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Abiotic Stress-Induced Programmed Cell Death in Plants: A Phytaspase Connection

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1. Introduction

The plants in the course of ontogenesis are frequently exposed to various unfavourable environmental stress factors (such as high and low temperatures, heavy metals, salt and anaerobic stress, biotic stresses, etc.). If extreme, these stress factors can bring about damage of agricultural crops and wild flora causing considerable environmental and economic losses worldwide.

In the course of evolution, and also due to targeted selection, many plant species have developed the capacity for adaptation to some extent to these unfavourable stresses. Under these circumstances adaptive processes in plants are aimed on sustainability of the plant cell and thus on survival of the whole organism. It is worth noting that plants have also developed alternative adaptive mechanisms which are aimed at selective death of the cells and tissues under abiotic and biotic stresses rather than on their survival. Such selective programmed cell death (PCD) eventually provides survival benefits for the whole organism under extreme conditions (Drew et al., 2000; Jackson & Armstrong, 1999).

This review focuses on abiotic stress-induced PCD in plants and highlights the role of a newly discovered plant protease, phytaspase, in this process. We describe the role of phytaspase in PCD execution, structure and properties of the enzyme, and its intriguing trafficking in response to stress stimuli. Parallels between the mechanisms of PCD in plants and animals are drawn, highlighting both similarities and differences of the apoptotic proteases involved and the strategies to keep them under control.

2. Abiotic stress-induced programmed cell death responses in plants

The two above mentioned distinct strategies of plant adaptation to unfavourable abiotic stresses are most pronounced under anaerobic stress (anoxia and hypoxia) during ontogenesis of rice (Vartapetian et al., 2008). Rice is a unique agricultural crop that readily inhabits waterlogged and flooded anaerobic soils, which occupy vast territories of our planet. In such soils anaerobic conditions are caused due to low solubility and slow

diffusion of molecular oxygen in water. Like other higher plants, rice is an obligate aerobe and needs a permanent supply of environmental oxygen. However, in contrast to the other agricultural crops, the evolution of this species has resulted in the two above mentioned opposite adaptive strategies to hypoxia and anoxia. The first adaptive strategy is aimed at the survival of the cells and the whole rice seedling during germination under hypoxia and even anoxia, when respiration, which is the main energy-supplying mechanism of aerobes, is entirely arrested. Under these extreme conditions, which virtually preclude aerobic respiration of the plant cells, the rice seeds have developed the capacity to support not only the germinating ability but also the active growth of the seedling due to stimulation of cell anaerobic energy metabolism (Magneschi & Perata, 2009).

However, the rice plant has also developed the mechanism of adaptation to anaerobic stress, which is paradoxically aimed on selective death of the root and shoot cells (Drew et al., 2000). This mechanism based on the pivotal role of the PCD starts up when anaerobically sprouted seedlings, having pushed through the water layer and come in contact with atmospheric air, start forming the root system. The cell death results in the formation of continuous air cavities (aerenchyma), which readily enable the oxygen transport from overground aerated organs to the roots and survival of the whole plant under these unfavourable conditions.

PCD, as a way of cell demise, is operative in plant responses to various other abiotic stresses such as salinity, cold and heat stress, UV, oxidative stress, etc. Therefore stress-induced PCD responses are of considerable interest, not only from the fundamental perspective but also in the context of applied agriculture, ecology and environmental protection. Elucidation of the mechanisms involving specific enzyme systems in these processes lays foundation for future development of biotechnological methods enabling the creation of plants that are more tolerant to these stress conditions.

3. Aspartate-specific proteases in programmed cell death

PCD is a fundamental process that operates during tissue development and homeostasis of a multicellular organism. This process employs suicide molecular machinery which is activated in order to rapidly destroy the cell. PCD helps maintain tissue balance by providing the means to eliminate redundant cells. It also plays a crucial role during a response to various abiotic stress factors by dismantling a limited number of affected cells to prevent severe damage to the whole organism. In animal organisms, one of the best characterized forms of PCD is apoptosis (Thompson, 1995). Essential molecular components of apoptosis are caspases – a family of highly specific cysteine proteases which execute apoptosis by cleaving specific cellular protein substrates after aspartate residues within a specific (usually tetrapeptide) recognition site (Cohen, 1997). Caspases are stored in the cytoplasm as latent zymogens (inactive precursors) and are processed in response to a variety of death stimuli to generate a mature active enzyme. Caspases divide in two groups: initiator and effector caspases. Initiator caspases (e.g. caspases-2, -8, -9 and -10) cleave inactive zymogens of effector caspases, thus activating them. Effector caspases (e.g. caspases-3, -6 and -7) in turn cleave other protein substrates within the cell, among which are proteins and enzymes of a crucial importance for the cellular homeostasis (Chang & Yang, 2000). Due to the supremely important role that caspases play in apoptosis, it is imperative that their activation is strictly controlled. Caspase activity is tightly regulated by

the caspase inhibitors, thereby inhibiting of the inappropriately activated caspases leads to suppression of apoptosis and survival of the cell (Brady & Duckett, 2009).

Animal and plant PCD have some features in common, such as nuclear DNA fragmentation (laddering) (Ryerson & Heath, 1996), release of cytochrome *c* from mitochondria, cytoplasm shrinkage (Levine et al., 1996), and cellular plasma membrane blabbing (Lam & del Pozo, 2000). However, bioinformatics studies of the plant genomes sequenced thus far have failed to reveal direct caspase homologues in plants. Plants do possess metacaspases – a family of distant caspase homologues, which may be involved in PCD, but possess specificity distinct from that of caspases (substrate cleavage after basic amino acid residues, Arg and Lys, not after Asp) (Aravind et al., 1999; Vercammen et al., 2006; Watanabe & Lam, 2005).

However, it is becoming increasingly evident, that plants employ the caspase-like (that is, Asp-specific) activity during the PCD. In various plant PCD models, application of synthetic peptide inhibitors based on the recognition sites of the specific members of the animal caspase family was reported to suppress PCD (Bonneau et al., 2008; del Pozo & Lam, 1998). Likewise, protein caspase inhibitors, such as p35 from baculovirus and IAP, having been synthesized in plants, counteract the PCD (Danon et al., 2004; del Pozo & Lam, 2003; Dickman et al., 2001). Conforming to these results, hydrolysis of peptide-based caspase substrates has been observed during the PCD in different plant systems. The absence of the caspase family genes in plant genomes therefore raises the possibility that plants possess functional, rather than structural, analogues of caspases.

Until recently, scarce information about plant PCD-related proteases with the 'caspase-like' activity was available, the unidentified putative caspase-like enzymes being named VEIDase, YVADase, VADase, DEVDase, etc., in accord with their peptide cleavage specificity (Bonneau et al., 2008). In rare cases, however, plant enzymes possessing some of the above mentioned 'caspase-like' activities have been identified. For example, vacuolar processing enzyme (VPE), which is an asparaginyl-specific cysteine protease distantly related to caspases, was shown to display an YVADase activity as well (Hatsugai et al., 2004; Rojo et al., 2004). Recently, a DEVDase activity was attributed to the *Arabidopsis* PBA1, a proteasome subunit (Hatsugai et al., 2009).

In this review, we will focus on the newly identified plant protease named phytaspase (for plant aspartate-specific protease) (Chichkova et al., 2010). Being structurally completely different from animal caspases, phytaspase possesses a 'caspase-like' cleavage specificity and its activity is essential for accomplishment of PCD induced by a variety of stress stimuli. Phytaspase thus appears to represent a functional analogue of the animal caspases.

4. Identification of the phytaspase

Nearly a decade ago, while studying a human protein prothymosin alpha (ProTa) it was noticed that ProTa is subject to caspase fragmentation in the course of apoptosis caused by a variety of death-inducing stimuli, including abiotic stresses (Evstafieva et al., 2000; Evstafieva et al., 2003). ProTa is a proliferation-related protein localizing in the nucleus where it is engaged in activity regulation of several stress-related transcription factors, such as p53 tumour suppressor and Nrf2 (Karapetian et al., 2005; Kobayashi et al., 2006; Zakharova et al., 2008). Caspase-3-mediated cleavage detaches a short C-terminal peptide of ProTa. This, however, exerts a profound effect on the fate of ProTa because this very region encompasses a nuclear localization signal (NLS) that drives protein import into the nucleus

(Rubtsov et al., 1997). As a result of caspase-mediated truncation, ProTa loses its ability to accumulate in the nucleus and, furthermore, becomes surface exposed by dying human cells (Evstafieva et al., 2003).

To learn whether some other proteins could follow this exciting scheme of relocalization in response to caspase-mediated fragmentation, a bioinformatics search for proteins containing a putative NLS lying close to a putative caspase cleavage site was performed. Among many hits obtained, one was particularly astonishing, predicting that a bacterial protein possesses an NLS and a caspase cleavage site nearby. It was realized however that this protein, VirD2, is encoded by *Agrobacterium tumefaciens*, a plant pathogen which causes the crown gall disease. Furthermore, one of the VirD2 functions is to guide a segment of bacterial DNA (T-DNA) into the plant cell nucleus to achieve transformation of the host cell (Pitzschke & Hirt, 2010). For this purpose, the bacterially encoded VirD2 protein is indeed equipped with an NLS positioned close to the C-terminus of the protein (Howard et al., 1992), which perfectly matched with the predicted one.

The second prediction came true as well. Caspase-3, the major human executioner caspase, has been shown to cleave VirD2 *in vitro* at a $TATD^{400}$ site close to the C-terminus of the protein, just upstream from the NLS. This observation has raised the possibility that such VirD2 fragmentation, if accomplished in plant cell by a plant caspase-like protease, could make sense to protect plants from transformation by limiting delivery of foreign DNA into the nucleus.

In planta studies involving a VirD2 C-terminal region (VirD2Ct)-based fluorescent reporter GFP-VirD2Ct have revealed that during the tobacco mosaic virus (TMV)-mediated PCD in *Nicotiana tabacum* leaves a caspase-like protease is activated which cleaves the VirD2 moiety at the same TATD motif (Chichkova et al., 2004). A substitution of D residue with A abolished the cleavage of GFP-VirD2Ct, testifying to the unique D-specificity of the newly discovered protease.

A synthetic peptide inhibitor designed based on the TATD-recognition motif has been shown to inactivate the enzyme and to suppress the development of the TMV-induced PCD in *Nicotiana tabacum* leaves, pointing to an essential role for this protease in PCD execution (Chichkova et al., 2004). The protease was named "phytaspase" in respect to its caspase-like activity, and phytaspase activity was detected in many plant species, both di- and monocotyledonous (Chichkova et al., 2008).

It was also found that healthy plant tissue wounding/disruption produces high level of phytaspase activity in extracts, indicating that mechanical stress, as well as PCD, causes rapid activation (or de-sequestration) of the enzyme.

5. Structure of phytaspases

Phytaspases from *Nicotiana tabacum* and *Oryza sativa* were purified, identified, and their cDNAs cloned (Chichkova et al., 2010). The enzymes from both species turned out to be homologous and belong to the S8A subtilisin-like (that is, Ser-dependent) protease family (as all known plant subtilases are). Alignment of phytaspases with known subtilases of bacterial and plant origin revealed a canonical triad of catalytic amino acid residues: Asp149, His220 and Ser537. Accordingly, mutating the predicted active site Ser^{537} produced an inactive enzyme. Analysis of the primary structure indicated that phytaspases are synthesized as precursor proteins, with the hydrophobic N-terminal signal peptide and the prodomain preceding the protease domain of the enzyme, which is typical for subtilases

(Schaller, 2004) – Figure 1. Phylogenetic comparison of tobacco and rice phytaspases with the *Arabidopsis* subtilisin-like proteins places phytaspases as a branch within the Subgroup 1 of *Arabidopsis* subtilases (Vartapetian et al., 2011).

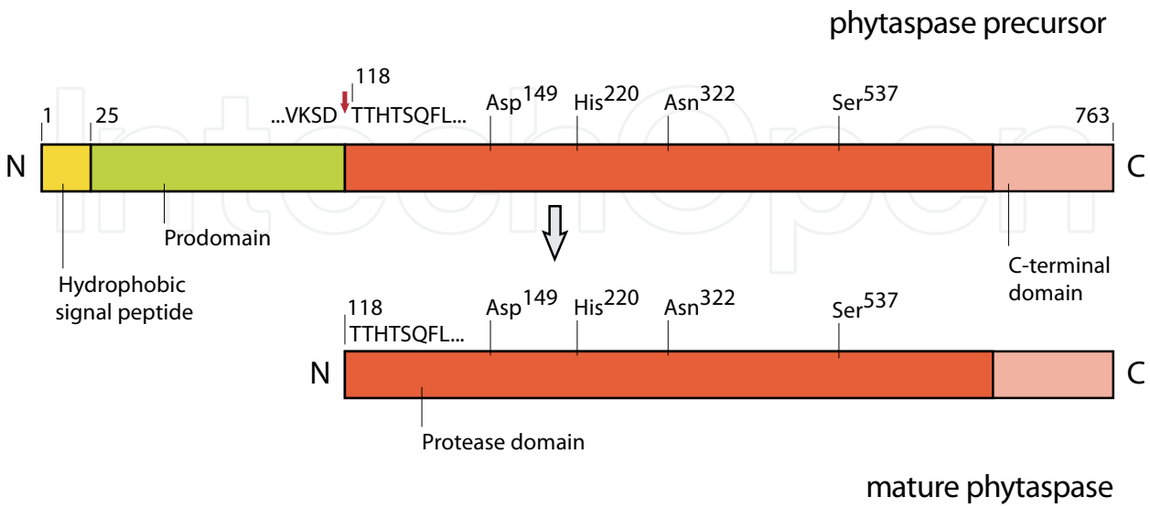

Fig. 1. Schematic representation of the pre-pro-phytaspase and the mature form of phytaspase. Amino acid numbering is given for tobacco phytaspase (MEROPS ID S08.150). Asp^{149}, His^{220} and Ser^{537} are active site residues; Asn^{322} is the oxyanion hole residue. Red arrow points to the prodomain cleavage site. Note that autocatalytic Asp-specific prodomain cleavage conforms to the caspase-like specificity of phytaspase.

6. Involvement of phytaspase in the abiotic stress response

In order to assess possible impact of phytaspase activity on plant development and response to stress factors, *Nicotiana tabacum* transgenic plants were constructed that either overproduced tobacco phytaspase, or possessed markedly decreased level of phytaspase gene expression due to the RNAi silencing. Notably, despite the significant variations in phytaspase activity levels achieved, under normal growth conditions neither overproduction, nor gene silencing has resulted in any discernible phenotype as compared to the wild type plants. Increased enzymatic activity could possibly undergo neutrally due to the tight regulation and a specific tissue localization of phytaspase under the normal conditions (described below). The silencing experiments suggest that functional redundancy may exist and another enzyme may compensate for the low level of phytaspase during plant growth and development.

Possible involvement of phytaspase in the PCD-related response to abiotic stresses, such as oxidative stress and osmotic stress, was assessed by treating leaf discs of transgenic tobacco plants with methyl viologen (MV) or with NaCl at elevated concentrations. Methyl viologen (paraquat) is known to be a toxic agent for both animals and plants. Upon contact with a cell, this redox-active compound intercalates into the intracellular electron transfer systems and causes uncontrollable formation of reactive oxygen species (ROS), leading to extensive damage of the macromolecules, including intracellular membrane components, and therefore destruction of the organelles of the cell. In plant cells, methyl viologen prevents the reduction of NADP to NADPH during photosynthesis, and thereby causes rapid

elimination of the chloroplasts and bleaching of the leaf tissues (Fujibe et al., 2004). The oxidative stress caused by methyl viologen leads to rapid development of PCD in plant cells. The effect of NaCl at concentrations exceeding the physiological rate represents a form of abiotic stress, similar to salinization of soil. Exposure to relatively high concentrations of NaCl causes a misbalance of cellular ion concentrations, osmotic shock and oxidative stress. Salt-induced PCD response, as well as treatment with methyl viologen, leads to rapid bleaching of the tissues and loss of viability (Huh et al., 2002).

Treatment with these stress-inducing agents produced characteristic molecular manifestations of PCD in plants, such as ROS accumulation (H_2O_2 production) and release of cytochrome *c* from mitochondria, which preceded the morphological changes (Chichkova et al., 2010). Both molecular and morphological changes have been shown to develop more rapidly in the phytaspase overexpressing line of tobacco plants, comparing to the wild type plants in response to treatment with 2.5 mM MV and with 75 mM NaCl. At the same time, even being exposed to the higher concentrations of stress-inducing agents (10 mM MV and at 250 mM NaCl), the phytaspase gene knockdown plants showed considerably improved viability comparing to the wild type plants. Expression of the active rice phytaspase in the RNAi knockdown tobacco plants restored the wild type phenotype (bleaching and the H_2O_2 levels), while expression of the catalytically inactive mutant did not, indicating a role for phytaspase proteolytic activity in the PCD execution.

7. Cleavage specificity of phytaspases

Phytaspase, like animal caspases, displays a unique specificity towards the Asp residue in the P_1 position of a substrate. Such a stringent specificity is quite unusual for subtilases. Most of the subtilisin-like proteases either display limited specificity, or possess only partial substrate selectivity towards different groups of amino acid residues. For instance, cleavage by bacterial subtilisin A typically occurs adjacent to large uncharged residues (Bryan et al., 1986). Likewise, proteinase K, a typical member of the subtilisin-like protease family, displays broad specificity towards aliphatic and aromatic amino acid residues and is frequently employed for complete protein digestion (Kraus & Femfert, 1976). Plant subtilases are usually also devoid of strict cleavage specificity (Beers et al., 2000). However, remarkable phytaspase specificity demonstrates that the structure of plant subtilisin-like proteins is able to support different levels of substrate selectivity, including the most rigorous and specific ones. It is also evident that phytaspases are processive, rather than digestive, proteolytic enzymes.

Besides a requirement for D at the P_1 position of a substrate, phytaspases display selectivity towards a preceding amino acid motif. By using peptide-based fluorogenic substrates of caspases, it was found that Ac-VEID-AFC (AFC is 7-amino-4-trifluoromethylcoumarin) is the optimal substrate (among the ones tested) for both tobacco and rice phytaspases. Several other substrates, such as YVAD-, VAD-, IETD- and LEHD-AFC are also cleaved albeit somewhat less efficiently, whereas the DEVD-AFC is not cleaved by phytaspases at all. Consistent with these results, peptide aldehyde caspase inhibitors based on the same amino acid motifs could reversibly inactivate phytaspases, Ac-VEID-CHO being the most potent inhibitor, whereas Ac-DEVD-CHO failed to inhibit phytaspases (Chichkova et al., 2010). Interestingly, the STATD motif representing an efficient phytaspase cleavage site in the VirD2 protein turned out to be a rather poor phytaspase substrate at the peptide level. This suggests that the activity of phytaspase may not only depend on the amino acid sequence of

the cleavage site, but also on the position, steric availability and (or) the overall context of the cleavage site within the protein molecule.
Of note, an unidentified subtilisin-like protease from oat (saspase) was reported to cleave various caspase peptide-based substrates as well, with the same exception of DEVD-AFC. However, cleavage specificities of phytaspases and saspases are markedly distinct, as VEID-AFC, an optimal phytaspase substrate was not cleaved by saspase at all (Coffeen & Wolpert, 2004).
Stringent hydrolytic specificity of phytaspases resembles that of animal and yeast subtilisin-like proteases called proprotein converting enzymes (convertases). Convertases belong to the S8B subfamily of subtilases which appear to be absent from plants (Tripathi & Sowdhamini, 2006). Convertases are involved in proteolytic processing of precursors to generate bioactive proteins and peptides (Seidah & Chretien, 1999). Unlike phytaspases, however, convertases introduce cleavage after a basic amino acid residue (Lys, Arg), not after Asp.

8. Phytaspase localization and processing

In healthy plant tissues, phytaspase was shown to accumulate in the apoplast in the processed form lacking the signal peptide and the prodomain. This was elucidated by biochemical fractionation and by tracing the phytaspase linked to a fluorescent protein in *N. tabacum* leaves (Chichkova et al., 2010) – Figure 2A. The apoplast separates the plasma membrane and the cellular wall and forms a continuous film of fluid on the cell exterior. It facilitates the transport of water and solutes across a tissue, and moreover, it is considered to play a key role in plant resistance to multiple abiotic stress factors, such as draught, low temperatures and salinization of soil. All of these stress conditions lead to a rapid alkalization of the apoplast with characteristic times of minutes after exposure to the stress factor. As long as the apoplastic fluid spreads from roots to the shoots of the plants, it seems to be capable of rapid transfer of the signal across the tissues (Felle et al., 2005). Apart from responding to various abiotic challenges, the apoplast also mediates plant hormone signaling and participates in plant organism homeostasis (Matsubayashi, 2003; Ryan et al., 2002). Secretory proteins are usually transferred and concentrate in the apoplast media. Many plant proteases undergo this secretory pathway, including those of the subtilisin-like proteases involved in plant response to stresses.
Application of brefeldin A, an inhibitor of secretion, resulted in retention of the phytaspase-mRFP fusion inside the plant cells indicating that the canonical secretory pathway mediates translocation of the de novo synthesized phytaspase to the apoplast (Chichkova et al., 2010). Interestingly, the brefeldin A-mediated arrest of secretion did not severely affect the processing of phytaspase suggesting that secretion is not required for this process. Although processing of phytaspase does not directly depend on the secretion, some molecular events during the transit through the secretory pathway may favour this process. In this case, proteolytic activity of phytaspase would remain shaded by the prodomain until phytaspase had reached the extracellular fluid, thus protecting the cell from undesirable induction of PCD.
It is thus evident that in healthy plant tissues phytaspase is constitutively processed and secreted into the apoplast. It turned out that the phytaspase processing (detachment of the prodomain) occurs autocatalytically, as revealed by the properties of the phytaspase Ser537Ala mutant. The loss of the enzymatic activity by the mutant led to its complete

inability to detach the prodomain, which was demonstrated by Western blot assay (the unprocessed enzyme displays lower electrophoretic mobility comparing to the wild type protein), and further unambiguously ascertained by Edman degradation sequencing of the N-terminal region of the Ser537Ala mutant.

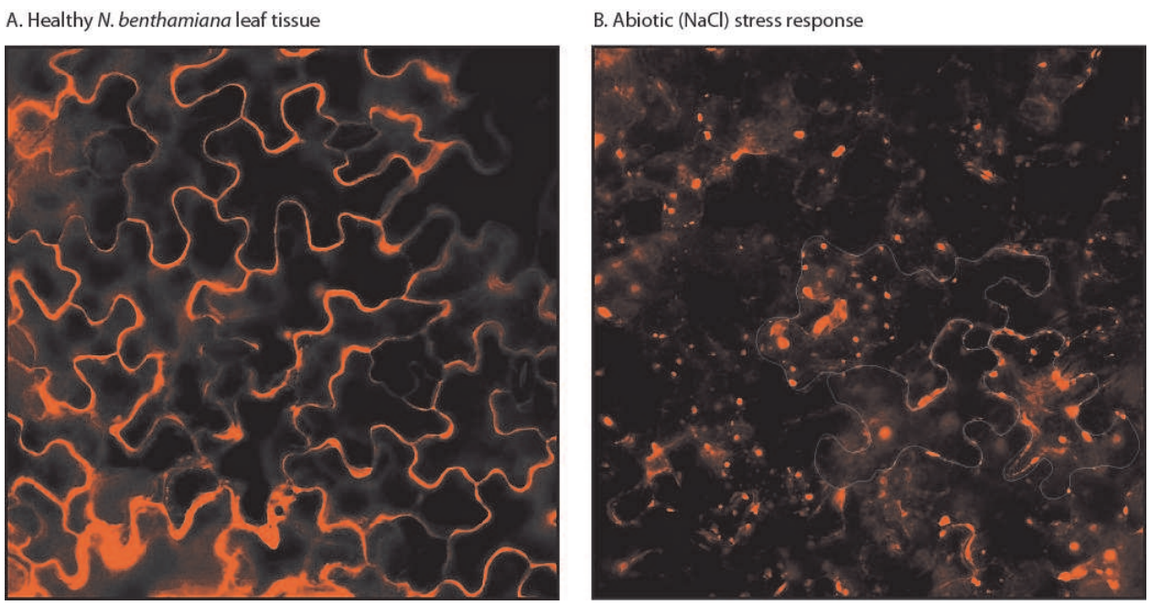

Fig. 2. Fluorescence microscopy visualization of phytaspase-EGFP in healthy (A) and osmotic stress-induced (B) *Nicotiana benthamiana* leaf tissues. PCD was induced in (B) by treatment with 300 mM NaCl for 6 h. The fluorescence colour has been changed to fit the colours given in Figures 1 and 3. White borders in (B) mark boundaries of the stressed cells, as visualized by the phase contrast.

In accordance with the phytaspase cleavage specificity, the C-terminal amino acid residue of the prodomain immediately preceding the prodomain cleavage site is D in both tobacco and rice phytaspases – Figure 1. Substitution of this Asp117 with Ala has impaired processing of the proenzyme (Chichkova et al., 2010). Of note, both of the phytaspase mutants with the processing defect, the Ser537Ala mutant and the Asp117Ala mutant, displayed impaired secretion into the apoplast, remaining mostly associated with the intracellular fraction. Cumulatively, these data suggest that the phytaspase processing, as a part of its normal maturation process, involves the enzymatic activity of phytaspase itself and occurs on the route of the enzyme to cell exterior.

9. Retrograde trafficking of phytaspase during the programmed cell death

Phytaspase is involved in the accomplishment of PCD in plants triggered by abiotic stresses. Furthermore, phytaspase-mediated proteolysis of an intracellular target protein was reported to occur in the course of PCD (Chichkova et al., 2004). It was however not immediately clear how phytaspase, being localized in the apoplast, could get access to cell interior.

An important observation, shedding light on this problem, was made using a phytaspase-mRFP fusion protein transiently produced in *N. tabacum* leaves subject to abiotic stresses,

such as treatment with MV or NaCl. It was found that phytaspase rapidly relocalizes from the apoplast to inside the cell in response to stress inducers (Chichkova et al., 2010) – Figure 2B. As the leaf treatment with cycloheximide, an inhibitor of protein biosynthesis, did not affect phytaspase relocalization, it appears likely that phytaspase accumulation within the dying cells originates from the redistribution of the presynthesized enzyme from the apoplast into the cytoplasm, rather than from the impairment of secretion of the newly synthesized protein.

The retrograde transport of the phytaspase to the cytoplasm appears to be specific, since another secreted protease, cathepsin B, under similar conditions retained its apoplastic localization.

A model describing phytaspase behaviour in healthy and dying plant cells has been suggested (Chichkova et al., 2010). According to this model (Figure 3), phytaspase is synthesized as a precursor protein which is constitutively secreted (due to the presence of a signal peptide) and autocatalytically processed on its route to the cell exterior. Mature

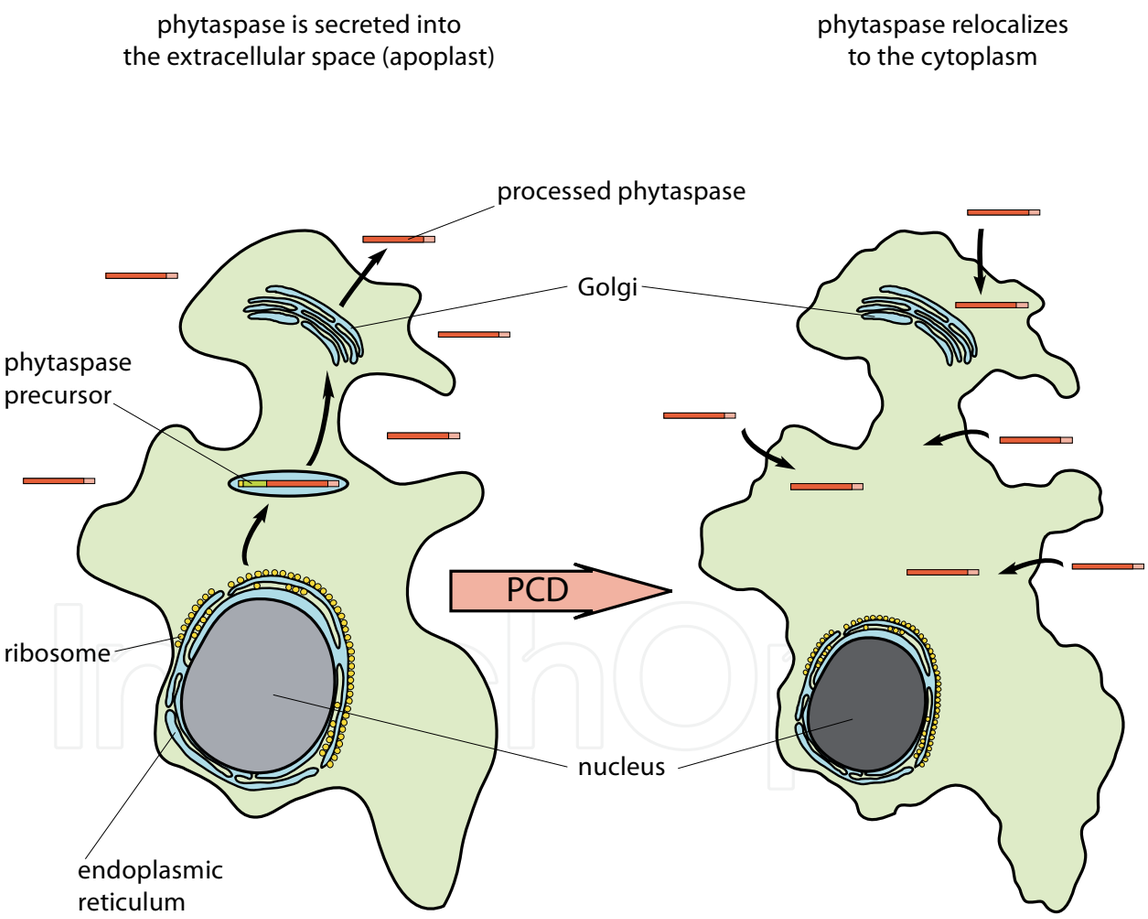

Fig. 3. Phytaspase trafficking in leaf tissue.

Left: Phytaspase is synthesized as an inactive precursor protein equipped with the N-terminal signal peptide and the prodomain. Within the secretory pathway, phytaspase undergoes autocatalytic processing, and the mature enzyme is accumulated in the apoplastic fluid.

Right: PCD-related abiotic stresses cause phytaspase relocalization from the apoplast to inside the cell.

phytaspase is stored within the apoplast without access to its intracellular targets, thus preventing unintended induction of cell death. Upon perception of PCD-inducing stimuli phytaspase becomes internalized via a yet undefined mechanism and participates in the accomplishment of PCD by introducing cleavages into target proteins.
The strategy employed by plant cells to control their death protease differs markedly from the one employed by animal cells. Animals store inactive (unprocessed) caspase precursors in the cytoplasm of healthy cells. Whenever an apoptogenic signal is received, caspases become activated via processing of their precursors which occurs in a form of a cascade. Caspase-mediated fragmentation of the target proteins ultimately causes cell death. The difference in the animal and plant strategies to manipulate with their PCD-related proteases may be indicative of additional role(s) played by phytaspase in the apoplast in the absence of cell death.

10. Role of phytaspase in biotic stress responses

In addition to its role in mediating plant PCD induced by abiotic stresses, phytaspase appears to be involved in protection of plants against pathogenic insults. One example is provided by the TMV infection of *N. tabacum* plants carrying the *N* gene. In this experimental system, hypersensitive response (HR, a form of plant PCD) is induced in infected plants due to the recognition of a viral protein by the *N* gene product (Erickson et al., 1999). This quick response serves to kill virus-infected cells and thus prevent virus multiplication and spread throughout the plant. It was demonstrated that the HR-associated cell death is markedly enhanced in phytaspase-overproducing plants and is suppressed in phytaspase-silenced plants (Chichkova et al., 2010). In accord with the protective role of phytaspase-mediated PCD, accumulation of TMV was suppressed in phytaspase overexpressors, whereas it was enhanced in phytaspase-silenced plants. Therefore, the PCD-promoting function of phytaspase confers resistance of tobacco plants to viral infection.
Another example of possible involvement of phytaspase in host-pathogen interactions comes from the initial observation that phytaspase is capable of introducing a cleavage into the C-terminal region of the VirD2 protein of *Agrobacterium tumefaciens* (Chichkova et al., 2004). This VirD2 region encompasses an NLS which allows VirD2 to direct the bacterial T-DNA complex into the plant cell nucleus to provide integration of bacterial DNA into the host genome and eventually plant cell transformation (Howard et al., 1992; Pitzschke & Hirt, 2010). Detachment of the NLS as a result of phytaspase-mediated VirD2 fragmentation is likely to interfere with the delivery of foreign DNA into the plant cell nucleus. In accordance with this scheme, an *Agrobacterium* strain encoding a mutant (phytaspase-resistant) VirD2 protein instead of the wild type one exhibited an enhanced capability to deliver and express foreign DNA in plant cell nucleus (Reavy et al., 2007) .
In general, apoplastic localization of phytaspase is consistent with a protective role which could be achieved by phytaspase-mediated fragmentation of the pathogen-encoded effector proteins. Examples of this kind are yet to be found.

11. Conclusions

PCD is an essential process which is frequently employed in animal and plant responses to abiotic stresses. Current data indicate that the role played by caspases in animal PCD is taken, at least in part, by plant subtilisin-like proteases, phytaspases. Caspases (Cys-dependent enzymes) and phytaspases (Ser-dependent proteases) are structurally very

different, yet they share Asp cleavage specificity and a role in PCD. Although both types of the proteolytic enzymes are synthesized as inactive precursors, plants and animals employ distinct strategies to further deal with their death proteases. Caspases are stored as latent precursors within the animal cells and become activated/processed in response to PCD-inducing stimuli to accomplish fragmentation of their target proteins. Unlike this scenario, phytaspase precursors are constitutively and autocatalytically processed even in the absence of PCD. To avoid unintended proteolysis, phytaspases are secreted out of the plant cells to physically separate the enzyme from its intracellular targets. Re-entering of phytaspases into the cell occurs upon the induction of PCD and is accompanied by cleavage of target proteins.

Therefore, PCD-related responses to abiotic stresses in animals and plants display both common and distinct features. Further studies aimed on the unravelling of the cellular proteins fragmented by phytaspase, as well as elucidation of the mechanism underlying the retrograde phytaspase trafficking in the course of PCD should provide important insights into molecular mechanisms of plant responses to stresses.

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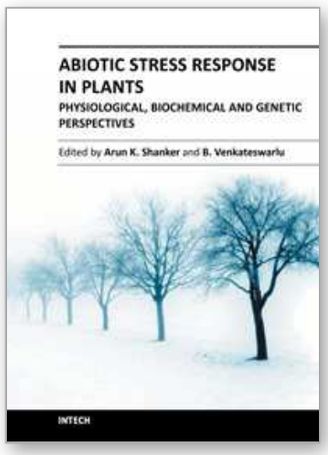

Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives

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Plants, unlike animals, are sessile. This demands that adverse changes in their environment are quickly recognized, distinguished and responded to with suitable reactions. Drought, heat, cold and salinity are among the major abiotic stresses that adversely affect plant growth and productivity. In general, abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that unfavorably affect plant growth, development and productivity. Drought, salinity, extreme temperatures (cold and heat) and oxidative stress are often interrelated; these conditions singularly or in combination induce cellular damage. To cope with abiotic stresses, of paramount significance is to understand plant responses to abiotic stresses that disturb the homeostatic equilibrium at cellular and molecular level in order to identify a common mechanism for multiple stress tolerance. This multi authored edited compilation attempts to put forth an all-inclusive biochemical and molecular picture in a systems approach wherein mechanism and adaptation aspects of abiotic stress are dealt with. The chief objective of the book hence is to deliver state of the art information for comprehending the effects of abiotic stress in plants at the cellular level.

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