REVIEW OF LITERATURE

The most important regulators controlling cell death in metazoan are the death inducing proteases known as cysteine aspartic-specific proteases (caspases). Caspases are composed of regulatory domains including an N-peptide region and a large catalytic p20 domain and a small catalytic p10 domain separated by a linker. Like caspases, metacaspases also contain a large subunit p20 and small p10 domains. The distribution of the caspase and caspase-like proteases showed that caspases and paracaspases are in metazoans and *Dictyostelium*, respectively, whereas metacaspases are found only in plants, protists and fungi. This suggests that MCs are likely the closest representative of eukaryotic caspases in plants, protists and fungi (Uren et al., 2000). Experimental data suggests that metacaspases play roles in regulating the various forms of PCD in these organisms via proteolytic activity of other metacaspases. The involvement of metacaspases is well established in response to oxidative stress, hypersensitive responses (HR), UV radiation, herbicide-induced stress, and flower senescence related cell death (He et al., 2008; Vercammen et al., 2007; Watanabe & Lam, 2011).

The organisms first utilized to explore the metacaspases and metacaspase-like proteases were prokaryotes wherein metacaspases and caspase-hemoglobinase fold identified (Uren et al., 2000). Some studies predicted the bacterial origin of metacaspase and also showed the role of metacaspases in various cellular activities including nuclear function, cellular proliferation and mitochondrial biogenesis (Szallies et al., 2002; Uren et al., 2000).

2.1 Metacaspases in protists and algae

Metacaspases are well characterized in some protists. *Trypanosoma*, which is a causative agent of trypanosomiasis was mainly targeted by various groups for metacaspase functional analysis. One of studies shows that *Trypanosoma* overexpressing metacaspases were more sensitive to H_2O_2-induced PCD (Lee et al., 2007). In another report, *Trypanosoma brucei* Metacaspase 2 (TbMCA2) was found to be a calcium-dependent cysteine peptidase (Moss et al., 2007) and its processing broadened its substrate specificity (Gilio et al., 2017). However, *Trypanosoma brucei* metacaspase 4 was found to be a pseudopeptidase and a virulence factor (Proto et al., 2011). In addition to these, antagonic activities of metacaspases affect the equilibrium between cell division, cell death and differentiation in *Trypanosoma cruzi* (Laverrière et al., 2012). The crystal structure of a metacaspase was also analysed in *Trypanosoma brucei* (McLuskey et al., 2012), in order to find potential targets for chemotherapy of the trypanosomiasis (Alvarez et al., 2013). Structural characterization of metacaspase from *Trypanosoma cruzi* was also

reported (De lima et al., 2022). *Leishmania* is another genus in trypanosomes that is responsible for the human disease *leishmaniasis*. Metacaspase, LmjMCA processes an active catalytic domain and is found to disrupt the mitochondria in *Leishmania major* (Zalila et al., 2011). However, the downregulation of LdMC1 and Hsp70 causes cell-division defects PCD in *Leishmania donovani* (Raina & Kaur, 2012). Functionally *Leishmania* metacaspase is found to be an arginine-specific peptidase (Martin et al., 2014). Furthermore, it has been reported that various metacaspase domain of *Leishmania major* are implicated in cell death and autophagy (Casanova et al., 2015). Characterization of metacaspases from malaria parasite *P. vivax* is also reported. Its metacaspase 1 (PvMCA1) catalytic domain is found to be highly conserved in different isolates (De souza et al., 2021). Metacaspase in *P. falciparum* have been implicated to be central mediators for essential parasitic activity in this more virulent malaria parasite species (Wu et al., 2003). In *Plasmodium falciparum,* PfMCA-1 triggers a z-VAD-fmk protease to regulate cell death (Meslin et al., 2011). The apoptotic role of metacaspase in *Toxoplasma gondii* which is an obligate intracellular parasitic protozoan have also been reported (Li et al., 2015).

Metacaspases are also discovered in different lineages of algae. The type I and II metacaspases are reported from green algae group chlorophyta (Choi & Berges, 2013). Type II-Metacaspases were shown to involved in cellular stress instead of cell death in alga *Dunaliella tertiolecta* (Mata et al., 2019) A newly discovered type III metacaspases is also reported in some algae (Klemenčič & Funk, 2018). Type III metacaspases are mostly found in Rhodophyta lineages such as Haptophyta, Heterokontophyta and Cryptophyta (Choi & Berges, 2013). Roles of metacaspases in protists and unicellular algae are listed in table 1.

Table 1: The roles of metacaspases in protists and unicellular algae

Protists and algae	Metacaspase name	Metacaspase function	References
Plasmodium falciparum	Metacaspase, Type I	Identified paracaspases and metacaspases	(Uren et al., 2000)
P. falciparum	Metacaspase	Metacaspase implicated to be mediators of essential parasitic activity	(Wu et al., 2003)
P. falciparum	Metacaspase	Overexpression of metacaspases results in more sensitivity of plasmodium to H_2O_2-induced cell death	(Lee et al., 2007)
P. falciparum	Metacaspase	Metacaspases exert the function of caspases in protists	(Deponte, 2008)
P. falciparum	PfMCA-1	PfMCA-1 triggers z-VAD-fmk protease in PCD	(Meslin et al., 2011)
Plasmodium vivax	PvMCA1	Catalytic domain of PvMCa1 is conserved in different strains	(De souza et al., 2021)
Trypanosoma brucei	metacaspase 4	metacaspase is a pseudo peptidases and act as a virulence factor	(Proto et al., 2011)
T. brucei	Metacaspase 2	Metacaspase 2 is a calcium-dependent cysteine peptidase	(Moss et al., 2007)
T. brucei	TbMCA2	Reported first inhibitors of TbMCA2	(Berg et al., 2010)
T. brucei	Metacaspase	Crystal structure of metacaspase reported	(McLuskey et al., 2012)
T. brucei	TbMCA2	Processing of TbMCA2 broadens its substrate specificity	(Gilio et al., 2017)
T. brucei	Metacaspase	Metacaspase as potential targets for chemotherapy of the trypanosomiases	(Alvarez et al., 2013)
Trypanosoma cruzi	Metacaspase	Antagonistic activity of metacaspase regulate the balance between cell division and cell death and differentiation	(Laverrière et al., 2012)
T. cruzi	TcMCAs	Structural characterization metacaspase	(De lima et al., 2022)
Leishmania donovani	LdMC1	Downregulation of LdMC1 results in cell-cycle defects and PCD	(Raina and Kaur, 2012)
Leishmania major	LmjMCA	LmjMCA process an active catalytic domain and hampers mitochondria	(Zalila et al., 2011)
L. major	Metacaspase	*Leishmania* metacaspase is an arginine-specific peptidase	(Martin et al., 2014)

L. major	Metacaspase	Metacaspase domains of the implicated in cell death and autophagy	(Casanova et al., 2015)
Dunaliella tertiolecta	Type II	Type II-Metacaspases are involved in cell stress unicellular green alga	(Mata et al., 2019)
Chlamydomonas reinhardtii and Volvox carteri	Type II	Biochemical properties of two type II metacaspases were analysed	(Fortin and Lam, 2018)
Guillardia theta	Type III	Introduction to type III metacaspases	(Klemenčič and Funk, 2018)

2.2 Metacaspases in fungi

Metacaspases are also found in fungi, but not in slime molds. Yeasts are widely studied model organisms for characterization of metacaspases. *Saccharomyces cerevisiae* (budding yeast) metacaspase named Yeast Caspase 1 (YCA1) is involved in programmed cell death and found to be essential for H_2O_2-induced aging and apoptosis. Its overproduction in yeast cells causes premature aging (Madeo et al., 2002). In budding yeast, YCA1 plays a role as a downstream executor of cell death (Mazzoni et al., 2005). Mutation of YCA1, abolishes apoptosis and increases quantity of oxidized proteins in cells (Khan et al., 2005) and its overexpression stimulates cell growth of cells and stress in fission yeast (Lim et al., 2007). It is also observed to alter cell cycle dynamics during cell division (Lee et al., 2008). It has also been reported that apoptotic death in fission yeast is dependent on the metacaspase (Guérin et al., 2009). Moreover, yeast YCA1 is also required for acetic acid-induced programmed cell death which can occur independent of cytochrome c release from mitochondria (Guaragnella et al., 2010). YCA1 is also found to control the production of insoluble protein aggregates. The loss of its function in yeast directly linked with insoluble protein aggregate formation during growth. This accumulation of the protein aggregates leads to induction of the autophagic pathway (Lee et al., 2010). Also, the presence of the poly-Q motif in the YCA1 prodomain dictates its role for localization in protein aggregates (Lee et al., 2010). YCA1 also regulates the composition of the insoluble proteome in yeast cells (Shrestha & Megeney, 2012). Differential proteome-metabolome profiling of YCA1-knockout revealed that the several cellular processes and metabolic pathways are dependent on YCA1 (Ždralević et al., 2015). It has also been suggested that YCA1 maintains cellular proteostasis via its interaction with the ubiquitination (Shrestha et al., 2014). In *Aspergillus fumigatus,* metacaspases are found to facilitate hyphal growth under endoplasmic reticulum (ER) stress. The, deletion of its two MC genes (*CasA and CasB*) causes hypersensitivity to ER stress, however, no effect on hyphal growth was found with these knockout mutants under induced oxidative stress conditions (Richie et al., 2007). These results show that some of the MCs may be involved in a clearing of the protein aggregates and enhancing the survival of organisms. Besides these, some MCs may play important role in cleavage of specific cellular targets during ER stress. However, contrary to its roles in PCD, the mechanism of killing by the proapoptotic molecules in yeast was reported not due to activation of the MCA1 (aka YCA1) (Guscetti et al., 2005) (table 2).

Table 2: The identification and function of metacaspases in fungi

Fungal species	Metacaspase name	Metacaspase function	References
Saccharomyces cerevisiae	Metacaspase, Type I	Identified paracaspases and metacaspases	(Uren et al., 2000)
S. cerevisiae	YCA1, Type I	Metacaspase YCA1 implicated in programmed cell death	(Rodriguez-Menocal and D'Urso, 2004)
S. cerevisiae	MCA1, Type I	Activation of MCA1 is not involved in proapoptotic molecule formation	(Guscetti et al., 2005)
S. cerevisiae	YCA1p, Type I	Yeast expressing the Arabidopsis AtMCP1b, AtMCP2b, or YCA1p exhibit arginine/lysine-specific endopeptidase activities	(Watanabe and Lam, 2005)
S. cerevisiae	YCA1	YCA1 act as a downstream executor of cell death in yeast	(Mazzoni et al., 2005)
S. cerevisiae	YCA1, Type I	Knockout of YCA1 abolishes apoptosis and elevates oxidized proteins in cells	(Khan et al., 2005)
S. cerevisiae	YCA1	*Leishmania major* metacaspase can restore the function of yeast metacaspase during PCD	(González et al., 2007)
S. cerevisiae	YCA1p	YCA1 is involved in cell cycle regulation	(Lee et al., 2008)
S. cerevisiae	YCA1	YCA1 is required for cell death induced by acetic acid-induced	(Guaragnella et al., 2010)
S. cerevisiae	YCA1	Role of YCA1 in insoluble protein aggregate clearance	(Lee et al., 2010)
S. cerevisiae	YCA1	Mutation of YCA1 resulted in the activation of a ROS-independent PCD	(Guaragnella et al., 2010)
S. cerevisiae	YCA1	GAPDH is found to be substrate of YCA1	(Silva et al., 2011)
S. cerevisiae	YCA1	Crystal structure of the YCA1 determined	(Wong et al., 2012)
S. cerevisiae	YCA1	YCA1 regulates the composition of the insoluble proteome	(Shrestha et al., 2013)
S. cerevisiae	YCA1	YCA1 function in proteostasis	(Shrestha et al., 2014)
S. cerevisiae	MCA1	Functions of MCA1 was reported	(Liu, 2014)

S. cerevisiae	MCA1	MCA1 involved in cytoprotective functions during aging	(Hill and Nyström, 2015)
S. cerevisiae	YCA1	Differential proteome-metabolome profiling of YCA1-knockout	(Ždralević et al., 2015)
S. cerevisiae	YCA1	YCA1 involved in proteostasis via ubiquitination mediated proteolysis	(Shrestha et al., 2019)
Schizosaccharomyces pombe	Pca1p	overexpression of a Pca1p promotes cell growth and stress response	(Lim et al., 2007)
S. pombe	Pca1p	Apoptotic death is dependent on the metacaspase Pca1p	(Guérin et al., 2009)
Candida albicans	CaMCA1, Type I	Mutation of CaMCA1 provide resistance during oxidative stress-induced death	(Cao et al., 2009)
Neurospora crasa	Metacaspase	ROS species but not metacaspases associated with PCD.	(Hutchison et al., 2009)
Aspergillus fumigatus	Type I	*Fungi* metacaspases facilitate growth under ER stress	(Richie et al., 2007)
Podospora anserina	Metacaspase	Poly ADP-ribose polymerase is found to be substrate of metacaspases	(Strobel and Osiewacz, 2013)
Toxoplasma gondii	Metacaspase	Cell death role of metacaspases characterized	(Li et al., 2015)

2.3 Plant Metacaspases

Unlike fungi and protists, both type I and type II metacaspases are found in the plants. Evolutionary all types of metacaspases found in plants, fungi and protists are predicted to be evolved through horizontal gene transfer events from ancestral mitochondrial endosymbionts (Koonin & Aravind, 2002). There is another possibility that some form of metacaspases may have evolved in various plant and algal lineages through cyanobacteria which is considered as ancestor of plastids (Vercammen et al., 2007). Plant metacaspases are grouped into type I and type II subgroups based on domain organization and sequence homology (Uren et al., 2000). Both this metacaspases have a conserved caspase-like domain which include p20 and p10 domains (Ojha et al., 2010). In both the metacaspases the p20 subunit contains the catalytic histidine/cysteine dyad domain. Unlike type II, type I metacaspases have an N-terminus extra domain that contains a zinc-finger domain and a proline-rich domain and can also have a glutamine rich region (Lam & Zhang, 2012). Type II metacaspases have no prodomain and but harbour a longer linker (Ojha et al., 2010). Longer linker region of type II metacaspases are predicted to interfere with its dimerization (Lam & Zhang, 2012).

Similar to caspases, both the type I and type II of metacaspases contain a histidine/cysteine (H::C) domain therefore placed into the CD clan cysteine dependent proteases (Koonin & Aravind, 2002, Vercammen et al. 2004). Based on the domain organization and substrate specificity, CD clan are divided into 7 families viz., C11, C13, C14, Members of the C14 family are grouped into two subfamilies which include C14A, consisting of caspases and C14B consisting of paracaspases and metacaspases (Minina et al., 2013). All peptidases including metacaspases grouped in CD clan uses a catalytic cysteine to hydrolyze peptide bond of targets (Cambra et al., 2010). Similar to caspases, metacaspases also have S1 pocket forming residues and maturation sites. Metacaspases, like caspases are mostly synthesized as zymogens, which are activated by autoprocessing or via other protein or protein complexes and the process is dependent on cysteine (Vercammen et al., 2004, Belenghi et al., 2007, Watanabe & Lam 2011). Although metacaspases share many structure features common to caspases, however both catalyze their substrate differently. Caspases are specialized to cleave their substrates at aspartic acid residues, whereas metacaspases cleave their substrate at either lysine or arginine residues (Vercammen et al., 2004, Watanabe & Lam 2005, Watanabe & Lam 2011). Also, plant metacaspases are unable to catalyse the known caspase substrates *in vitro* therefore do not have typical caspase-like activity (He et al., 2008; Bonneau et al., 2008). Despite of this, metacaspases along with metazoans caspases and paracaspases are placed in clan CD of

cysteine dependent proteases (Vercammen et al., 2006). Therefore, metacaspase are now considered as analogous to caspases (Vercammen et al., 2004, Bonneau et al., 2008).

The first plant metacaspase characterized was MCII-Pa during developmental cell death in Norway spruce (*Picea abies*). Silencing of MCII-Pa resulted in suppression of PCD in suspensor during embryogenesis (Suarez et al., 2004). In *A. thaliana*, nine metacaspases (AtMC1-9) have been identified and some of these are found to be involved in cell death regulation. Arabidopsis AtMC8 is found to involve in the regulation of PCD induced by oxidative stress (He et al., 2008). Knockout of AtMC8 significantly reduced the cell death phenotypes in response to oxidative stresses (He et al., 2008). Arabidopsis AtMC1 has been shown to be a positive regulator of hypersensitive response induced cell death (Coll et al., 2010). Its closest homolog AtMC2 is shown to negatively regulate cell death in Arabidopsis. It has been found that knockout of these type I metacaspases can nearly abolish the HR responses induced by plant intracellular receptor LSD1 (Coll et al., 2010). Besides these Arabidopsis AtMC4 is found to be a positive regulator of cell death in abiotic and biotic stress (Watanabe & Lam, 2011). Another Arabidopsis AtMC9 is found to be involved in cell corpus clearance of xylem vessel elements. Experimental data suggests that it is part of a proteolytic cascade during xylem cell autolysis (Bollhoner et al., 2013). In addition, metacaspases have also been discovered in other plants and indicated to have roles in cell death, stress and hypersensitive responses (HR). In *Nicotiana benthamiana,* type II metacaspases were involved in the resistance against *Colletotrichum destructivum* (Hsiang, 2007). In *Capsicum annuum,* CaMC9 function during cell death induced by pathogen (Kim et al., 2013). Wheat TaMCA4 shown to function in PCD induced by a fungal pathogen (Wang et al., 2012). In tomato, metacaspase LeMCA1 was found to be upregulated in *Botrytis cinerea* infected leaves (Hoeberichts et al., 2003).

A very few substrates of metacaspase have been identified. The first substrate of plant metacaspase identified was Tudor staphylococcal nuclease (Tsiatsiani et al., 2011. In *in vitro* conditions, it act as a common substrate for both *picea abies* metacaspase mcII-Pa and *Homo sapience* caspase-3, suggesting that MCA2pa can catalyse and cause cell death similar to caspases (Tsiatsiani et al., 2011). Arabidopsis type II metacaspase AtMC9 has also found to cleave AtSerpin1 (Vercammen et al., 2006).

Several abiotic, biotic factors and ROS molecules have also been demonstrated to upregulate plant metacaspases (Dubey et al., 2019). Cell death was reduced in the AtMC8 knockout

mutant after H_2O_2 treatment indicating that AtMC8 is triggered and regulate oxidative stress induced cell death in Arabidopsis (He et al., 2008). Arabidopsis AtMC4 has been shown to protect cell death triggered by fungal toxin (Watanabe and Lam, 2011). Type I AtMC1 is found to interact with hypersensitive response mutant LSD1 and regulate hypersensitive responses (HR) in Arabidopsis (Coll et al., 2010).

Various reports as highlighted in review of literature and summarized in Tables 1-3 to show the importance of plant metacaspases in development regulated programmed cell death and in cell death induced by various abiotic and biotic stress. However, despite various work done so far there is still fragmentary evidence to suggest the exact roles and molecular mechanisms of these metacaspases in various cellular processes in plants. We need to determine the roles of metacaspases in pro-death and pro-survival responses, vegetative and reproductive development, senescence, protein aggregate clearance and also their cross-talk with autophagic and other related pathways. In the tomato genome, eight metacaspases have been identified, of which six are type I and two are type II metacaspases and function of none these have been characterized in plants (Dubey et al., 2019; Liu et al., 2016). There are some reports to show that metacaspases are differentially expressed during plant development and also induced by various abiotic and biotic stresses (Hoeberichts et al., 2003; Liu et al., 2016). In the present work, functional role of one of the most important typeII metacaspase named as SolycMC4, a close homolog of Arabidopsis AtMC4 is characterized in plant development using various molecular genetics and biochemical approaches.

Table 3: Identification and the functional roles of metacaspases in plants

Plant species	Metacaspase name	Metacaspase function	References
A. thaliana	Metacaspase, Type I, II	Identified paracaspases and metacaspases	(Uren et al., 2000)
Picea abies	Type II, MCII-Pa	MCII-Pa is essential for suspensor cell death during embryo formation	(Suarez et al., 2004)
P. abies	Type II, MCII-Pa	Established metacaspase role as an executioner of PCD during embryo formation	(Bozhkov et al., 2005)
P. abies	Type II, MCII-Pa	Indicated vacuolar cell death in plants	(Minina et al., 2013)
A. thaliana	Type I, II	Identified type I and Type II metacaspases in Arabidopsis	(Watanabe and Lam, 2004)
A. thaliana	AtMC4, AtMC9, Type II	AtMC4 and AtMC9 cleave substrates after arginine and lysine residue	(Vercammen et al., 2006)
A. thaliana	Type I, II	Plant protease inhibition by a plant serpin	(Vercammen et al., 2006)
A. thaliana	AtMC9, Type II	S-nitrosylation involved in regulation of AtMC9 proteolytic activity	(Belenghi et al., 2007)
A. thaliana	AtMC8, Type II	AtMC8 regulates PCD induced by oxidative stress	(He et al., 2008)
A. thaliana	AtMCP1b, Type I	AtMCP1b expressed in vascular tissue after pathogen infection and wounding	(Castillo-Olamendi et al., 2008)
A. thaliana	Metacaspase, Type II	type II metacaspase is linked with viability of cell	(Woltering, 2010)
A. thaliana	Type I, AtMC1, AtMC2	AtMC1 and AtMC2 control HR cell death	(Coll et al., 2010)
A. thaliana	AtMCP2d, Type II	AtMCP2d is found to be positive regulator of PCD induced during biotic and abiotic stresses and Calcium-dependent activation and autolysis of AtMCP2d	(Watanabe and Lam, 2011) Click or tap here to enter text.

A. thaliana	AtMC9, Type II	AtMC9 plays a role during post mortem function in xylem development	(Bollhöner et al., 2013)
A. thaliana	AtMC9, Type II	AtMC9 degradome analysed	(Tsiatsiani et al., 2011)
A. thaliana	AtMC1, Type I	AtMC1 involved in pathogen-triggered programmed cell death and aging	(Coll et al., 2010)
A. thaliana	AtMC9, Type II	AtMC9 modulates autophagy during xylem differentiation	(Escamez et al., 2016)
A. thaliana	AtMC1, Type I	AtSERPIN1 inhibits AtMC1-mediated PCD in plants	(Lema Asqui et al., 2018)
A. thaliana	AtMC4, Type II	The AtMC4 regulates the stem cell homeostasis in Arabidopsis	(Huang et al., 2018)
A. thaliana	AtMC4, Type II	wounding activates Ca^{2+}-dependent metacaspase for release of immunomodulatory peptides	(Hander et al., 2019)
A. thaliana	AtMC4, Type II	Metacaspases prepare peptides for cleavage	(Hou et al., 2019)
A. thaliana	Metacaspase, Type II	Type-II MCs mediate the processing of plant elicitor peptides	(Shen et al., 2019)
A. thaliana	AtMC4, Type II	Structural analysis of MCs for its Ca^{2+}-dependent activation	(Zhu et al., 2020)
A. thaliana	Type I, II	Programmed cell death (PCD) control in Arabidopsis	(Valandro et al., 2020)
Solanum lycopersicum	LeMCA1, Type II	Expression levels of LeMCA1 rapidly increased upon infection with fungal pathogen *Botrytis cinerea*.	(Hoeberichts et al., 2003)
S. lycopersicum	LeMCA1, Type II	Biochemical characterization of LeMCA1	(Wen et al., 2013)
S. lycopersicum	Type I, II	Identification and analysis of the metacaspases in tomato.	(Liu, et al., 2016)
Nicotiana Benthamiana	Type II, NbMCA1	NbMCA1 involved in stress adaptation	(Hsiang, 2007)
Nicotiana tabacum	NtMC1, Type II	Two aspartate residues of p10 domain contribute to substrate-binding	(Acosta-Maspons et al., 2014)
Triticum vulgare	TaeMCAII, Type II	Characterization of type II Metacaspase, TaeMCAII	(Piszczek et al., 2012)
Triticum aestivum	TaMCA4, Type II	TaMCA4 plays role in PCD triggered by *Puccinia striiformis*	(Wang et al., 2012)

T. aestivum	TaMCA1, Type I	TaMCA1 contribute to *Puccinia striiformis infection in wheat*	(Hao et al., 2016)
T. aestivum	TaMCAII	Expression of TaMCAII under high temperature stress	(Topchieva et al., 2017)
T. aestivum	Metacaspase	Caspase-Like and Metacaspase-like activities during biotic stress and its proteomics	(Balakireva et al., 2018)
Zea mays	Type II	Ozone and aging up-regulates metacaspase expression and its activity	(Ahmad et al., 2012)
Z. mays	Type I	Maize metacaspases modulate the defence response	(Luan et al., 2021)
Z. mays	Type I, II	Potential roles of different metacaspases in maize defence response	(Ma et al., 2021)
Capsicum annuum	CaMC9, Type II,	Role of CaMC9 characterised in cell death induced by pathogen	(Kim et al., 2013)
Vitis vinifera	Type I, II	Identification and expression analysis of Metacaspases in grape	(Zhang et al., 2013)
Oryza sativa	Type I, II	Identification and characterization of metacaspases in rice	(Wang and Zhang, 2014)
O. sativa	Type I, II	Characterization of metacaspases in cultivated and wild rice	(Bansal et al., 2020)
Hevea brasiliensis	Type I and II	Identification of the metacaspases in rubber plant	(Liu, Deng, et al., 2016)
H. brasiliensis	HbMC1, Type I	HbMC1 mediate PCD in rubber plant	(Liu et al., 2019)
Litchi chinensis	LcMCII-1	Characterization of LcMCII-1 in the ROS induced leaf senescence	(Wang et al., 2016)
Populus tremula × *tremuloides*	PttMC13, PttMC14, Type II	Characterization of PttMC13 and PttMC14 in xylem formation	(Bollhöner et al., 2018)
Hordeum vulgare	Type I, II	Identification and role of metacaspases in boron-induced PCD	(Bostancioglu et al., 2018)
Cucumis sativus	Type I, II	Identification and expression analysis of the metacaspase	(Zhou et al., 2018)
Rosaceae family (*Fragaria vesca, Prunus mume, Prunus persica, Pyrus communis, Pyrus*	Type I, II	Comparative genomic and expression analysis of metacaspases during pear pollen tube and fruit development in Rosaceae plants	(Cao et al., 2019)

bretschneideri and *Malus domestica*)			
Solanum tuberosum	Type I, II	Identification and expression analysis of the metacaspases in potato	(Dubey et al., 2019)
Gossypium raimondii, *Gossypium barbadense*, *Gossypium hirsutum*, *Gossypium arboretum*	Type I, II	Identification of the Metacaspases in cotton	(Fan et al., 2019)
Pyrus bretschneideri	PbMC1a/1b	PbMC1a/1b involved in lignin formation in fruit stone cell formation n	(Gong et al., 2020)
Brassica napus	Metacaspase	Metacaspase involved in autophagy-dependent cell death in *brassica* microspore embryogenesis	(Berenguer et al., 2021)

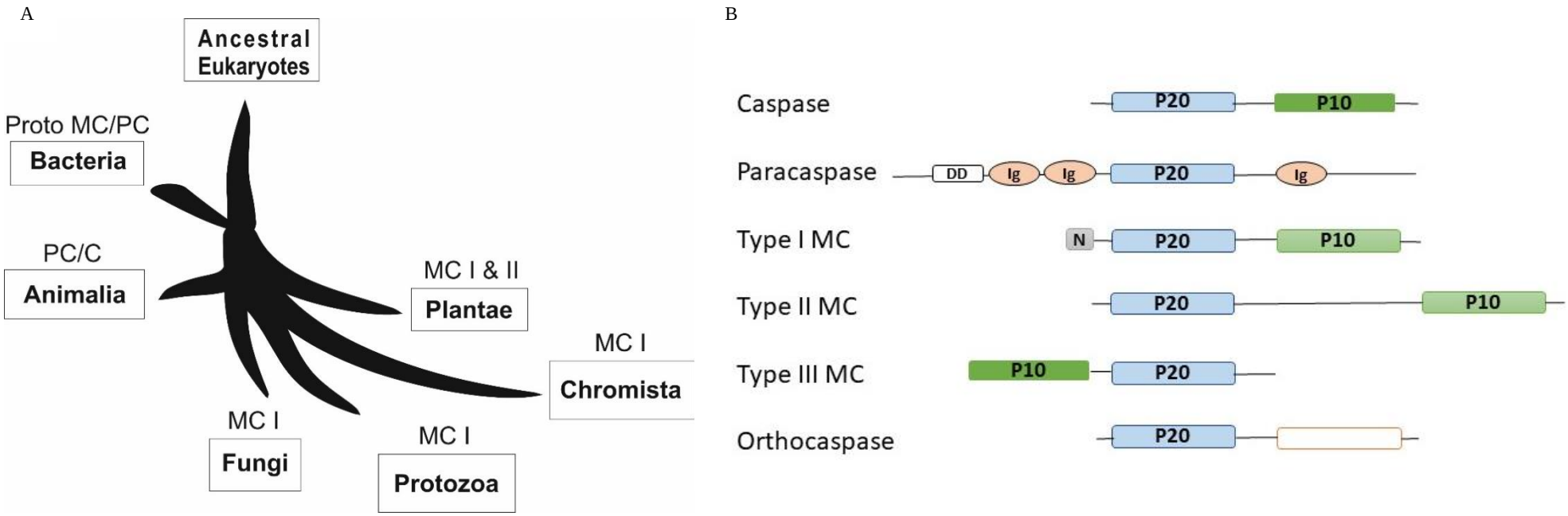

Figure 1: Evolutionary relationship (A) and distribution and domain structure (B) of caspases, paracaspases and metacaspases in different kingdoms.