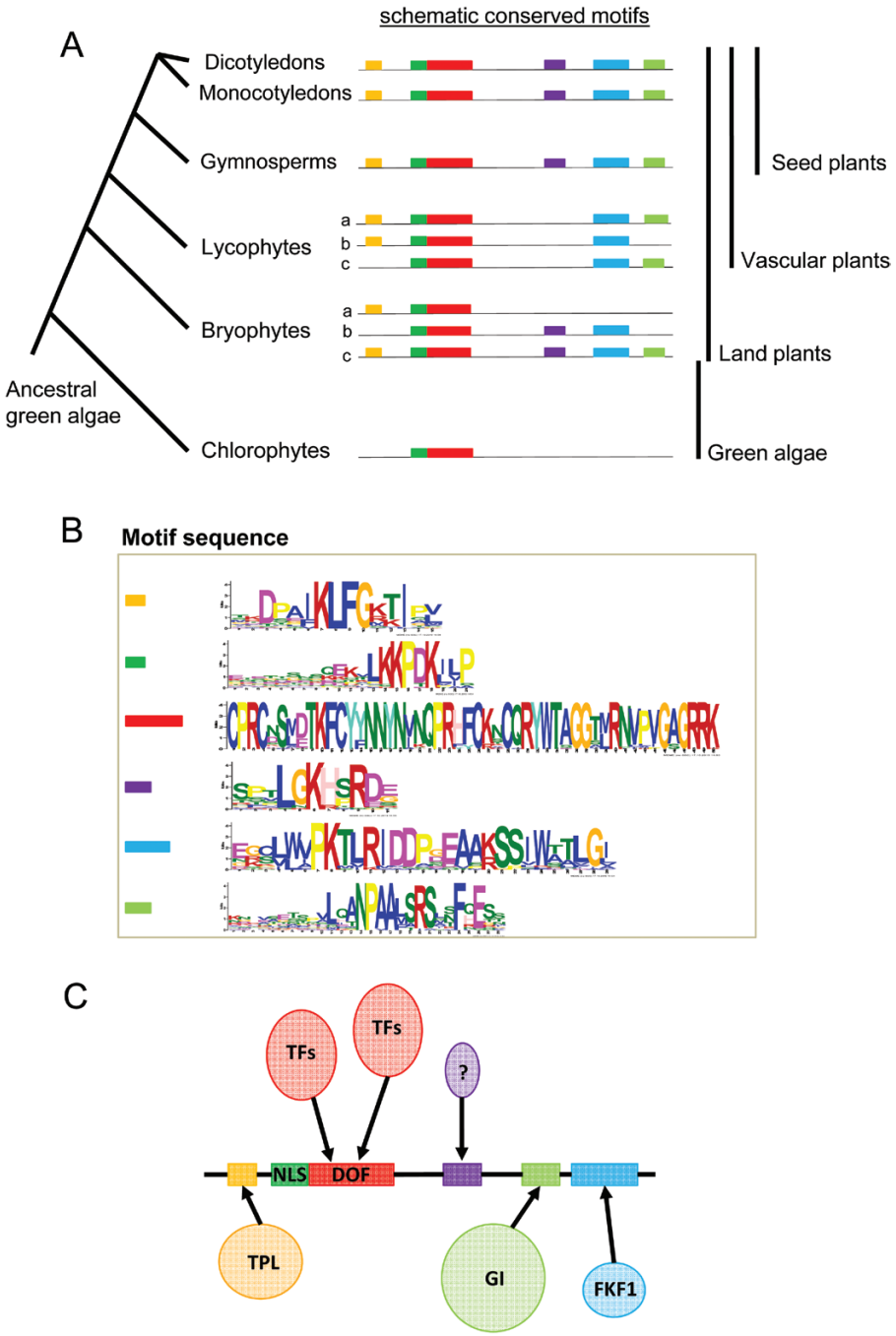

Fig. 2. Phylogenetic cladogram of CDF proteins and schematic representation of the conserved motifs identified. The diversity of motif compositions in the CDF proteins identified in representative model plants and algae species such as *C. reinhardtii*, *O. tauri*, *V. carteri*, *M. polymorpha*, *P. patens*, *S. moelendorffii*, *P. pinaster*, *P. trichocharpa*, *O. sativa*, Arabisopsis, and *S. lycopersium* genomes was assessed using the MEME program (Bailey *et al.*, 2009), and a total of six conserved motifs were identified. (A) Phylogenetic cladogram of CDF proteins in the plant lineage and representation of the most representative motifs from those identified by means of MEME software. CDF proteins from the chlorophyte division, including *C. reindhartii*, *O. tauri*, and *O. carteri*, only share in their protein sequences the DOF DNA-binding domain (marked in red); CDF factors from angiosperm species contain all six conserved motifs in addition to the DOF domain. Three classes of CDF factors with different domain composition, labelled as 'a', 'b', and 'c', have been identified in bryophyte and lycophyte clades. (B) Conserved amino acid sequences of the identified motifs. Only domains with a significant score in the MEME program are shown. (C) Domain structure of CDF transcription factors subfamily. The conserved domains in CDFs are marked with different colours: TPL interaction domain (yellow), nuclear localization signal (NLS; green), DOF–DNA binding domain (red), GI interaction (light green), FKF1 interaction domain (blue), and the conserved domain with unknown function (purple).

The approximate average copy number of *CDF* genes in the genome in a vascular plant is five. However, the genome of the moss *Physcomitrella patens* encodes six *CDF* genes and that of the spikemoss *Selaginella* encodes four *CDF* genes, which both are highly variable in amino acid sequence and domain structure. The fact that *Physcomitrella* and *Selaginella* genomes contain a similar number of *CDF* genes to the one found in vascular seed plants, higher than the number of *CDF* genes

Table 1. Numbers of *CDF* genes in a variety of plant species

Species	No. of *DOF* genes	No. of *CDF* genes	Reference
Green algae			
Chlamydomonas reinhardtii	1	1	Lucas-Reina *et al.* (2015)
Ostreococcus tauri	1	1	Lucas-Reina *et al.* (2015)
Volvox carteri	1	1	Lucas-Reina *et al.* (2015)
Liverwort			
Marchantia polymorpha	1	1	Lucas-Reina *et al.* (2015)
Moss			
Physcomitrella patens	19	6	Shigyo *et al.* (2007), Sugiyama *et al.* (2012)
Spikemoss			
Selaginella moellendorffii	8	4	Moreno-Risueno *et al.* (2007)
Gymnosperm			
Pinus pinaster	10	3	Rueda-López *et al.* (2013)
Monocotyledons			
Brachypodium distachon	27	5	Hernando-Amado *et al.* (2012)
Hordeum vulgare	26	5	Moreno-Risueno *et al.* (2007)
Oryza sativa	30	5	Lijavetzky *et al.* (2003)
Sorghum bicolor	28	5	Kushwaha *et al.* (2011)
Dicotyledons			
Arabidopsis	36	5	Lijavetzky *et al.* (2003)
Capsicum annuum	33	6	Wu *et al.* (2016)
Cucumis sativus	36	5	Wen *et al.* (2016)
Daucus carota	46	5	Huang *et al.* (2016)
Glycine max	78	15	Guo and Qiu (2013)
Jatropha curcas	24	4	Wang *et al.* (2018)
Populus trichocarpa	41	4	Wang *et al.* (2017)
Pyrus bretschneideri	45	5	Liu *et al.* (2019)
Solanum lycopersicum	34	5	Corrales *et al.* (2014)
Solanum melongena	29	5	Wei *et al.* (2018)
Solanum tuberosum	35	5	Venkatesh and Park (2015)
Vitis vinifera	25	4	da Silva *et al.* (2016)

found in the green algae clade, indicates that the ancestral *CDF*-like gene arose before the evolution and divergence of green algae from other multicellular green lines. Thus, the actual CDFs might have evolved from at least one *CDF*-like ancestral gene by duplication and subsequent divergence of a common ancestor of green algae and non-vascular plants.

Phylogenetic analyses performed using available sequences of CDF members using the genome information from representative plant species of the major clades have provided a new perspective on the evolution of this gene family (Corrales *et al.*, 2014, 2017; Yanagisawa, 2016). The phylogenetic tree obtained by these analyses indicates that CDFs can be clustered in four different groups, which are correlated with the major plant groups (Fig. 3). Among them, two large groups can be identified that contain all the CDF factors from chlorophytes and angiosperms, which are quite distant from the others. Besides, there are two clusters that include CDFs from the bryophyte and lycophyte lineages, which are contiguous with one another (Fig. 3). These results suggest that CDFs significantly changed their structure during evolution by gradual divergence and acquisition of new functional domains.

Comparative analysis of the complete amino acid sequences within the CDF subgroups in the different evolutionary clusters reveals that only the DOF domain has been highly conserved (Fig. 3). However, the amino acid residues in the region outside the DOF domain have significantly changed during plant evolution, sharing a similar structure within specific groups of phylogenetically related species (Fig. 3). While CDF proteins from the chlorophyte division, including *C. reindhartii*, *O. tauri*, and *O. carteri*, only share in their protein sequences the 50 amino acid DNA-binding domain (marked in red; Fig. 3), CDF factors from angiosperm species contain all five conserved motifs in addition to the DOF domain (Fig. 3). In sharp contrast, in bryophytes and lycophytes, intermediate forms have been identified, in which the identified CDFs contain different combinations of the conserved domains outside the DOF domain (Fig. 3).

The identified *CDF* gene in the liverwort *Marchantia polymorpha* shows all the described motifs present in CDF proteins of seed plants, including the TPL binding site and the motifs required for the interaction with GI and FKF1, in accordance with the reported identification of *GI* and *FKF1* homologous genes in these species (Kubota *et al.*, 2014). However, sequence analyses of CDFs identified in the moss *P. patens* (PpCDFs) showed two different domain structures (Sugiyama *et al.*, 2012). While the putative homologues of AtCDF1 and AtCDF3, PpDof3 and PpDof4, conserve the GI but not the FKF1 and TPL motifs, the two other CDF groups only maintain the TPL motif (Sugiyama *et al.*, 2012). Similarly, in the case of the spikemoss *Selaginella* (a lycophyte), the identified CDFs show three different structures and combination of domains. While several SmCDFs present TPL, GI, and FKF1

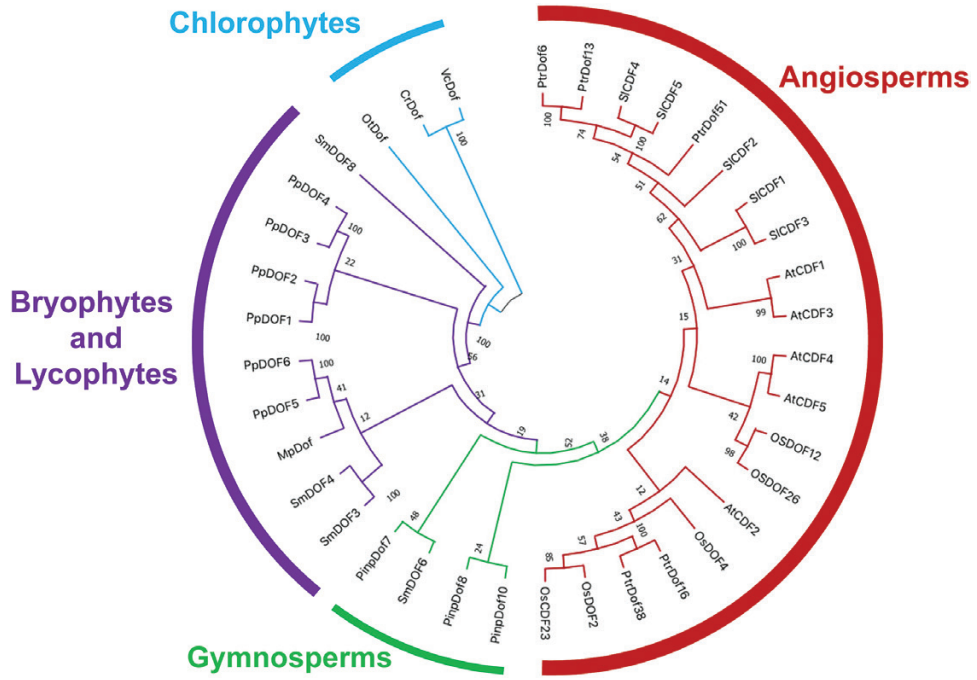

Fig. 3. Phylogenetic tree of CDF TFs. The phylogenetic tree was inferred using the complete amino acid sequences of the CDFs from *C. reinhardtii* (*CrDOF*), *O. tauri* (*OtDOF*), *V. carteri* (*VcDOF*), *M. polymorpha* (*MpDOF*), *P. patens* (*PpDOF*), *S. moellendorffii* (*SmDOF*), *P. pinaster* (*PinpDOF*), *P. trichocarpa* (*PtrDOF*), *O. sativa* (*OsDOF*), Arabidopsis (*AtCDF*), and *S. lycopersicum* (*SlCDF*). CDF protein sequences were aligned by MUSCLE using MEGA X 10.1 (Kumar *et al.*, 2018) and their phylogenetic relationships were deduced using the neighbour-joining method with the substitution model JTT (Jones *et al.*, 1992). The names of major plant clades are shown outside of the circle. The scale bar corresponds to 0.2 substitutions per site. The bootstrap number was 500.

motifs, some exhibit only GI and FKF and others TPL and GI motifs (Fig. 2). Since bryophytes and lycophytes are representatives of the earliest land plants, which possess a number of complex traits that enable them to survive new environmental stresses (Banks *et al.*, 2011; Bowman *et al.*, 2017; Rensing *et al.* 2008), the gradual appearance of different new GI, FKF, and TPL binding domains in the CDF TF protein sequences might be related to the acquisition of new functions and adaptation processes to such new terrestrial conditions. Further functional domain swapping studies are still needed to establish the specific functions of these domains in plant responses to different environmental stress conditions.

Role of CDF transcription factors in abiotic stress responses

The role of DOF TFs in the responses to several environmental stress conditions in different plant species, including crops such as maize, wheat, rice, potato, and banana, has been described in a number of studies (reviewed in Le Hir and Bellini, 2013; Yanagisawa, 2016). Recent data indicate that the CDFs are especially involved in the control of different abiotic stress responses (Corrales *et al.*, 2014, 2017; Fornara *et al.*, 2015; Xu and Dai, 2016; Renau-Morata *et al.*, 2017).

Arabidopsis CDFs

Despite the Arabidopsis CDFs being initially characterized by their cyclical diurnal transcript levels, additional expression analyses of *AtCDF1–5* genes showed that all change their expression levels in respond to different abiotic stresses, including drought, salinity osmotic stress, cold, and high temperatures (Fornara *et al.*, 2015; Corrales *et al.*, 2017). Among them, CDF3 has been studied in more detail, and functional analyses have shown that it plays a crucial role in controlling plant response to drought, osmotic, and low temperature stress (Corrales *et al.*, 2017). The overexpression of *AtCDF3* in Arabidopsis enhances plant tolerance to drought, cold, and osmotic stress, while down-regulation of *CDF3* (*cdf3*-KO) leads to reduced resistance (Corrales *et al.*, 2017). Moreover, transcriptomic analyses of *35S::AtCDF3* overexpressor plants revealed increased expression of a group of genes encoding heat shock proteins, peroxidases, catalases, thioredoxins, and DNAJ proteins, involved in cellular osmoprotection and ROS homeostasis (Corrales *et al.*, 2017). In addition, increased expression of key stress tolerance transcriptional regulators such as *CBF1-2* and *-3*, *DREB2A*, and *ZAT10-12* was observed (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017), suggesting that CDF3 has important functions in the regulation of mechanisms against cellular damage caused by osmotic and low temperature stresses (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). In agreement with these results, detailed expression analyses of the *gi* mutant, which exhibits increased stability and accumulation of CDF proteins, showed higher expression of *COR stress-regulated* genes and increased protection against low temperatures (Fornara *et al.*, 2015). However, further transcriptomic analyses revealed a limited overlap between stress-responsive genes regulated by GI and CDF3 (Corrales *et al.*, 2017), indicating that GI and CDF3 display specific

functions in low temperature and osmotic stress conditions. Additional system and functional analyses are necessary to establish the specific functions of these factors in plant responses to the different environmental stress conditions.

Tomato CDFs

In a similar way to Arabidopsis CDFs, SlCDF1–5 tomato homologs have been demonstrated to be transcriptional regulators involved in responses to salinity and drought conditions as well as in the control of flowering time (Corrales *et al.*, 2014; Renau-Morata *et al.*, 2017). Notably, quantitative PCR (qPCR) and *in silico* expression analyses uncovered that all *SlCDFs* respond to different abiotic stresses such as salt, drought, and high and low temperatures with different expression patterns in roots and shoots, supporting a role of tomato SlCDFs in abiotic stress responses (Corrales *et al.*, 2014; Renau-Morata *et al.*, 2017). In addition, overexpression of the tomato genes *SlCDF1* and *SlCDF3* in Arabidopsis resulted in increased tolerance to both salt and drought stress (Corrales *et al.*, 2014) and induced expression of recognized abiotic stress-responsive genes such as *COR15*, *RD29A*, and *ERD10* (Corrales *et al.*, 2014). Furthermore, higher tolerance to salinity stress has been also reported in tomato plants overexpressing *AtCDF3* or *SlCDF3* genes (Renau-Morata *et al.*, 2017). Altogether, these reports support that like Arabidopsis CDFs, tomato CDFs might function as key upstream regulators in salinity and drought response pathways.

Rapeseed CDFs

As with Arabidopsis CDF1, BnCDF1, a CDF homolog from rapeseed (*Brassica napus*), has been found to play an important role in the control of low temperature stress responses (Xu and Dai, 2016). Although *BnCDF1* was not regulated by salinity or low temperature stress treatments, the overexpression of *BnCDF1* in Arabidopsis enhanced freezing tolerance and induced the expression of *cold-responsive genes* including *COR15A*, *RD29A*, *COR47*, and *CBF1* (Xu and Dai, 2016). These various findings reveal the roles of CDFs in several plant species in the regulation of plant responses to different abiotic stress conditions, which are likely conserved across plants.

In silico analysis, using available expression data of *CDF* genes from representative plant species of the major clades, has provided additional evidence that supports the functions of the *CDF* gene family in plant responses to adverse environmental conditions (Corrales et al., 2014, 2017; Huang *et al.*, 2016; Khraiwesh *et al.*, 2015; Wu *et al.*, 2016; Li *et al.*, 2020). A heat map representation obtained from these expression analyses (Fig. 4) showed that *CDF* genes in a number of plant species including dicots like tomato, potato, pepper, carrot, and poplar, and monocots like rice, as well as green algae like *Chlamydomonas* (*CrDOF*) and mosses like *Physcomitrella* (*PpDOF2*) are differentially expressed in response to diverse abiotic stress conditions such as drought, salinity, osmotic stress, and extreme temperatures, but with different expression patterns. All these data revealed that *CDF* genes from different plant species might play important roles in the regulation of plant responses to particular environmental stress conditions.

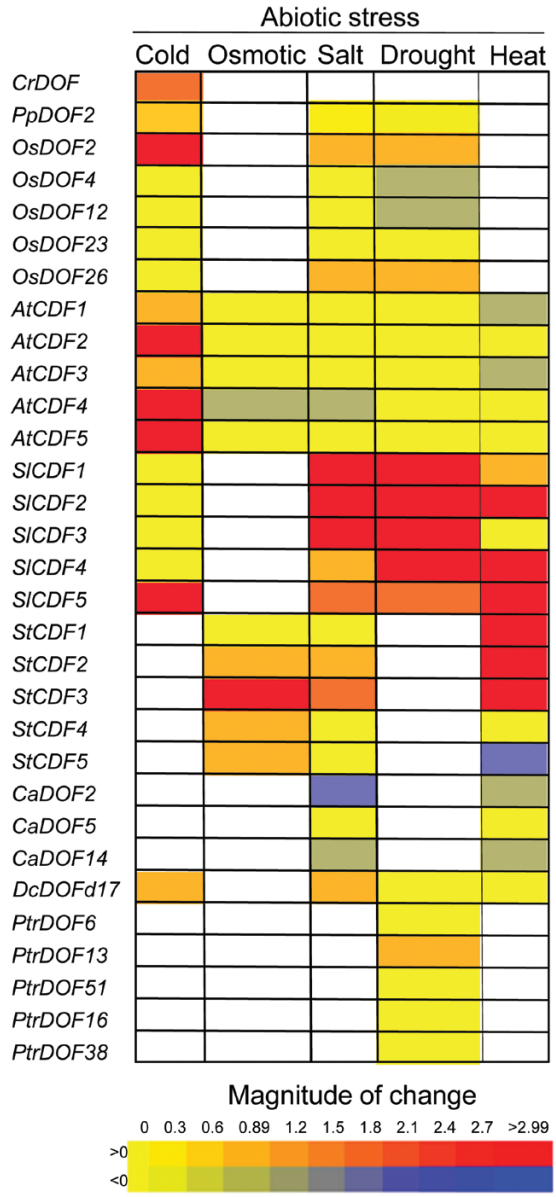

Fig. 4. Expression analysis of *CDFs* under different abiotic stress treatments in *Chlamydomonas*, *Physcomitrella*, rice, Arabidopsis, tomato, potato, carrot, pepper, and poplar. Heat map representation for the expression patterns of *CDF* genes after cold, osmotic, salt, drought, and heat stress treatments: expression levels under stress versus control; the available expression data of *OsDOF*, *AtCDF*, *StCDF*, and *PtrDOF* genes were collected from BAR (http://bar.utoronto.ca/) and *CrDOF*, *PpDOF2*, *SlCDF*, *CaDOF*, and *DcDOFd1-7* genes were obtained from Li *et al.* (2019), Khraiwesh *et al.* (2015), Corrales *et al.* (2014), Wu *et al.* (2016), and Huang *et al.* (2016), respectively. The heat map was performed using BAR Heat Mapper Plus software. Bar at the bottom represents log2 FC values.

New functions of CDF TFs in the regulation of plant growth and metabolism

Since the disclosure that ZmDOF1 activates the expression of several genes associated with carbohydrate metabolism, such as *PEPC1-2* and *PK1-2*, it has been proposed as a central regulator of carbon metabolism (Yanagisawa, 2004). The overexpression of *ZmDOF1* in Arabidopsis and rice leads to the up-regulation of genes involved in carbon-skeleton production and increased nitrogen assimilation, displaying a rise in amino

acid levels, especially glutamine and glutamate (Yanagisawa, 2004). Notably, the transgenic plants showed improved growth under low-nitrogen conditions (Yanagisawa, 2004; Kurai *et al.*, 2011). Thus, DOF1 was proposed to be a key factor controlling the assimilation and balance of C/N metabolism in plants (Yanagisawa, 2004, 2016). Moreover, it has been shown that other DOF factors might also play important roles in other physiological processes such as light responses (Yanagisawa and Sheen, 1998; Yanagisawa, 2000; Papi *et al.*, 2002), photosynthesis (Yanagisawa, 2004; Shaw *et al.*, 2009; Wang *et al.*, 2018), phytochrome signalling (Park *et al.*, 2016), and responses to plant hormones including auxins (De Paolis *et al.*, 1996; Kisu *et al.*, 1998) and gibberellins (GAs) (Washio, 2001; Mena *et al.*, 2002; Rojas-Gracia *et al.*, 2019).

Arabidopsis CDFs

Recent reports indicate that among the CDFs, CDF3 might play important roles in the control of primary metabolism and growth in Arabidopsis and tomato (Corrales *et al.*, 2014; Fornara *et al.*, 2015; Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). The overexpression of *AtCDF3* in Arabidopsis and tomato enhanced biomass production and photosynthetic capacity under both control and osmotic stress conditions (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017), resulting in increased sucrose availability and growth promotion. Consistently, *CDF3* overexpressors showed higher stomatal (g_s) and mesophyll (g_m) conductance values, as well as higher Rubisco carboxylation and triose utilization rates under osmotic stress, indicating lower diffusional and biochemical limitations to photosynthesis, respectively (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). Further expression analysis of both Arabidopsis and tomato *AtCDF3*-overexpressing plants revealed induced expression of key genes of primary metabolism, including *PK*, *GS*, and *glutamate decarboxylase* (*GAD*) (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017), which are correlated with higher amounts of amino acids such as glutamine, asparagine, and γ-aminobutyric acid (GABA) and altered quantities of organic acids such as succinate and malate (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). Remarkably, these metabolites are synthesized during abiotic stress conditions to act as compatible osmolytes, in protection of membranes and ROS scavenging, as precursors for secondary metabolites, or as storage forms of organic nitrogen (Hoekstra *et al.*, 2001; Rizhsky *et al.*, 2004; Shinozaki and Yamaguchi-Shinozaki, 2007; Nishiyama *et al.*, 2012; Zhu, 2016). Particularly, the levels of GABA, asparagine, and glutamine are consistent indicators of nitrogen use efficiency (Stitt and Krapp, 1999; Yanagisawa, 2004; Foyer *et al.*, 2006). In fact, GABA is involved in nitrogen storage through the GABA shunt metabolic pathway that converts glutamate to succinate, which has a great impact on the nitrogen economy of plants (Shelp *et al.*, 1999). Based on these observations, it was proposed that CDF3 plays a central role in amino acid metabolism during abiotic stress responses (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). As mentioned above, *CDF3*-overexpressing plants also showed higher content of sucrose (Corrales *et al.*, 2017). Due to the fine balance between carbon and nitrogen metabolism, it has been hypothesized that CDF3 stimulates CO_2 fixation to maintain this balance (Corrales *et al.*, 2017; Renau-Morata *et al.*, 2017). These reports indicate a key role of CDF3 in the regulation of primary metabolism under specific environmental stress conditions. Additional global transcriptomic analysis using tomato *AtCDF3*-overexpressing plants showed that CDF3 regulates a set of genes related to plant growth including *trehalose-6P synthase* (*TPS*), *expansins* (*EXP*), *xyloglucan endotransglycosylase/hydrolase* (*XTH*), *cellulose synthase* (*CESA*), as well as *phytochrome-interacting HLH transcriptional factors* (*PIFs*) (Renau-Morata *et al.*, 2017). Notably, this set of genes display key functions in growth promotion and the regulatory programme that integrates internal (e.g. sucrose and gibberellins) and environmental signals (Leivar and Monte, 2014). Besides, metabolic analyses of the *CDF3*-overexpressing tomato plants showed higher levels of active gibberellins and a subsequent increased number of fruits with larger size (Renau-Morata *et al.*, 2017). Higher bioactive GA levels are reported to increase tomato fruit set and early fruit development through cell expansion (Mariotti *et al.*, 2011; Ariizumi *et al.*, 2013), which highlights the potential role of CDF3 in the regulation of C/N metabolism, photosynthetic efficiency, and growth.

Tomato CDFs

Tomato *SlCDF1–5* genes exhibit different expression patterns during development and among organs. Thus it was proposed that these genes may control the expression of particular subsets of genes involved in specific metabolic processes (Corrales *et al.*, 2014). The overexpression of *SlCDF3* in tomato promoted a similar metabolic profile to that promoted by its Arabidopsis orthologue, *CDF3* (Renau-Morata *et al.*, 2017). This suggested that both factors share conserved functions in the control of C and N assimilation and, as described above, in stress tolerance. Besides, the *SlCDF3*-overexpressing tomato plants exhibit increased photosynthetic rate and biomass production, resulting in higher plant yield, under both control and salinity stress conditions (Renau-Morata *et al.*, 2017). Moreover, altered sugar and organic acid profiles in fruits that are related to higher levels of sucrose equivalents and to other variables associated with sweetness perception and acceptability were also observed (Baldwin *et al.*, 1998; Bucheli *et al.*, 1999), supporting its role in the regulation of primary metabolism reflected in the quality of the fruit (Renau-Morata *et al.*, 2017). These results confirmed common features of *CDF3* genes in plant responses to environmental stress conditions (Fig. 5). Accordingly, the overexpression of tomato *SlCDF1* or *SlCDF3* genes in Arabidopsis promoted higher shoot and root growth under salinity and drought stress conditions (Corrales *et al.*, 2014). All these data underline the potential role of CDF3 in the regulation of the genes involved in the control of C/N metabolism, photosynthetic efficiency, and consequently plant growth and yield under control and stress conditions

CDF like factors in non-vascular plant species

Up to now, there are available only two functional studies on CDF factors in non-flowering plants: the unicellular alga *C. reinhardtii* and the moss *P. patens*. As mentioned above, the

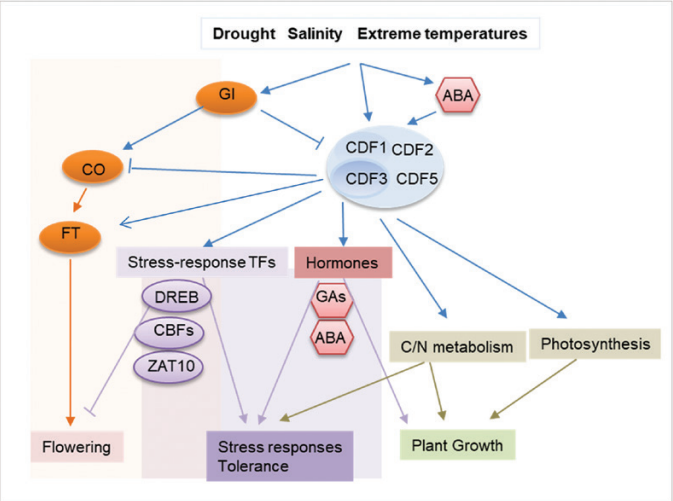

Fig. 5. Proposed model of CDF functions in plant responses to abiotic stresses. *CDF* expression is regulated by different environmental stress conditions including salinity, drought, and extreme temperatures. CDFs regulate CBF, DREB, and ZAT10 abiotic stress modules, which are involved in osmotic regulation, protein folding, autophagy, and protection of cellular structures. In addition, CDF proteins also control plant growth through the regulation different aspects of primary C/N metabolism and photosynthesis. CDFs control gibberellin and ABA levels, which modulate plant growth and stress responses. CDFs are involved in the crosstalk between abiotic stress and flowering time by controlling the GI/CO/FT module. Positive and negative regulation are indicated by arrows and blunted lines, respectively.

P. patens genome encodes six *CDF*-like genes (Shigyo *et al.*, 2007). Among them, *PpDof3* and *PpDof4*, exhibit diurnal expression patterns in a similar fashion to their Arabidopsis or tomato homologues (Sugiyama *et al.*, 2012). However, loss-of-function mutants for both *PpDof3* and *PpDof4* obtained using targeted mutagenesis by homologous recombination did not affect diurnal expression of the three putative *CO*-like target genes of *P. patens* (Ishida *et al.*, 2014). On the other hand, the disruption of an alternative *PpCDF* gene, *PpCDF1*, promoted slow growth and delay in gametophore formation as well as reduced branching of protonemal filaments, depending on the ratio or total amount of carbon and nitrogen nutrients available. Consequently, a role of PpDof1 in the control of nutrient-dependent growth of filaments has been proposed (Sugiyama *et al.*, 2012). In a similar way, functional analyses of the unique *CrCDF* gene in the chlorophyte *C. reinhardtii* revealed an important role in fatty acid metabolism. Indeed, the overexpression of *CrCDF* in *C. reinhardtii* enhanced the accumulation of lipids (Ibáñez-Salazar *et al.*, 2014). Therefore, the data available on CDFs of green algae and bryophyte lineages indicate that these factors have functions related to several aspects of plant growth and metabolism, which explains why some of them are conserved between vascular and non-vascular plants.

Interplay between CDFs, flowering time, and abiotic stress responses

Altering the time of flowering is a reproductive strategy of plants growing under environmental stress conditions. Flowering is regulated by a complex network of pathways that respond to endogenous and environmental stimuli (photoperiod, vernalization, age, GAs, and autonomous pathways). These pathways converge on a few floral integrator genes that activate floral meristem identity genes promoting floral transition (Andrés and Coupland, 2012; Blümel, 2014). Stress-regulated flowering is not formally recognized as a floral transition pathway *per se*. However, different studies suggest that abiotic stress factors play key roles in controlling the transition to flowering (reviewed by Kazan and Lyons, 2016; Park *et al.*, 2016; Takeno, 2016). A tradeoff between stress avoidance and resource allocation to growth and reproduction is crucial for plant fitness. Emerging evidence suggests that GI is a pivotal component of abiotic stress response and serves as a major hub that connects abiotic stress responses, sugar and light signalling, and photoperiodic control of flowering time (Park *et al.*, 2016; Takeno, 2016; Jose and Bánfalvi, 2019). GI affects plant tolerance to different abiotic stresses including drought, salinity, and low temperatures (Cao *et al.*, 2005; Han *et al.*, 2013; Riboni *et al.*, 2013; Kim *et al.*, 2013; Fornara *et al.*, 2015), and it can be hypothesized that GI diverts resources away from stress responses and towards flower development. Under drought stress, GI enables a drought escape response via ABA-dependent activation of florigen genes *FT* and *TWIN SISTER OF FT* (*TSF*) (Riboni *et al.*, 2013). Besides, *gi* mutants show enhanced tolerance to salt and low temperature stress as well as delayed flowering (Kim *et al.*, 2013; Fornara *et al.*, 2015).

Abiotic stress signalling cascades appear to influence the transcription of floral integrators in order to promote floral pathways or delay flowering. Some molecular interactions between flowering time and abiotic stress pathways have been partially elucidated in Arabidopsis, and it is known that GI integrates photoperiod signalling with drought, cold, and salt stress responses (reviewed by Park *et al.*, 2016; Takeno, 2016), involving in some cases CDFs. It was demonstrated that GI and CDFs display antagonistic effects in the expression of a set of genes involved in photoperiodic modulation of flowering (Imaizumi *et al.*, 2005; Sawa *et al.*, 2007; Fornara *et al.*, 2009; Corrales *et al.*, 2017), as well as in a large group of oxidative stress- and drought-responsive genes (Fornara *et al.*, 2009; Corrales *et al.*, 2017). Thus, *gi* mutants exhibit higher stability and accumulation of CDF proteins, which consequently resulted in a higher expression of stress-regulated genes and increased tolerance to oxidative and low temperatures stress (Kurepa *et al.*, 1998; Cao *et al.*, 2005; Fornara *et al.*, 2015). Notably, similar functions have been described for CDFs from other plant species such as *Brassica napus* (Xu and Dai, 2016), suggesting that CDFs act as key regulators of plant stress responses as well in flowering-time control.

The work of Corrales *et al.* (2017) has unveiled a role of CDF3 in the link between flowering time and abiotic stress. As previously noted, CDF3 regulates the expression of genes involved in abiotic stress responses including a set of key abiotic stress TFs such as *CBF1-2* and *-3*, *DREB2A*, and *ZAT10-12* (Fig. 5), which are involved in both GI-dependent and GI-independent pathways. Interestingly, the overexpression of these transcriptional regulators also results in late flowering (Gilmour *et al.*, 2004; Vogel *et al.*, 2005; Sakuma *et al.*, 2006;

Achard *et al.*, 2008). Furthermore, there is increasing evidence indicating that in temperate legume species CDFs appear to participate in the regulation of additional *FT*-like genes and photoperiodic flowering by a CO-independent mechanism (Putterill *et al.*, 2013; Weller and Ortega, 2015; Zhang *et al.*, 2019). This suggests that in these plant species CDFs show additional functions in photoperiodic regulation of flowering and eventually in abiotic stress responses that deserve to be explored in more detail. Finally, the results of metabolite analyses from the work of Corrales *et al.* (2017) in Arabidopsis and Renau-Morata *et al.* (2017) in tomato allow it to be hypothesized that changes in sugar and nitrogen metabolism induced by up-regulation of CDFs under stress may also influence the control of flowering time (Roldán *et al.*, 1999; Bolouri Moghaddam and Van den Ende, 2013; Yuan *et al.*, 2016; Cho *et al.*, 2018; Wingler, 2018).

Taken together these data indicate that CDFs are involved in the connection between flowering and abiotic stress responses through GI/CO/FT-dependent and -independent pathways. In addition, alternative TFs up-regulated by CDFs under abiotic stress conditions, as well as metabolic changes induced by CDFs under environmental stress, might be part of the signalling components regulating flowering time. The occurrence of all these events under different environmental stresses, as well as the integration of the diverse pathways, still has to be clarified.

Conclusion and perspectives

Although a lot of information has been gathered about the CDF factors, there are still many open questions about their functions. In this review, we have provided evidence that this group of factors appear to display more multifaceted roles than previously expected, in agreement with their complex protein structure during plant evolution. Nevertheless, only a few plant CDF proteins have been functionally characterized. Members of the CDFs family are likely to participate in the fine-tuning of responses to the different abiotic stresses and also in various aspects of plant development such as flowering time and root development. By modulating a variety of TFs in their protein–DNA and/or protein–protein interactions, the CDF TFs could therefore act in the cross-talk of various signalling pathways directly or indirectly linked to C and N metabolism.

It would be very interesting to analyse the molecular mechanisms by which CDFs orchestrate metabolic homeostasis, plant stress responses, and plant growth and development. In this respect, in the near future we would expect significant advances in connecting the structure and functions of the different domains found in the proteins of this family of plant TFs.

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