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Review

Twenty years of research on cerato-platanin family proteins: clues, conclusions, and unsolved issues

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ARTICLE INFO

Article history:
Received 11 April 2019
Received in revised form
15 July 2019
Accepted 8 October 2019

Keywords:
Elicitor
Expansin
Fungal growth
Induced resistance
PAMP
Snodprot

ABSTRACT

Twenty years of research on cerato-platanin family proteins (CPPs) have led to some clear conclusions: CPPs are exclusively present in the fungal kingdom and possess an outstanding capacity to stimulate the immune system of plants. Recent discoveries have highlighted remarkable structural and functional similarities between CPPs and expansins, a class of non-enzymatic proteins found in both plants and microbes possessing loosening ability on the cell wall structure. Nevertheless, the determination of a biological role for CPPs in fungi is becoming a complicated puzzle to solve, since experimental data are often divergent and point to functional diversification. A general consensus appears however possible: CPPs from pathogenic and beneficial fungi may be considered as microbe-associated molecular patterns (MAMPs) and likely play a dual role, exerting functions in the fungal cell wall and/or in plant colonization. In this review, which celebrates 20 y of research on CPPs, we trace the history of these proteins and highlight experimental evidence and still unsolved issues.

1. Historical aspects: from cerato-platanin to cerato-platanin family

The study of proteins hereafter referred to as cerato-platanin family proteins (CPPs, or "cerato-platanins") officially starts in the mid-nineties in Italy, where a lethal fungus indigenous to North America had been destroying since the late sixties the so-called London planetrees (*Platanus hybrida* Brot., also known as *Platanus* x *acerifolia*). The fungus responsible for the disease, named "canker stain disease", was *Ceratocystis fimbriata* f. sp. *platani*, nowadays *C. platani* (Walter) Engelbr. & Harr. (Tsopelas et al., 2017).

Since the molecular mechanisms underlying the fungus–plant interaction were poorly understood (Panconesi, 1999), at the University of Florence Prof. Aniello Scala from the Plant Pathology Section, and Prof. Gianni Cappugi from the Biochemistry Section, joined their efforts to identify putative virulence factors secreted by *C. platani*.

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https://doi.org/10.1016/j.fbr.2019.10.001
1749-4613/

From culture filtrates of this ascomycete fungus it was purified a protein of about 12.4 kDa, 120-amino-acid long, which was named cerato-platanin (CP) (Pazzagli et al., 1999).

For some aspects CP seemed to show partial similarity to the hydrophobin-like protein cerato-ulmin from *Ophiostoma (Ceratocystis) ulmi* (Pazzagli et al., 1999). Curiously, some years before, in France, another protein from *C. platani* resembling cerato-ulmin had been partially characterized and had been named fimbriatan (Ake et al., 1992). However, on the basis of the limited information existing on fimbriatan, CP seemed to be a different protein (Pazzagli et al., 1999). After its first discovery no further characterization was carried out for fimbriatan, while CP was thoroughly studied in the following years and proved to be one of the most abundant proteins in the culture filtrates of *C. platani* during *in vitro* growth conditions. Were CP and fimbriatan the same protein which, given their abundance, had been accidentally purified twice by different research groups?

At the same time, despite the high similarity between CP and cerato-ulmin in their N-terminal region (40 % amino acidic identity in the 1–25 stretch), there were differences in length, spacing between cysteine residues, and overall hydrophobicity (lower for CP), suggesting that the newly purified protein was not a novel hydrophobin-like protein (Pazzagli et al., 1999). Accordingly, the protein was named cerato-platanin.

As a confirmation of those initial clues, some years later a new protein family was created by the European Bioinformatics Institute (EBI) at the European Molecular Biology Laboratory (EMBL): the "cerato-platanin family" (InterPro entry IPR010829). The family initially included five more secreted fungal proteins with close homology to CP: SnodProt1 from *Phaeosphaeria nodorum*, Ccg-14 from *Neurospora crassa*, Aspf13 from *Aspergillus fumigatus*, CS–Ag from *Coccidioides immitis*, and Sp1 from *Leptosphaeria maculans* (Pazzagli et al., 2006). In a short time, on the basis of the sequence similarity, the moderate hydrophobicity, and the presence of four cysteine residues forming two disulfide bridges (Pazzagli et al., 2014), many other proteins were added to the newly established protein family and studies specifically addressed to the characterization of CPPs in fungi started to bloom (Fig. 1).

As reported in the next paragraphs, to date we possess a large amount of data concerning biochemical features and biological roles for CPPs. The study of CPPs has been greatly improved by the availability of recombinant proteins (mainly produced in *Pichia pastoris* - Carresi et al., 2006; Buensanteai et al., 2010; Frias et al., 2011; Yu et al., 2012; Quarantin et al., 2019) and of fungal strains silenced for the expression of CPP encoding genes (Fig. 2).

2. Cerato-platanin family proteins in fungi: an ever-expanding world

CPPs appear exclusive to the fungal kingdom, and recent bioinformatic analyses have indicated their presence in almost a hundred of fungal species (Kim et al., 2016). However CPPs might not be ubiquitous in fungi. So far, in fact, CPP homologs have been found in Dikarya in the subphyla Pezizomycotina (Ascomycota) and Agaricomycotina (Basidiomycota), excluding Tremellomycetes and Dacrymycetes (Agaricomycotina) (Chen et al., 2013; Kim et al., 2016). As reported by Chen et al. (2013), CPPs seem to have been lost in fungi with yeast or yeast-like forms in their life cycle, as well as in jelly fungi belonging to Tremellomycetes and Dacrymycetes.

The presence of these proteins does not appear to be correlated to any particular fungal lifestyle, since saprotrophs, symbionts, and parasites of plants and animals all possess CPPs (Chen et al., 2013; Kim et al., 2016). Molecular dating analyses suggest that CPP encoding genes originated with saprotrophism and evolved with the development of parasitic features (Yu and Li, 2014).

The number of CPP genes within the genome greatly varies between Ascomycota and Basidiomycota. Ascomycota generally possess 1 homolog, with only few species having 2 and up to 4 homologs; Basidiomycota generally possess multiple CPP encoding genes, and their overall number may raise up to 12 (Chen et al., 2013; Kim et al., 2016). Within the same organism, different CPP homologs may be characterized by biochemical and functional differences (de O. Barsottini et al., 2013; Baccelli et al., 2015; Crutcher et al., 2015; Gaderer et al., 2015; Quarantin et al., 2019) (Fig. 2).

In 1999, when CP from *C. platani* was purified and characterized for the first time, similar gene sequences had been already reported in two human pathogenic fungi: *C. immitis* and *A. fumigatus* (Pan and Cole, 1995). Nevertheless, the study of CPPs in this research area has never taken off, and to date we possess an extensive literature (more than 70 research papers) exclusively devoted to CPPs from plant-interacting fungi and their effects on plants. An overview of the most characterized CPPs and some key steps concerning their study over the last 20 y is shown in Fig. 1.

3. Biochemical and structural properties of cerato-platanin family proteins

CPPs are small cysteine-rich proteins mainly composed of 105–134 amino acid residues. All CPP sequences confirmed at the protein level contain a signal peptide of 14–18 residues that targets them to the secretory pathway. Accordingly, CPPs are usually found as the most abundant proteins in the secretome of fungi (Pazzagli et al., 2014; Ashwin et al., 2017) (Fig. 2). The distinctive feature of CPPs is the presence of four cysteines in a conserved spacing forming two disulfide bridges, a biochemical feature conferring to CPPs extreme stability to high temperatures and acidic media (Pazzagli et al., 2014; de Oliveira et al., 2011).

The first tertiary (3D) structure was determined by NMR technique in 2011 for CP from *C. platani* (de Oliveira et al., 2011). CP presented a globular fold containing two α-helices and six β-strands forming a double $\psi\beta$-barrel fold (de Oliveira et al., 2011). Subsequently, a double $\psi\beta$-barrel fold was also found in MpCP1, MpCP2, MpCP3, and MpCP5 from *Moniliophthora perniciosa* (de O. Barsottini et al., 2013), and Sm1 from *Trichoderma virens* (Protein Data Bank, 3m3g). Sm1 and MpCP3 possess a 3D structure that can be perfectly superposed to the one from CP; conversely, MpCP1, MpCp2, and MpCP5 show some structural differences that have been

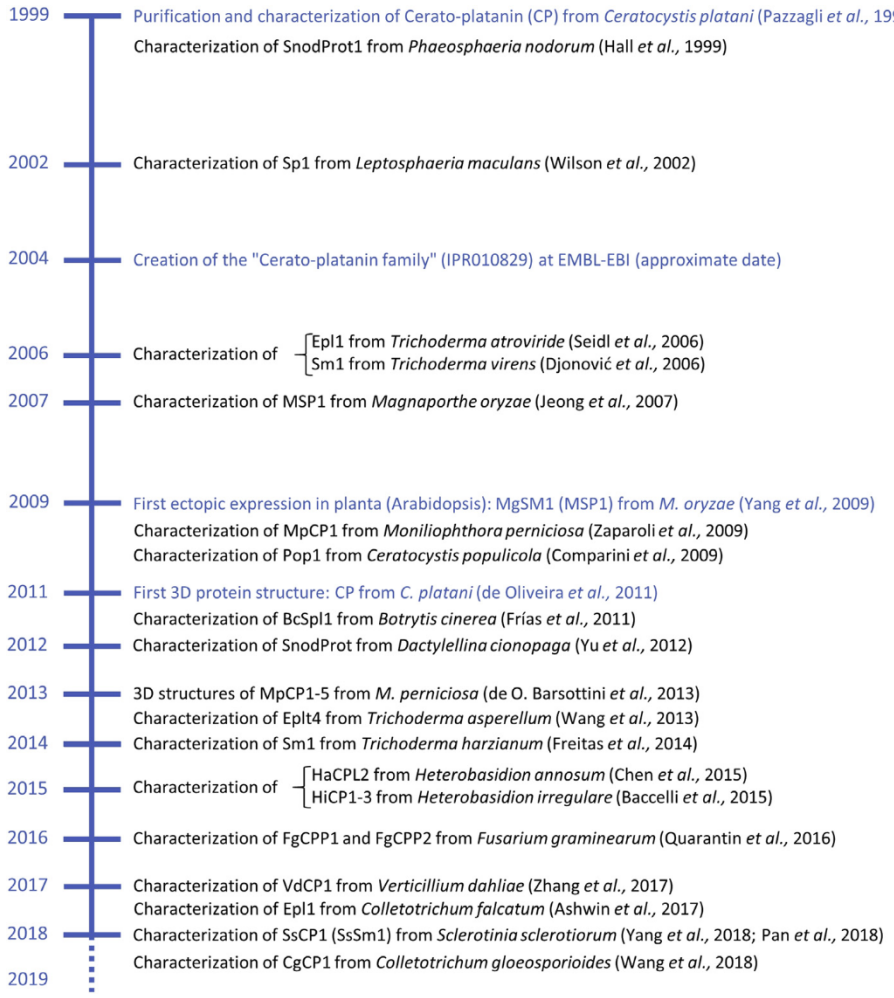

Fig. 1 – Twenty years of research on cerato-platanin family proteins (CPPs). The timescale shows the temporal order in which CPPs have been studied. Per each fungal species, only the first paper specifically addressed to the study of CPPs has been generally reported. Some key steps concerning the study of CPPs are highlighted in blue. It is worth noting that all fungi in which CPPs have been studied are plant-interacting fungi (beneficial or pathogenic), with the exception of the nematophagous fungus *Dactylellina cionopaga*. We apologize with the authors whose work has not been reported in this figure (Comparini et al., 2009; Freitas et al., 2014; Hall et al., 1999; Seidl et al., 2006; Wang et al., 2013).

associated to some peculiar functions (Pazzagli et al., 2014; de O. Barsottini et al., 2013) (see also Fig. 2 and next paragraphs).

Importantly, while CPPs show significant sequence similarity only to proteins belonging to the cerato-platanin family (de Oliveira et al., 2011), their 3D structure shows a certain level of similarity with plant and bacterial expansins, fungal endoglucanases, and the plant defense proteins barwins, all proteins possessing a double ψβ-barrel fold and involved in polysaccharide recognition and modification (de Oliveira et al., 2011; de O. Barsottini et al., 2013). To the list of structurally similar proteins it has been recently added the kiwellin protein ZmKWL1 from *Zea mays* (Han et al., 2019). In maize, ZmKWL1 specifically blocks the activity of the chorismate mutase Cmu1 produced by the fungus *Ustilago maydis*, and is involved in pathogen defense (Han et al., 2019). However CPPs apparently lack all regions that in ZmKWL1 are necessary for the interaction with Cmu1 (Han et al., 2019). In contrast, the similarity between CPPs and expansins seems relevant for the understanding of the role that CPPs may play in fungi, including the interaction with plants, as described in the following paragraphs.

Despite it is now well established that CPPs and hydrophobins belong to different protein families, it is worth reporting that their similarity was for a long time the object of several studies addressed, in particular, to the self-assembling ability. For instance, both *in vitro* and during the interaction with hydrophobic surfaces, CP from *C. platani* was found to form ordered aggregates that were more effective than the soluble form in activating defense responses when applied to plants (Pazzagli et al., 2009). Protein aggregation in MpCP2 from *M. perniciosa* facilitated instead germ tube elongation during basidiospore germination *in vitro* (de O. Barsottini et al., 2013). On the other hand, Sm1 and Epl1 from *T. virens* and *T. atroviride*, respectively, were detected both as a monomer and a dimer, but only the monomeric form showed biological activity as elicitor on plants (Frischmann et al., 2013; Bonazza

effect observed	✔
no effect observed	X
not evaluated	-

FUNGI	Gene expression		Secretome		Fungal cell wall interaction	NAG binding	Chitin binding	Scavenging chitin fragments	Cellulose binding	Cellulose loosening	PR1 targeting	Necrotizing activity	KO/silenced mutants	Virulence reduction	Growth involvement	Spores formation	Sclerotia formation	Protective role against CWDE	Stress tolerance
	in vitro	*in planta*	*in vitro*	*in planta*															
C. platani **(CP)**	✔	✔	✔	✔	✔	✔	✔	-	X	✔	-	✔	-	-	-	-	-	-	-
C. populicola **(Pop1)**	-	-	-	-	-	-	✔	-	X	✔	-	✔	-	-	-	-	-	-	-
B. cinerea **(BcSpl1)**	✔	✔	✔	✔	✔	-	-	-	-	-	-	✔	✔	✔	X	X	-	-	-
S. sclerotiorum **(SsCP1/SsSM1)**	✔	✔	✔	✔	-	-	-	-	-	-	✔	✔	✔	✔	✔	-	✔	-	✔
M. oryzae **(MgSM1/MSP1/MoSM1)**	✔	✔	-	-	X	-	-	-	-	-	-	X ✔	✔	✔	X	-	-	-	-
F. graminearum **(FgCPP1-2)**	✔	✔	✔	✔	-	-	X	-	✔	✔	-	✔ (FgCPP2)	✔	X	X	X	-	✔	X
P. nodorum **(SP1)**	-	✔	✔	✔	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-
V. dahliae **(VdCP1)**	✔	✔	✔	✔	-	-	✔	-	-	X	-	✔	✔	✔	X	X	-	✔	-
C. gloeosporioides **(CgCP1)**	-	-	-	-	-	-	-	-	-	-	-	✔	✔	✔	X	✔	-	-	-
M. perniciosa **(MpCP1-12)**	✔	✔	✔	-	-	✔ (MpCP3-5)	✔ (MpCP1-2-3-5)	✔ (MpCP5)	-	✔ (MpCP2 aggregates)	-	✔ (MpCP1)	-	-	-	-	-	-	-
L. maculans **(Sp1)**	✔	✔	-	-	-	-	-	-	-	-	-	✔	✔	X	-	-	-	-	-
H. hannosum **(HaCPL2)**	✔	✔	-	-	-	-	-	-	-	-	-	✔	-	-	-	-	-	-	-
T. atroviride **(EPL1-2)**	✔	-	✔ (EPL1)	-	-	-	✔ (EPL1)	-	X (EPL1)	-	-	-	✔	-	X	X	-	-	X
T. harzianum **(Epl-1)**	✔	-	-	-	✔	-	-	-	-	-	-	-	✔	-	-	-	-	-	-
T. virens **(SM1-2)**	✔	✔ (SM1)	✔ (SM1)	✔ (SM1)	-	-	-	-	-	-	-	X (SM1)	✔	-	X	X	-	-	X

Fig. 2 – Understanding the role of cerato-platanin family proteins (CPPs) in fungi. The figure summarizes experimental data concerning the most studied CPPs: gene expression and protein secretion (grey and blue columns); chemical and biochemical properties (orange columns); knock-out/silenced strains for CPPs obtained so far, and phenotypic analyses of the mutant strains (green columns). CPPs which were named differently within different studies, but belong to fungal species possessing a single CPP encoding gene within their genome, have been reported in the figure with all the existent names separated by slashes.

et al., 2015). The ability to form dimers seems to be inversely related to the presence of a sugar moiety that may be required to prevent oligomerization and to maintain the protein in the monomeric form (Vargas et al., 2008; Zaparoli et al.,2009, Bonazza et al., 2015). Sequence analyses have also shown that an N-glycosylation site is present in most CPPs, although CP is not glycosylated *in vivo* (Pazzagli et al., 2009) and the role of glycosylation for CPPs is still far to be understood (Yu and Li, 2014; Crutcher and Kenerley, 2019).

An important feature of CPPs resides in their ability to interact with carbohydrates, as reported for CP, Epl1, MpCPs, VdCP1 and FgCPPs (Baccelli et al., 2014a; Frischmann et al., 2013; de O Barsottini et al., 2013; Zhang et al., 2017; Quarantin et al., 2019). CP and MpCP3-5, for instance, were found to interact with N-acetylglucosamine tetramers (GlcNAc-4) at the level of their flat and shallow groove located on a face of the β-barrel, which is rich in polar and aromatic residues and thus is suitable for sugar binding (de O. Barsottini et al., 2013). Remarkably, the residues involved in GlcNAc-binding are among the most conserved in CPPs, suggesting that a biological role for CPPs may be accomplished through chitin binding (see following paragraphs). As shown in Fig. 2, some CPPs have been proven to bind chitin (Baccelli et al., 2014a; Zhang et al., 2017; Frischmann et al., 2013; de O Barsottini et al., 2013), while FgCPPs from *Fusarium graminearum* showed apparent binding to a soluble cellulose derivative (Quarantin et al., 2019).

Further details on the biochemical features of CPPs are provided in Pazzagli et al. (2014) and Gaderer et al. (2014).

4. Why fungi produce cerato-platanin family proteins: a complex enigma

CPP encoding genes are generally highly expressed in fungi, both when they grow in planta and *in vitro*. *In vitro*, their expression has been frequently reported during hyphal growth, mycelial development, sporulation, and spore maturation (Fig. 2). CPPs have been shown to be abundantly secreted into different culture media and in plant hosts (Fig. 2). In a few studies, CPPs have been found to be embedded in the fungal cell wall. This is the case of CP from *C. platani*, which was found in the cell wall of ascospores, conidia and hyphae (Boddi et al., 2004), and BcSpl1 from *Botrytis cinerea*, which was detected in the cell wall of hyphae and especially in septa between adjacent cells (Frias et al., 2014). In order to establish a biological role for CPPs, gene-disruption and silenced mutants have been obtained from several fungal species, and the involvement of CPPs in growth, conidiation, and virulence has been thoroughly analyzed over the years (Fig. 2). Although in most cases no significant phenotypic differences were observed between the mutant and the wild-type strain in term of mycelial and conidial morphology, growth rate, and conidial or appressoria production during *in vitro* growth conditions (Fig. 2), mutants for SsCP1/SsSm1 from *Sclerotinia sclerotiorum* and CgCP1 from *Colletotrichum gloeosporioides* have been reported to show altered phenotypes. In particular, RNA-silenced mutants for SsCP1/SsSm1 exhibited lagged and abnormally branched hyphal growth, malformed sclerotia, a lower number of infection cushions, and reduced tolerance to NaCl, sorbitol and SDS (Pan et al., 2018). Knockout mutants for CgCP1 from *C. gloeosporioides* showed reduced conidiation but no difference in hyphal growth (Wang et al., 2018). These results are thus in accordance with the remarkable correlation found in *C. platani* between the expression of the *cp* gene and the growth of hyphae or the formation of chlamydospores (Baccelli et al., 2012), and overall suggest that some CPPs may be involved in different stages of fungal growth and development possibly acting as expansins in the fungal cell wall (paragraph 5).

In contrast, several experimental data support a role for CPPs in the interaction between fungi and host plants. In fact, pathogenicity assays performed on a variety of host plants with mutants impaired for the expression of CPPs have led to the clear conclusion that CPPs are important for fungal virulence, as in the case of *B. cinerea*, *S. sclerotiorum*, *Magnaporthe oryzae*, *Verticillium dahliae*, and *C. gloeosporioides* (Frías et al., 2011; Yang et al., 2018; Pan et al., 2018; Jeong et al., 2007; Zhang et al., 2017; Wang et al., 2018). An intriguing observation can be made by looking at Fig. 2, in which a reduced virulence of the mutants generally appears associated to the protein ability to induce cell death in the plant (Frías et al., 2011; Yang et al., 2018; Zhang et al., 2017; Wang et al., 2018). For what concerns fungi with a necrotrophic behavior this relationship would actually make perfect sense, as observed with BcSpl1 from *B. cinerea* (Frías et al., 2011), since cell death could benefit the pathogen. Nevertheless contrasting data are not missing. In fact, Sp1 from *L. maculans* (Wilson et al., 2002) and FgCPP2 from *F. graminearum* (Quarantin et al., 2016; 2019), although expressed during infection and able to induce necrosis in host plants, were found to be dispensable for virulence. Therefore, the necrosis-inducing ability of CPPs seems to be more reasonably the result of the widespread capacity that plants have to sense CPPs and trigger defense responses that culminate in programmed cell death (PCD, paragraph 6). PCD may benefit some necrotrophic fungi, but the reason why CPPs are produced by both plant pathogenic and non-pathogenic species must be searched elsewhere.

The study of the chemical properties possessed by CPPs could thus aid to decipher their biological role (Fig. 2). For instance CP from *C. platani* and some MpCPs from *M. perniciosa* were able to bind N-acetylglucosamine oligomers and chitin (de Oliveira et al., 2011; de O. Barsottini et al., 2013; Baccelli et al., 2014a). N-acetylglucosamine oligomers are potent defense elicitors when released during fungal colonization (Boller, 1995), and by binding to them MpCP5 was able to impede the activation of the plant's immune reaction (de O. Barsottini et al., 2013). Unfortunately this ability has not been investigated further, and it is not known whether this capacity is actually widespread within the protein family, but scavenging free chitin fragments during infection to suppress the plant's immune response might be a role played by some CPPs. The cell wall localization of CPPs could however be indicative of a different/additional role. Remarkably, VdCP1 from *V. dahliae* and FgCPP1-2 from *F. graminearum* were able to protect the fungal cell wall from enzymatic degradation operated by chitinases (Quarantin et al., 2016; Zhang et al., 2017), and FgCPPs were also able to protect from β-1,3-glucanases (Quarantin et al., 2016). Although

FgCPPs did not appear to bind chitin nor directly inhibit chitinases (Quarantin et al., 2019), these results overall reinforce the hypothesis that some CPPs, by scavenging free cell wall polysaccharide oligomers, or by protecting them from enzymatic degradation by plant's enzymes, can facilitate the infection process. However these hypotheses should imply that some CPPs, such as MpCP5, possess null or lower eliciting activity compared to the molecule they scavenge. These CPPs could possess a peculiar protein structure that partially or totally blurs the eliciting epitopes. A protective role in pathogenic fungi would be instead allowed by effectors able to suppress the immune reaction that CPPs, and other MAMPs, can elicit.

An interesting discovery showing an additional protective role has been recently made with SsCP1/SsSm1 from *S. sclerotiorum* (Yang et al., 2018). SsCP1/SsSm1 was able to interact with pathogenesis-related protein 1 (PR1), a well-known plant's defense protein associated with the establishment of systemic acquired resistance (SAR) (Van Loon and Van Strien, 1999). The interaction between SsCP1/SsSm1 and PR1 occurs in the plant cell apoplast and has been suggested to contribute to fungal virulence by inhibiting the antifungal activity of PR1 (Yang et al., 2018).

5. Cerato-platanin family proteins as expansins: is this the overwhelming evidence?

Expansins are wall-loosening proteins firstly discovered in plants and subsequently identified in bacteria, fungi and other organisms (Georgelis et al., 2015). Canonical expansins are small proteins of about 26 kDa consisting of two domains: an N-terminal domain (D1) forming a double ψβ-barrel fold (the domain similar to the CP domain) resembling family-45 glycosyl hydrolases; and a C-terminal domain (D2) which forms an Ig-like β-sandwich fold resembling carbohydrate-binding modules (CBMs) (Cosgrove, 2017).

In plants, expansins can loosen the rigid carbohydrate matrix of the cell wall through a non-lytic mechanism, and play a role in modifying the cell wall during growth, vascular differentiation, fruit ripening, seed germination, and leaf development (Sampedro and Cosgrove, 2005). In addition, they are involved in abiotic stress tolerance and pathogen resistance in various plant species (Zhang et al., 2019; Abuqamar et al., 2013; Chen et al., 2018).

In microbes, expansins have been mainly studied in bacteria: firstly in *Bacillus subtilis*, and subsequently in a wide array of plant-associated bacteria and fungi (Cosgrove, 2017). Microbial expansins are involved in the interaction with plants, can facilitate microbial digestion of plant cell walls, and share loosening ability on cellulose fibrils in spite of a distantly related phylogenetic origin (Cosgrove, 2017).

A similarity between CPPs and expansins was noticed soon after the determination of the tertiary structure of CP from *C. platani*: superposition of CP to EXLX1 from *B. subtilis* showed a significant Z-score with D1 of EXLX1 (de Oliveira et al., 2011). CP is embedded in the fungal cell wall of *C. platani* (Boddi et al., 2004), and gene expression data showed that the *cp* gene was regulated in a growth-dependent manner in *C. platani* (Baccelli et al., 2012). For the above-mentioned reasons it was suggested that CP, similarly to expansins, might be involved in cell wall remodeling and enlargement during hyphal growth and spore formation in *C. platani* (Baccelli et al., 2012). In accordance with this hypothesis, MpCP2 was reported to promote the elongation of the germ tube of *M. perniciosa*'s basidiospores *in vitro* (de O. Barsottini et al., 2013), suggesting an activity on hyphal development. Recent findings of mutants actually affected in hyphal growth or conidiation, such as SsCP1/SsSm1 in *S. sclerotiorum* (Pan et al., 2018), and CgCP1 in *C. gloeosporioides* (Wang et al., 2018), respectively (see previous paragraph), further support the hypothesis that CPPs behave as expansin-like proteins and loosen chitin-containing fungal cell walls (Baccelli, 2015). The observation that many other CPP knock-out mutants do not show phenotypic differences in hyphal growth and conidiation could be explained by a possible compensation effect caused by the presence of "true" expansins in the fungal genomes, as highlighted elsewhere (Baccelli, 2015).

Further data argue in favor of a functional similarity between CPPs and expansins. In particular, it appears intriguingly the ability of CPPs to weaken cellulose derivate substrates without hydrolytic activity, as reported for CP from *C. platani*, Pop1 from *Ceratocystis populicola* (Baccelli et al., 2014a), MpCP2 from *M. perniciosa* (the latter only in the aggregated form) (de O Barsottini et al., 2013), and recently for FgCPPs from *F. graminearum* (Quarantin et al., 2019). FgCPPs enhanced the activity of fungal cellulases on different substrates, including wheat cell walls, probably by increasing the accessibility of cellulose to hydrolytic enzymes through their loosening ability (Quarantin et al., 2019). This synergistic effect, which is commonly reported for other microbial expansin-like proteins (Yan et al., 2012), was not however detected with CP from *C. platani* (Baccelli et al., 2014a). Interestingly, the knock-out mutant for FgCPPs showed significantly more cellulase activity than the wild-type strain, likely compensating the loss of expansin-like activity and further supporting a role for FgCPPs as expansins on plant cellulose (Quarantin et al., 2019).

The expansin-like activity of CPPs, in combination or not with a synergistic effect with fungal hydrolytic enzymes, could be exploited by fungi during their colonization process to facilitate hyphal advancement into plant tissues, thereby being of benefit to both plant-pathogenic and non-pathogenic fungi (Baccelli, 2015; Quarantin et al., 2019). Indeed, the plant root colonization efficiency of *Trichoderma* spp. has been reported to be remarkably increased by swollenin, a protein with expansin-like activity (Brotman et al., 2008), while expansin-like proteins in mycorrhizal fungi have been reported to play a role in cell wall remodeling during hyphal penetration and accommodation inside the plant's cortical cells (Plett and Martin, 2011; Balestrini et al., 2005). In CPPs, the finding that knock-out mutants for Sm2 in *T. virens* showed reduced ability to colonize maize roots (Crutcher et al., 2015) seems to support this hypothesis.

The similarity between expansins and CPPs, and in particular with CP from *C. platani*, is also supported by structural motifs present on a side of the β-barrel where, similarly to D1 of EXLX1 from *B. subtilis*, several polar and aromatic residues suitable for carbohydrate interaction are located (de Oliveira et al., 2011; Georgelis et al., 2011). Of particular

significance is the residue Asp-77, which is conserved within CPPs (de Oliveira et al., 2011) similarly to Asp-82 within expansins (Georgelis et al., 2011). In EXLX1 from *B. subtilis*, Asp-82 is located within D1, which is involved in twisting glucan chains and loosing cellulose fibrils (Silveira and Skaf, 2016). In CPPs, Asp-77 is located in a polar pocket interacting with substrates, and a site-directed mutagenesis replacing Asp-77 with Ala-77 (D77A mutation) impaired the ability of CP to weaken cellulose fibrils similarly to the analogous mutation D82A in EXLX1 (Georgelis et al., 2015; Luti et al., 2017). Considering that both Asp-77 and other residues located in the polar pocket involved in the interaction with cellulose are conserved within CPPs (Luti et al., 2017), we can assume that the expansin-like activity may be a widespread feature in CPPs. As reported in Fig. 2, CP, Pop1, MpCP2 and FgCPPs have actually showed expansin-like cellulose loosening ability *in vitro*, while this specific function was not observed with VdCP1 from *V. dahliae* (Zhang et al., 2017). Interestingly, CPPs show a peculiar feature: while they can fragment both microcrystalline cellulose and filter paper without lytic activity as do canonical expansins, they are unable to bind these substrates (Baccelli et al., 2014a). In fact, only FgCPPs from *F. graminearum* showed the ability to interact with a soluble cellulase derivative and, partially, with 1,3-β-glucan (Quarantin et al., 2019).

In conclusion, considering CPPs as expansins which act on non-covalent interactions in plant and fungal cell walls may be the keystone to finally explain many experimental data produced in this first 20 y of study.

6. How plants respond to the perception of cerato-platanin family proteins

Since early the first purification of CP from *C. platani*, it became evident that plants were responsive to the application of CPPs: when infiltrated into tobacco leaves, pure CP induced leaf necrosis and production of fluorescence compounds (likely phytoalexins, i.e. plant secondary metabolites with antimicrobial activity) (Pazzagli et al., 1999). Nowadays, given the well-established capacity of CPPs to elicit defenses in plants, the production of phytoalexin, and the induction of necrosis or cell death (in some studies also described as hypersensitive response, HR), have become popular methods to assay CPPs on plants (Table 1). In addition, it seems possible to conclude that leaf necrosis caused by CPPs is the result of PCD, as clearly demonstrated by the typical apoptotic-like DNA fragmentation occurring in plane tree leaves treated with CP from *C. platani* or Pop1 from *C. populicola*, and in rice cell suspensions treated with MgSM1/MSP1/MoSM1 from *M. oryzae* (Lombardi et al., 2013; Wang et al., 2016).

CPPs have been reported to induce very early responses in plants. The production of reactive oxygen species (ROS) was observed for the first time in rice and cotton leaves after treatment with Sm1 from *T. virens* (Djonović et al. 2006), and since then many other CPPs have been found to induce ROS burst in plants (Table 1). Interestingly, within few minutes after treatment of Arabidopsis leaves, CP from *C. platani* was able to induce a ROS burst spreading from stomata (in the form of H_2O_2 production) and mitogen-activated protein kinase (MAPK) phosphorylation (i.e. activation) (Baccelli et al., 2014b). A quick MAPK phosphorylation has been also reported to occur with MgSM1/MSP1/MoSM1 from *M. oryzae* in rice (Meng et al., 2018), and Pop1 from *C. populicola* in plane tree leaves (Lombardi et al., 2013).

Responses reflecting the status of plant cell membranes, such as the leakage of electrolytes and the alkalinization of the extracellular pH, have also been detected. In tobacco, electrolyte leakage occurred within the first hours after leaf infiltration with BcSpl1 from *B. cinerea* (Frías et al., 2011), or VdCP1 from *V. dahliae* (Zhang et al., 2017); in rice, a similar response was detected in leaves transiently expressing MgSM1/MSP1/MoSM1 from *M. oryzae* (Hong et al., 2017). Extracellular alkalinization was instead reported to occur within minutes after treatment of sugarcane suspension cells with Epl1 from *Colletotrichum falcatum* (Ashwin et al., 2017).

The majority of CPPs have been found to stimulate the activation of the salicylic acid (SA)-signaling pathway, as demonstrated by several observations reporting the up-regulation of SA-dependent genes and the increase of SA levels in plants (Table 1). This evidence was nicely corroborated by Frías et al. (2013), who demonstrated that BcSpl1 from *B. cinerea* was able to induce systemic acquired resistance (SAR) in tobacco through the generation of SA waves spreading from the point of infiltration to neighboring leaves. However, CPPs can also activate different signaling pathways: MgSM1/MSP1/MoSM1 from *M. oryzae* and Sm1 from *T. virens* were found to up-regulate jasmonic acid (JA)-signalling genes in Arabidopsis and maize, respectively (Yang et al., 2009; Djonović et al., 2007); in rice, MgSM1/MSP1/MoSM1 led to an increase in JA levels (Hong et al., 2017). Remarkably, Arabidopsis and rice leaves elicited by MgSM1/MSP1/MoSM1 showed a simultaneous activation of both SA and JA signaling (Yang et al., 2009; Hong et al., 2017), whereas Arabidopsis leaves treated with CP from *C. platani*, as well as FgCPPs from *F. graminearum*, showed a simultaneous up-regulation of both SA- and ET-signaling genes, while JA-signaling genes turned out to be down-regulated (Baccelli et al., 2014b; Quarantin et al., 2019).

As the outcome of their eliciting activity, CPPs have been frequently demonstrated to be highly effective as resistance inducers against plant pathogens (Table 1). Their protective effect is unequivocally due to the ability to stimulate the plant's immune system, since CPPs have never been found to be directly toxic to microbes *in vitro*. As reported in Table 1, CPPs have been reported to protect plants against both fungal and bacterial infections, while oomycete infections have never been tested so far. The protection conferred by CPPs is broad-spectrum, i.e. effective against pathogens with different lifestyles. This has been shown, for instance, with CP from *C. platani*, or MgSM1/MSP1/MoSM1 from *M. oryzae*: either proteins were able to induce resistance against both necrotrophic (*B. cinerea*) and hemibiotrophic (*Pseudomonas syringae* pv. *tomato*) pathogens in Arabidopsis (Yang et al., 2009; Baccelli et al., 2014b); or with SsCP1/SsSM1 from *S. sclerotiorum*, which protected Arabidopsis plants from both necrotrophic (*B. cinerea* and *Alternaria brassicicola*) and biotrophic (*Golovinomyces orontii*) pathogenic fungi (Yang et al., 2018).

It is worth reporting here that when CPPs are applied as pure proteins on plants, they are generally active in the micro

Table 1 – Eliciting activity of cerato-platanin family proteins (CPPs) on plants.

Plant response	Protein name(s)[a]	Producing fungus	Reference
Necrosis/cell death/HR	CP	*C. platani*	Pazzagli et al. (1999); Lombardi et al. (2013)
	Pop1	*C. populicola*	Lombardi et al. (2013)
	BcSpl1	*B. cinerea*	Frías et al. (2011)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009); Wang et al. (2016)
	MpCP1	*M. perniciosa*	Zaparoli et al. (2009)
	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018); Pan et al. (2018)
	HaCPL2	*H. annosum*	Chen et al. (2015)
	Epl1	*C. falcatum*	Ashwin et al. (2017)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
	CgCP1	*C. gloeosporioides*	Wang et al. (2018)
	ChEC5[b]	*C. higginsianum*	Kleemann et al. (2012)
	FgCPP2	*F. graminearum*	Quarantin et al. (2019)
Reactive oxygen species	CP	*C. platani*	Baccelli et al. (2014b)
	Pop1	*C. populicola*	Lombardi et al. (2013)
	BcSpl1	*B. cinerea*	Frías et al. (2011)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009); Wang et al. (2016)
	Sm1	*T. virens*	Djonović et al. (2006)
	SsCP1/SsSM1	*S. sclerotiorum*	Pan et al. (2018)
	Epl1	*C. falcatum*	Ashwin et al. (2017)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
	CgCP1	*C. gloeosporioides*	Wang et al. (2018)
	FgCPP2	*F. graminearum*	Quarantin et al. (2019)
Nitric oxide	CP	*C. platani*	Lombardi et al. (2013)
	Pop1	*C. populicola*	Lombardi et al. (2013)
Electrolyte leakage	BcSpl1	*B. cinerea*	Frías et al. (2011)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Hong et al. (2017)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
Extracellular alkalinisation	Epl1	*C. falcatum*	Ashwin et al. (2017)
Phytoalexins/fluorescence	CP	*C. platani*	Pazzagli et al. (1999); Baccelli et al. (2014b)
	Pop1	*C. populicola*	Martellini et al. (2013)
	BcSpl1	*B. cinerea*	Frías et al. (2011)
	Sm1	*T. virens*	Djonović et al. (2006)
	HaCPL2	*H. annosum*	Chen et al. (2015)
MAPK activation	CP	*C. platani*	Baccelli et al. (2014b)
	Pop1	*C. populicola*	Lombardi et al. (2013)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Meng et al. (2018)
Gene up-regulation			
Salicylic acid signaling	CP	*C. platani*	Baccelli et al. (2014b)
	BcSpl1	*B. cinerea*	Frías et al. (2011)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009)
	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018)
	HaCPL2	*H. annosum*	Chen et al. (2015)
	Epl1	*T. harzianum*	Gomes et al. (2017)
	Epl1-Tas	*T. asperellum*	Yu et al. (2018)
	FgCPP1-2	*F. graminearum*	Quarantin et al. (2019)
Jasmonic acid signalling	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009)
	Sm1	*T. virens*	Djonović et al. (2007)
Ethylene signalling	CP	*C. platani*	Baccelli et al. (2014b)
	FgCPP1-2	*F. graminearum*	Quarantin et al. (2019)
Phytohormones			
Salicylic acid	BcSpl1	*B. cinerea*	Frías et al. (2013)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Hong et al. (2017)
	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
Jasmonic acid	MgSM1/MSP1/MoSM1	*M. oryzae*	Hong et al. (2017)
Proteomic changes	CP	*C. platani*	Luti et al. (2016)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Meng et al. (2019)
Enzymatic activities	Epl1-Tas	*T. asperellum*	Yu et al. (2018)
Volatile Organic Compounds	CP	*C. platani*	Luti et al. (2016)
Stomatal closure	CP	*C. platani*	Baccelli et al. (2014b)
Callose accumulation	CP	*C. platani*	Taiti et al. (2016)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Wang et al. (2016)

Table 1 (*continued*)

Plant response	Protein name(s)[a]	Producing fungus	Reference
Resistance to pathogens			
Bacteria			
Pseudomonas syringae pv. *tomato*	CP	*C. platani*	Baccelli et al. (2014b)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009)
	Sm1	*T. virens*	Salas-Marina et al. (2015)
P. syringae pv. *tabaci*	BcSpl1	*B. cinerea*	Frías et al. (2013)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
Xanthomonas oryzae pv. *oryzae*	MgSM1/MSP1/MoSM1	*M. oryzae*	Hong et al. (2017)
Fungi			
Botrytis cinerea	CP	*C. platani*	Baccelli et al. (2014b)
	BcSpl1	*B. cinerea*	Frías et al. (2013)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009)
	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
	Epl1	*T. atroviride*	Salas-Marina et al. (2015)
	Sm1	*T. virens*	Salas-Marina et al. (2015)
	VdCP1	*V. dahliae*	Zhang et al. (2017)
	FgCPP1-2	*F. graminearum*	Quarantin et al. (2019)
Alternaria brassicicola	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018)
	MgSM1/MSP1/MoSM1	*M. oryzae*	Yang et al. (2009)
A. alternata	Epl1-Tas	*T. asperellum*	Yu et al. (2018)
A. solani	Epl1	*T. atroviride*	Salas-Marina et al. (2015)
Golovinomyces orontii	SsCP1/SsSM1	*S. sclerotiorum*	Yang et al. (2018)
Colletotrichum spp.	Sm1	*T. virens*	Djonović et al. (2006)
C. graminicola	Sm1	*T. virens*	Djonović et al. (2007)
Magnaporthe oryzae	MgSM1/MSP1/MoSM1	*M. oryzae*	Wang et al. (2016); Hong et al. (2017)
Verticillium dahliae	VdCP1	*V. dahliae*	Zhang et al. (2017)

a CPPs which were named differently within different studies, but belong to fungal species possessing a single CPP encoding gene within their genome, have been reported in this review with all the existent names separated by slashes.
b In the case of ChEC5, the authors report a cell-death suppressing effect.

molar range. For instance, a minimum concentration of 75 μM was needed to induce a ROS burst in Arabidopsis leaves when drops of CP from *C. platani* were applied on the leaf surface (Baccelli et al., 2014b), while 10 μM VdCP1 from *V. dahliae* was active in tobacco, tomato, or cotton infiltrated leaves (Zhang et al., 2017).

7. Concluding remarks: cerato-platanins as MAMPs that may be involved in plant colonization

After 20 y of study it seems now to exist a general consensus in considering CPPs as microbe-associated molecular patterns (MAMPs). CPPs, in fact, possess several features that usually define typical MAMPs (Pel and Pieterse, 2013): they are widespread in fungi including non-pathogenic species, have a conserved structure, are abundantly secreted, and elicit defenses in several host and non-host plant species indicating the presence of a widespread plant perception system. Moreover, Frías et al. (2014) identified two short peptides derived from BcSpl1 that were sufficient to elicit defenses, strongly supporting the existence of a plant receptor that recognizes specific epitopes on the protein surface. In contrast, CPPs appear not to be always essential for fungal life, they can help the fungus to colonize the plant, and their absence has been often shown to negatively impact virulence of plant pathogens. We might thus wonder whether it is fair to consider CPPs as MAMPs, rather than effectors.

To solve this dilemma, we should first remind that in natural contexts a strict distinction between MAMPs and effectors is clearly unrealistic (Thomma et al., 2011). In fact several MAMPs have been reported to play a role in pathogen virulence (Thomma et al., 2011). A remarkable example is represented by bacterial flagellin, the most studied MAMP overall: flagellin mutants impaired in eliciting activity were negatively affected in bacterial motility and virulence (Naito et al., 2008). In conclusion CPPs can definitely be considered closer to MAMPs, rather than to effectors: fungi (pathogenic and beneficials) seem to produce CPPs to exert functions in the fungal cell wall (loosening or protection) and/or in plant colonization (plant cell wall loosening or protection from plant's defenses). Plants, being CPPs so highly secreted and widespread in the fungal kingdom, have generally evolved the capacity to sense these proteins and activate defense responses.

Declaration of Competing Interest

None.

Acknowledgments

The authors wish to thank Prof. Aniello Scala for critically reading this review ahead of submission to the journal.

REFERENCES

Abuqamar, S., Ajeb, S., Sham, A., Enan, M.R., Iratni, R., 2013. A mutation in the expansin-like A2 gene enhances resistance to necrotrophic fungi and hypersensitivity to abiotic stress in *Arabidopsis thaliana*. Mol. Plant Pathol. 14, 813–827.

Ake, S., Darbon, G., Grillet, L., Lambert, C., 1992. Fimbriatan, a protein from *Ceratocystis fimbriata*. Phytochemistry 31, 1199–1202.

Ashwin, N.M.R., Barnabas, L., Ramesh Sundar, A., Malathi, P., Viswanathan, R., Masi, A., Agrawal, G.K., Rakwal, R., 2017. Comparative secretome analysis of *Colletotrichum falcatum* identifies a cerato-platanin protein (EPL1) as a potential pathogen-associated molecular pattern (PAMP) inducing systemic resistance in sugarcane. J. Proteomics 169, 2–20.

Baccelli, I., Gonthier, P., Bernardi, R., 2015. Gene expression analyses reveal a relationship between conidiation and cerato-platanin in homokaryotic and heterokaryotic strains of the fungal plant pathogen *Heterobasidion irregulare*. Mycol. Prog. 14, 40.

Baccelli, I., Comparini, C., Bettini, P.P., Martellini, F., Ruocco, M., Pazzagli, L., Bernardi, R., Scala, A., 2012. The expression of the cerato-platanin gene is related to hyphal growth and chlamydospores formation in *Ceratocystis platani*. FEMS Microbiol. Lett. 327, 155–163.

Baccelli, I., Luti, S., Bernardi, R., Scala, A., Pazzagli, L., 2014a. Cerato-platanin shows expansin-like activity on cellulosic materials. Appl. Microbiol. Biotechnol. 98, 175–184.

Baccelli, I., Lombardi, L., Luti, S., Bernardi, R., Picciarelli, P., Scala, A., Pazzagli, L., 2014b. Cerato-platanin induces resistance in Arabidopsis leaves through stomatal perception, overexpression of salicylic acid- and ethylene-signalling genes and camalexin biosynthesis. PLoS One 9, e100959.

Baccelli, I., 2015. Cerato-platanin family proteins: one function for multiple biological roles? Front. Plant Sci. 5, 769.

Balestrini, R., Cosgrove, D.J., Bonfante, P., 2005. Differential location of α-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus. Planta 220, 889–899.

Boddi, S., Comparini, C., Calamassi, R., Pazzagli, L., Cappugi, G., Scala, A., 2004. Cerato-platanin protein is located in the cell walls of ascospores, conidia and hyphae of *Ceratocystis fimbriata* f. sp. *platani*. FEMS Microbiol. Lett. 233, 341–346.

Boller, T., 1995. Chemoperception of microbial signals in plant cells. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 189–214.

Bonazza, K., Gaderer, R., Neudl, S., Przylucka, A., Allmaier, G., Druzhinina, I.S., Grothe, H., Friedbacher, G., Seidl-Seiboth, V., 2015. The fungal cerato-platanin protein EPL1 forms highly ordered layers at hydrophobic/hydrophilic interfaces. Soft Matter 11, 1723–1732.

Brotman, Y., Briff, E., Viterbo, A., Chet, I., 2008. Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. Plant Physiol. 147, 779–789.

Buensanteai, N., Mukherjee, P.K., Horwitz, B.A., Cheng, C., Dangott, L.J., Kenerley, C.M., 2010. Expression and purification of biologically active *Trichoderma virens* proteinaceous elicitor Sm1 in *Pichia pastoris*. Protein Expr. Purif. 72, 131–138.

Carresi, L., Pantera, B., Zoppi, C., Cappugi, G., Oliveira, A.L., Pertinhez, T.A., Spisni, A., Scala, A., Pazzagli, L., 2006. Cerato-platanin, a phytotoxic protein from *Ceratocystis fimbriata*: expression in *Pichia pastoris*, purification and characterization. Protein Expr. Purif. 49, 159–167.

Chen, H., Quintana, J., Kovalchuk, A., Ubhayasekera, W., Asiegbu, F.O., 2015. A cerato-platanin-like protein HaCPL2 from *Heterobasidion annosum* sensu stricto induces cell death in *Nicotiana tabacum* and *Pinus sylvestris*. Fungal Genet. Biol. 84, 41–51.

Chen, H., Kovalchuk, A., Keriö, S., Asiegbu, F.O., 2013. Distribution and bioinformatic analysis of the cerato-platanin protein family in Dikarya. Mycologia 105, 1479–1488.

Chen, L.J., Zou, W.S., Fei, C.Y., Wu, G., Li, X.Y., Lin, H.H., Xi, D.H., 2018. α-Expansin EXPA4 Positively Regulates Abiotic Stress Tolerance but Negatively Regulates Pathogen Resistance in *Nicotiana tabacum*. Plant Cell Physiol. 59, 2317–2330.

Comparini, C., Carresi, L., Pagni, E., Sbrana, F., Sebastiani, F., Luchi, N., Santini, A., Capretti, P., Tiribilli, B., Pazzagli, L., 2009. New proteins orthologous to cerato-platanin in various *Ceratocystis* species and the purification and characterization of cerato-populin from *Ceratocystis populicola*. Appl. Microbiol. Biotechnol. 84, 309–322.

Cosgrove, D.J., 2017. Microbial Expansins. Annu. Rev. Microbiol. 71, 479–497.

Crutcher, F.K., Moran-Diez, M.E., Ding, S., Liu, J., Horwitz, B.A., Mukherjee, P.K., Kenerley, C.M., 2015. A paralog of the proteinaceous elicitor SM1 is involved in colonization of maize roots by *Trichoderma virens*. Fungal Biol. 119, 476–486.

Crutcher, F.K., Kenerley, C.M., 2019. Analysis of a putative glycosylation site in the *Trichoderma virens* elicitor SM1 reveals no role in protein dimerization. Biochem. Biophys. Res. Commun. 509, 817–821.

de, O., Barsottini, M.R., de Oliveira, J.F., Adamoski, D., Teixeira, P.J., do Prado, P.F., Tiezzi, H.O., Sforça, M.L., Cassago, A., Portugal, R.V., de Oliveira, P.S., de, M., Zeri, A.C., Dias, S.M., Pereira, G.A., Ambrosio, A.L., 2013. Functional diversification of cerato-platanins in *Moniliophthora perniciosa* as seen by differential expression and protein function specialization. Mol. Plant Microbe Interact. 26, 1281–1293.

de Oliveira, A.L., Gallo, M., Pazzagli, L., Benedetti, C.E., Cappugi, G., Scala, A., Pantera, B., Spisni, A., Pertinhez, T.A., Cicero, D.O., 2011. The structure of the elicitor Cerato-platanin (CP), the first member of the CP fungal protein family, reveals a double ψβ-barrel fold and carbohydrate binding. J. Biol. Chem. 286, 17560–17568.

Djonović, S., Pozo, M.J., Dangott, L.J., Howell, C.R., Kenerley, C.M., 2006. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol. Plant Microbe Interact. 19, 838–853.

Djonović, S., Vargas, W., Kolomiets, M., Horndeski, M., Wiest, A., Kenerley, C., 2007. A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol. 145, 875–889.

Freitas, R.S., Steindorff, A.S., Ramada, M.H., de Siqueira, S.J., Noronha, E.F., Ulhoa, C.J., 2014. Cloning and characterization of a protein elicitor Sm1 gene from *Trichoderma harzianum*. Biotechnol. Lett. 36, 783–788.

Frías, M., Brito, N., González, M., González, C., 2014. The phytotoxic activity of the cerato-platanin BcSpl1 resides in a two peptide motif in the protein surface. Mol. Plant Pathol. 15, 342–351.

Frías, M., González, C., Brito, N., 2011. BcSpl1, a cerato-platanin family protein, contributes to *Botrytis cinerea* virulence and elicits the hypersensitive response in the host. New Phytol. 192, 483–495.

Frías, M., Brito, N., González, C., 2013. The *Botrytis cinerea* cerato-platanin BcSpl1 is a potent inducer of systemic acquired resistance (SAR) in tobacco and generates a wave of salicylic acid expanding from the site of application. Mol. Plant Pathol. 14, 191–196.

Frischmann, A., Neudl, S., Gaderer, R., Bonazza, K., Zach, S., Gruber, S., Spadiut, O., Friedbacher, G., Grothe, H., Seidl-Seiboth, V., 2013. Self-assembly at air/water interfaces and carbohydrate-binding properties of the small secreted protein EPL1 from the fungus *Trichoderma atroviride*. J. Biol. Chem. 288, 4278–4287.

Gaderer, R., Bonazza, K., Seidl-Seiboth, V., 2014. Cerato-platanins: a fungal protein family with intriguing properties and application potential. Appl. Microbiol. Biotechnol. 98, 4795–4803.
Gaderer, R., Lamdan, N.L., Frischmann, A., Sulyok, M., Krska, R., Horwitz, B.A., Seidl-Seiboth, V., 2015. Sm2, a paralog of the *Trichoderma* cerato-platanin elicitor Sm1, is also highly important for plant protection conferred by the fungal-root interaction of *Trichoderma* with maize. BMC Microbiol. 15, 2.
Georgelis, N., Nikolaidis, N., Cosgrove, D.J., 2015. Bacterial expansins and related proteins from the world of microbes. Appl. Microbiol. Biotechnol. 99, 3807–3823.
Georgelis, N., Tabuchi, A., Nikolaidis, N., Cosgrove, D.J., 2011. Structure-function analysis of the bacterial expansin EXLX1. J. Biol. Chem. 286, 16814–16823.
Gomes, E.V., Ulhoa, C.J., Cardoza, R.E., Silva, R.N., Gutiérrez, S., 2017. Involvement of *Trichoderma harzianum* Epl-1 protein in the regulation of *Botrytis* virulence- and tomato defense-related genes. Front. Plant Sci. 8, 880.
Hall, N., Keon, J.P.R., Hargreaves, J.A., 1999. A homologue of a gene implicated in the virulence of human fungal diseases is present in a plant fungal pathogen and is expressed during infection. Physiol. Mol. Plant Pathol. 55, 69–73.
Han, X., Altegoer, F., Steinchen, W., Binnebesel, L., Schuhmacher, J., Glatter, T., Giammarinaro, P.I., Djamei, A., Rensing, S.A., Reissmann, S., Kahmann, R., Bange, G., 2019. A kiwellin disarms the metabolic activity of a secreted fungal virulence factor. Nature 565, 650–653.
Hong, Y., Yang, Y., Zhang, H., Huang, L., Li, D., Song, F., 2017. Overexpression of MoSM1, encoding for an immunity-inducing protein from *Magnaporthe oryzae*, in rice confers broad-spectrum resistance against fungal and bacterial diseases. Sci. Rep. 7, 41037.
Jeong, J.S., Mitchell, T.K., Dean, R.A., 2007. The *Magnaporthe grisea* snodprot1 homolog, MSP1, is required for virulence. FEMS Microbiol. Lett. 273, 157–165.
Kim, K.T., Jeon, J., Choi, J., Cheong, K., Song, H., Choi, G., Kang, S., Lee, Y.H., 2016. Kingdom-wide analysis of fungal small secreted proteins (SSPs) reveals their potential role in host association. Front. Plant Sci. 7, 186.
Kleemann, J., Rincon-Rivera, L.J., Takahara, H., Neumann, U., Ver Loren van Themaat, E., van der Does, H.C., Hacquard, S., Stüber, K., Will, I., Schmalenbach, W., Schmelzer, E., O'Connell, R.J., 2012. Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. PLoS Pathog. 8e1002643.
Lombardi, L., Faoro, F., Luti, S., Baccelli, I., Martellini, F., Bernardi, R., Picciarelli, P., Scala, A., Pazzagli, L., 2013. Differential timing of defense-related responses induced by cerato-platanin and cerato-populin, two non-catalytic fungal elicitors. Physiol. Plant. 149, 408–421.
Luti, S., Caselli, A., Taiti, C., Bazihizina, N., Gonnelli, C., Mancuso, S., Pazzagli, L., 2016. PAMP Activity of cerato-platanin during plant interaction: an -omic approach. Int. J. Mol. Sci. 17, 866.
Luti, S., Martellini, F., Bemporad, F., Mazzoli, L., Paoli, P., Pazzagli, L., 2017. A single amino acid mutation affects elicitor and expansins-like activities of cerato-platanin, a non-catalytic fungal protein. PLoS One 12, e0178337.
Martellini, F., Faoro, F., Carresi, L., Pantera, B., Baccelli, I., Maffi, D., Tiribilli, B., Sbrana, F., Luti, S., Comparini, C., Bernardi, R., Cappugi, G., Scala, A., Pazzagli, L., 2013. Cerato-populin and cerato-platanin, two non-catalytic proteins from phytopathogenic fungi, interact with hydrophobic inanimate surfaces and leaves. Mol. Biotechnol. 55, 27–42.
Meng, Q., Gupta, R., Min, C.W., Kim, S.T., 2018. Label-free quantitative proteome data associated with MSP1 and flg22 induced signaling in rice leaves. Data Brief 20, 204–209.
Meng, Q., Gupta, R., Min, C.W., Kim, J., Kramer, K., Wang, Y., Park, S.R., Finkemeier, I., Kim, S.T., 2019. A proteomic insight into the MSP1 and flg22 induced signaling in *Oryza sativa* leaves. J. Proteomics 196, 120–130.
Naito, K., Taguchi, F., Suzuki, T., Inagaki, Y., Toyoda, K., Shiraishi, T., Ichinose, Y., 2008. Amino acid sequence of bacterial microbe-associated molecular pattern flg22 is required for virulence. Mol. Plant Microbe Interact. 21, 1165–1174.
Pan, S.C., Cole, G.T., 1995. Molecular and biochemical characterization of a *Coccidioides immitis*-specific antigen. Infect. Immun. 63, 3994–4002.
Pan, Y., Wei, J., Yao, C., Reng, H., Gao, Z., 2018. SsSm1, a Cerato-platanin family protein, is involved in the hyphal development and pathogenic process of *Sclerotinia sclerotiorum*. Plant Sci. 270, 37–46.
Panconesi, A., 1999. Canker stain of plane trees: a serious danger to urban plantings in Europe. J. Plant Pathol. 81, 3–15.
Pazzagli, L., Cappugi, G., Manao, G., Camici, G., Santini, A., Scala, A., 1999. Purification, characterization, and amino acid sequence of cerato-platanin, a new phytotoxic protein from *Ceratocystis fimbriata* f. sp. *platani*. J. Biol. Chem. 274, 24959–24964.
Pazzagli, L., Pantera, B., Carresi, L., Zoppi, C., Pertinhez, T.A., Spisni, A., Tegli, S., Scala, A., Cappugi, G., 2006. Cerato-platanin, the first member of a new fungal protein family: cloning, expression, and characterization. Cell Biochem. Biophys. 44, 512–521.
Pazzagli, L., Zoppi, C., Carresi, L., Tiribilli, B., Sbrana, F., Schiff, S., Pertinhez, T.A., Scala, A., Cappugi, G., 2009. Characterization of ordered aggregates of cerato-platanin and their involvement in fungus-host interactions. Biochim. Biophys. Acta 1790, 1334–1344.
Pazzagli, L., Seidl-Seiboth, V., Barsottini, M., Vargas, W.A., Scala, A., Mukherjee, P.K., 2014. Cerato-platanins: elicitors and effectors. Plant Sci. 228, 79–87.
Pel, M.J., Pieterse, C.M., 2013. Microbial recognition and evasion of host immunity. J. Exp. Bot. 64, 1237–1248.
Plett, J.M., Martin, F., 2011. Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. Trends Genet. 27, 14–22.
Quarantin, A., Glasenapp, A., Schäfer, W., Favaron, F., Sella, L., 2016. Involvement of the *Fusarium graminearum* cerato-platanin proteins in fungal growth and plant infection. Plant Physiol. Biochem. 109, 220–229.
Quarantin, A., Castiglioni, C., Schäfer, W., Favaron, F., Sella, L., 2019. The *Fusarium graminearum* cerato-platanins loosen cellulose substrates enhancing fungal cellulase activity as expansin-like proteins. Plant Physiol. Biochem. 139, 229–238.
Salas-Marina, M.A., Isordia-Jasso, M.I., Islas-Osuna, M.A., Delgado-Sánchez, P., Jiménez-Bremont, J.F., Rodríguez-Kessler, M., Rosales-Saavedra, M.T., Herrera-Estrella, A., Casas-Flores, S., 2015. The Epl1 and Sm1 proteins from *Trichoderma atroviride* and *Trichoderma virens* differentially modulate systemic disease resistance against different life style pathogens in *Solanum lycopersicum*. Front. Plant Sci. 6, 77.
Sampedro, J., Cosgrove, D.J., 2005. The expansin superfamily. Genome Biol. 6, 242.
Seidl, V., Marchetti, M., Schandl, R., Allmaier, G., Kubicek, C.P., 2006. Epl1, the major secreted protein of *Hypocrea atroviridis* on glucose, is a member of a strongly conserved protein family comprising plant defense response elicitors. FEBS J. 273, 4346–4359.
Silveira, R.L., Skaf, M.S., 2016. Molecular dynamics of the *Bacillus subtilis* expansin EXLX1: interaction with substrates and structural basis of the lack of activity of mutants. Phys. Chem. Chem. Phys. 18, 3510–3521.
Taiti, C., Giorni, E., Colzi, I., Pignattelli, S., Bazihizina, N., Buccianti, A., Luti, S., Pazzagli, L., Mancuso, S., Gonnelli, C.,

2016. Under fungal attack on a metalliferous soil: ROS or not ROS? Insights from *Silene paradoxa* L. growing under copper stress. Environ. Pollut. 210, 282–292.
Thomma, B.P., Nürnberger, T., Joosten, M.H., 2011. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23, 4–15.
Tsopelas, P., Santini, A., Wingfield, M.J., Wilhelm de Beer, Z., 2017. Canker stain: a lethal disease destroying iconic plane trees. Plant Dis. 101, 645–658.
Van Loon, L.C., Van Strien, E.A., 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol. 55, 85–97.
Vargas, W.A., Djonović, S., Sukno, S.A., Kenerley, C.M., 2008. Dimerization controls the activity of fungal elicitors that trigger systemic resistance in plants. J. Biol. Chem. 283, 19804–19815.
Wang, Y., Song, J., Wu, Y., Odeph, M., Liu, Z., Howlett, B.J., Wang, S., Yang, P., Yao, L., Zhao, L., Yang, Q., 2013. Eplt4 proteinaceous elicitor produced in *Pichia pastoris* has a protective effect against *Cercosporidium sofinum* infections of soybean leaves. Appl. Biochem. Biotechnol. 169, 722–737.
Wang, W., An, B., Feng, L., He, C., Luo, H., 2018. A *Colletotrichum gloeosporioides* cerato-platanin protein, CgCP1, contributes to conidiation and plays roles in the interaction with rubber tree. Can. J. Microbiol. 64, 826–834.
Wang, Y., Wu, J., Kim, S.G., Tsuda, K., Gupta, R., Park, S.Y., Kim, S.T., Kang, K.Y., 2016. *Magnaporthe oryzae*-secreted protein MSP1 induces cell death and elicits defense responses in rice. Mol. Plant Microbe Interact. 29, 299–312.
Wilson, L.M., Idnurm, A., Howlett, B.J., 2002. Characterization of a gene (sp1) encoding a secreted protein from *Leptosphaeria maculans*, the blackleg pathogen of *Brassica napus*. Mol. Plant Pathol. 3, 487–493.
Yan, Z., Ming-Xiong, H., Bo, W., Qi-Chun, H., Qing, L., Jian, Z., 2012. Recombinant EXLX1 from *Bacillus subtilis* for enhancing enzymatic hydrolysis of corn stover with low cellulase loadings. Afr. J. Biotechnol. 11, 11126–11131.
Yang, Y., Zhang, H., Li, G., Li, W., Wang, X., Song, F., 2009. Ectopic expression of MgSM1, a Cerato-platanin family protein from *Magnaporthe grisea*, confers broad-spectrum disease resistance in Arabidopsis. Plant Biotechnol. J. 7, 763–777.
Yang, G., Tang, L., Gong, Y., Xie, J., Fu, Y., Jiang, D., Li, G., Collinge, D.B., Chen, W., Cheng, J., 2018. A cerato-platanin protein SsCP1 targets plant PR1 and contributes to virulence of *Sclerotinia sclerotiorum*. New Phytol. 217, 739–755.
Yu, H., Li, L., 2014. Phylogeny and molecular dating of the cerato-platanin-encoding genes. Genet. Mol. Biol. 37, 423–427.
Yu, H., Duan, J., Wang, B., Jiang, X., 2012. The function of snodprot in the cerato-platanin family from *Dactylellina cionopaga* in nematophagous fungi. Biosci. Biotechnol. Biochem. 76, 1835–1842.
Yu, W., Mijiti, G., Huang, Y., Fan, H., Wang, Y., Liu, Z., 2018. Functional analysis of eliciting plant response protein Epl1-Tas from *Trichoderma asperellum* ACCC30536. Sci. Rep. 8, 7974.
Zaparoli, G., Cabrera, O.G., Medrano, F.J., Tiburcio, R., Lacerda, G., Pereira, G.G., 2009. Identification of a second family of genes in *Moniliophthora perniciosa*, the causal agent of witches' broom disease in cacao, encoding necrosis-inducing proteins similar to cerato-platanins. Mycol. Res. 113, 61–72.
Zhang, Y., Gao, Y., Liang, Y., Dong, Y., Yang, X., Yuan, J., Qiu, D., 2017. The *Verticillium dahliae* SnodProt1-like protein VdCP1 contributes to virulence and triggers the plant immune system. Front. Plant Sci. 8, 1880.
Zhang, H., Liu, H., Yang, R., Xu, X., Liu, X., Xu, J., 2019. Overexpression of PttEXPA8 gene showed various resistances to diverse stresses. Int. J. Biol. Macromol. 130, 50–57.