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PLANT CHITINASES – REGULATION AND FUNCTION

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Abstract: The aim of this review is to present the current state of knowledge on plant chitinases and their regulation and function. Chitinases are up-regulated by a variety of stress conditions, both biotic and abiotic, and by such phytohormones as ethylene, jasmonic acid, and salicylic acid. Like other PR proteins, chitinases play a role in plant resistance against distinct pathogens. Moreover, by reducing the defence reaction of the plant, chitinases allow symbiotic interaction with nitrogen-fixing bacteria or mycorrhizal fungi. However, recent investigations have shown that these enzymes are also involved in numerous physiological events. The involvement of chitinases in development and growth processes is also described.

Key Words: Chitinases, Nod Factors, Chitoooligosaccharides

INTRODUCTION

Chitinases (EC 3.2.1.14) catalyse the hydrolytic cleavage of the β -1,4-glycoside bond present in biopolymers of *N*-acetylglucosamine, mainly in chitin. Chitinases are present in various organisms [1]. Depending on the organism of origin, these enzymes have different functions [1]. Bacterial chitinases are mainly involved in nutrition processes – they degrade chitin, delivering carbon and nitrogen to the cells [1, 2]. In yeast and various fungi, these enzymes participate in morphogenesis – they take part in remodelling cell wall structure and daughter cell separation, and also in some pathogenesis processes [1-3]. Chitinolytic activity was found in viruses, snails, fish, amphibians, mammals and also in gymnosperms and angiosperms, despite the fact that chitin is not

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Abbreviations used: 2,4-D - 2,4-dichlorophenoxyacetic acid; ACC - 1-aminocyclopropane-1-carboxylic acid; AGPs - arabinogalactan proteins; AVG - aminoethoxyvinylglycine; CHRK1 - chitinase-related receptor-like kinase; EP - extracellular protein; ERE - ethylene-responsive elements; EREBPs - ethylene-responsive element binding proteins; Nod - nodulation; OBF - *ocs* element binding factor; PCD - programmed cell death; PR - pathogenesis-related; ts - temperature-sensitive.

present in these organisms [1, 2]. In animals and plants, chitinases mainly play a role in the defence of the organism against pathogen attack [1, 2, 4]. The results of recent investigations indicate that in both animals and plants, chitinases may be involved not only in defence-related processes or general stress response but also in growth and development processes [5-9].

Tab. 1. Differences between the chitinases of glycoside hydrolase family 18 and 19.

Glycosidase family	Class of chitinase	Catalytic mechanism	Intermediate	Anomeric configuration of product	Inhibitors
18	III and V	substrate-assisted	oxazolinium ion	β	allosamidin
19	I, II, IV, VI and VII	acidic	oxocarbenium ion	α	amidines amidrazones

A number of proteins demonstrating chitinolytic activity were identified in plants [2, 5, 10, 11]. It is possible to find them in all organs and plant tissues, in both the apoplast and the vacuole. These proteins present a large and diverse group of enzymes; they differ not only in spatial and temporal localisation, but above all in their molecule structure and substrate specificity [5, 10]. Chitinases can be divided into two categories: exochitinases, demonstrating activity only for the non-reducing end of the chitin chain; and endochitinases, which hydrolyse internal β -1,4-glycoside bonds. Many plant endochitinases, especially those with a high isoelectric point, exhibit an additional lysozyme or lysozyme-like activity [5, 10, 12, 13]. Chitinases use two different hydrolytic mechanisms (Tab. 1) [14]. Substrate-assisted catalysis, characteristic for chitinases belonging to family 18, leads to retention of conformation at the anomeric carbon of the product [15]. The reaction of hydrolysis carried out by chitinases from family 19, using the mechanism of acid catalysis, inverts the anomeric configuration [16]. Higher plants synthesise 7 different classes of chitinases, which differ in protein structure, substrate specificity [10], mechanisms of catalysis and sensitivity to inhibitors; they probably evolved from different "protochitinase" coding sequences [11]. This diversity of plant chitinases raises the question of their functions. It seems that the role of plant chitinases does not solely consist of defence against pathogen attacks, especially since many of these enzymes do not show any antifungal properties in *in vitro* assays. Therefore, it would be interesting to know for what reason plants need so many and so diversified enzymes to hydrolyse polymers of *N*-acetylglucosamine.

SUBSTRATES OF CHITINASES

The main substrate of chitinases is chitin – a natural homopolymer of β -1,4-linked *N*-acetylglucosamine residues. Long, unbranched chains of chitin have a helical conformation [17]. One in six aminosaccharide residues can be devoid of an acetyl group. Deacetylation is a common process involved in the chitin-protein interaction [17]. Chitin deacetylation leads to chitosan formation. Chitin and chitosan can be found in fungal and algal cell walls, in bacteria, and in invertebrates (crustaceans, arachnids, insects); they are the components of the exoskeleton together with proteins and salts of calcium and magnesium [17].

Chitinases can also hydrolyse the lipochitooligosaccharides (Nod factors) produced by nitrogen-fixing bacteria [7, 12, 18, 19]. The Nod factors consist of the *N*-acetylglucosamine tetra- or pentamer backbone with an *N*-linked fatty acid moiety replacing the *N*-acetyl group on the non-reducing end. Moreover, the reducing end of the Nod factor aminosaccharide backbone undergoes, depending on the organism of origin, different types of modifications (e.g. acetylation, fucosylation, methylation, sulphurylation) [7, 18].

Many plant chitinases with additional lysozyme or lysozyme-like activity are able to cleave bacterial peptidoglycan, a polymer of β -1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues [5, 12].

An endogenous substrate for plant chitinases has not yet been found. However, there is strong evidence that these enzymes catalyse the hydrolytic decomposition of arabinogalactan proteins (AGPs) present in plants [20, 21]. Moreover, it is supposed that other *N*-acetylglucosamine-containing glycoproteins occurring in cell walls can be endogenous substrates for plant chitinases [12, 22-24].

CHITINASES – PR PROTEINS

With regard to physicochemical properties and enzymatic activity type, plant chitinases are classified as pathogenesis-related proteins (PR) [5]. First, it was known that the expression of genes encoding PR proteins are induced by pathogens: viruses, bacteria and fungi, as well as by pest attack. Subsequently, it was found that PR proteins (including chitinases) are induced by various stress factors, i.e. drought, salinity, wounding, heavy metals in their environment, endogenous and exogenous elicitor treatment, and plant growth regulators [19, 25-29]. Acidic PR proteins are induced by salicylic acid, whereas basic PR proteins are induced by ethylene or jasmonic acid [4, 27, 30-33]. The expression of some PR genes is constitutive, developmentally regulated, and tissue- and organ-specific. Based on biological properties, enzyme activity and coding sequence similarities, PR proteins are divided into 14 classes. Chitinases belong to 3 classes [34]. Class PR-3 includes chitinases of class Ia, Ib, II, IV, VI and VII, chitinases of class III belong to PR-8, and chitinases of class V to PR-11. Additionally, in class PR-4, some proteins with low endochitinase activity were

found among the chitin-binding proteins [35]. Most of class Ia, III and VI chitinases have a high pI and are located in vacuoles. Acidic chitinases belonging to class Ib, II, III, IV and VI are secreted to the apoplast [36].

REGULATION OF CHITINASE ACTIVITY

Constitutive forms of chitinases

In healthy plants, some forms of chitinases, both vacuolar and apoplastic, are synthesised constitutively [5, 9]. High, constitutive expression of class I chitinases was found in the roots and floral tissues of numerous plants [4, 5, 33, 37-39]. Class III chitinase transcripts are constitutively present in the vascular bundles, hydathodes and guard cells of *Cucumis sativus* and *Arabidopsis thaliana* [40, 41]. Moreover, the constitutive expression of genes encoding chitinases increases with the plant's age [33, 40]. Generally, higher chitinolytic activity is detected in old leaves than in young tissues. In *Cucumis sativus*, the expression of gene encoding class III chitinase increases gradually during the plant's growth. The level of gene transcript accumulation decreases from the base (the oldest part) to the top of the shoot (the youngest part) [40]. During the ageing progress, the plant has higher a competence for factors inducing the expression of chitinase-encoding genes; e.g. in *Arabidopsis thaliana* the level of basic chitinase gene expression under ethylene treatment conditions increases with plant age [33].

The pattern of expression of chitinases is developmentally and tissue- and organ-specifically regulated. The enzymes are present in the hydathodes, anthers, styles, ripening fruit, swelling seeds and micropyle of germinating seeds, and in the developing embryo and tuber buds [4, 9, 25, 37, 38, 42-47].

The induction of chitinases upon infection

Stimulation or induction of chitinase gene expression by pathogen attack is often observed [5, 48]. The character of this expression can be systemic or local [31, 49]. It depends on the infecting pathogen, its virulence and also on the particular chitinase class. In *Arabidopsis thaliana* plants, infection by an incompatible pathogen causes a rapid accumulation of class IV chitinase mRNA [31], whereas infection by a compatible pathogen switches on chitinase class III expression around necrosis caused by the progress of the disease [41]. The local induction of class IV chitinase genes in the place of infection by a compatible pathogen was observed in *Beta vulgaris* plants [50]. However, the enzymes had no influence on the disease's progress; in *in vitro* tests they showed no antifungal properties and the plants were pathogen sensitive [50]. In *Phaseolus vulgaris* roots infected with the compatible pathogen *Fusarium solani* f. sp. *phaseoli*, proteolytic processing of chitinase class IV occurred [51]. This processing was not detected in incompatible or symbiotic interactions. By contrast, in *Cucumis sativus*, a systemic induction of class III chitinases was correlated with systemic acquired resistance (SAR) progress. The appearance of these enzymes was also

caused by salicylic acid treatment [40].

Pathogen attack leads to an increase in the endogenous salicylic acid and jasmonic acid content in plants [27]. These compounds induce two different gene pools coding pathogenesis-related proteins, among them various chitinases [25, 27, 32]. Moreover, intensive ethylene production and tissue wounding are simultaneous with infection [4], and these facts additionally complicate the understanding of the mechanism of chitinase induction during disease development. In general, it seems that infection by an incompatible pathogen leads to faster, higher and often systemic induction and accumulation of chitinases. However, the attack of virulent pathogens in general induces chitinases to a lesser extent and more slowly, and often is not accompanied by their induction [49]. This is not a rule for all plant-pathogen interactions, nor for all chitinase isoforms.

Induction by ethylene

Plant exposure to ethylene usually leads to systemic induction of basic chitinases. In the promoter regions of some chitinase genes, especially members of class I, conservative motif GCC-box was found. This ethylene responsive element (ERE) binds transcription factors, EREBPs (Ethylene-Responsive-Element-Binding-Proteins), which activate the expression of ethylene response genes [52]. The GCC-box element is present in the promoters of numerous pathogen-inducible PR genes. However, this element has not been found in other ethylene regulated genes, e.g. those involved in fruit ripening [53].

It is supposed that the level of ethylene synthesis and the time of ethylene exposure affect wounding-inducible chitinase expression. Many authors observed that similar isoforms of ethylene-inducible chitinases were produced upon wounding [4, 25]. Such a correlation was not observed in other reports, [30, 33]. The reason for this contradiction can be the difference in the timing of wound-stimulated ethylene synthesis – some of these time intervals might not be long enough for chitinase gene induction [30]. There is also a possibility that chitinases synthesised upon wounding are not induced by ethylene derived from the damaged tissues, but that their expression is triggered by another wound signal transduction molecule (i.e. systemin) [54].

The induction of chitinases in *in vitro* conditions

The chitinases' induction in *in vitro* cultures revealed a very complicated pattern. In a *Nicotiana* sp. culture, the presence of auxin and cytokinin in the medium repressed the class I chitinases genes. After passage of the explants on the medium without these phytohormones, the induction of chitinases was triggered immediately [39]. In *Cucurbita* sp. callus and suspension culture, the basic chitinase gene was expressed both with and without 2,4-D presence in the nutrient medium [36]. The application of exogenous ethylene elevated the chitinase activity in *Discorea japonica* callus culture [53]. However, the relationship between the level of this gas and the chitinolytic activity is not so

clear. Firstly, there are contradictory reports on ethylene production and its level under *in vitro* conditions – the elevated production of this hormone was not found in all the types of culture [53]. Secondly, Siefert *et al.* [55] observed no positive, stimulating influence of ethylene, applied alone, on the induction of chitinases. It seems that in *Helianthus annuus* suspension culture, an increase in the ratio of ethylene precursor (ACC) to endogenous ethylene triggers the induction of chitinases. Moreover, the application of exogenous ethylene in this model system inhibited chitinase induction and synthesis [55]. Thirdly, the high activity of genes encoding OBF (*ocs* element binding factor) transcription factors was found in *in vitro* culture [53]. It is probably not the level of ethylene nor its proportion to ACC, but the increased activity of OBF transcription factors (which triggers the defence genes) is responsible for the induction of chitinase genes under *in vitro* conditions. [53].

Other inductive factors

In addition to the factors described above, the transcription of chitinase genes or an increase in chitinase activity may be induced by other external stimuli; e.g. wounding, drought, cold, ozone, heavy metals, excessive salinity and UV light [1, 4, 5, 26]. Cold acclimation and dehydration induce chitinase class II gene expression in *Cynodon* sp. [56]. In *Cucurbita* sp., the synthesis of basic extracellular chitinase is induced by wounding and osmotic stress [36]. Wounding also induces the expression of class I chitinase in *Brassica napus* roots [4], and leaf senescence triggers class IV chitinase gene expression [26]. Ozone treatment caused a rapid increase in intracellular chitinases in *Nicotiana tabacum* plants [57].

THE ROLE OF CHITINASES

Since the majority of chitinases are induced by stress factors, mainly by infection, and some isoforms show antifungal properties in *in vitro* assays, the role of chitinases is usually considered to be an active or passive defence mechanism against pathogens [4, 9, 43]. Developmentally-regulated induction of these enzymes in healthy tissues, e.g. in germinating seeds or ripening fruits, has been interpreted as a plant defence mechanism against possible pathogen attacks on sensitive or mechanically-unprotected organs [4, 32, 37, 38, 46]. However, it is now supposed that chitinases in healthy plant tissues can play a role other than that in defence functions (Tab. 2). Chitinases may regulate processes of growth and development by generating or degrading signal molecules, as during the nodulation process, where the bacterial lipochitooligosaccharides (Nod factors) are degraded by chitinases [7, 18, 24].

It is also suggested that the enzymes take part in programmed cell death (PCD) [45, 47]. This conclusion is supported by the observation that the chitinase (*EP3*) gene is activated earlier in *Daucus carota* cells which are in an apoptosis-preceding stage [45]. It seems that in *Arabidopsis thaliana*, class IV chitinase,

Tab. 2. Chitinase functions and processes.

Process / function	Chitinase type	Plant species	References
pathogenesis	intracellular, class IV	<i>Nicotiana tabacum</i>	[35]
	basic, class I and acidic, class II	<i>Arabidopsis thaliana</i>	[27,41,48]
	class IV	<i>Arabidopsis thaliana</i>	[31]
	intracellular endochitinase	<i>Phaseolus vulgaris</i>	[30, 49]
	class IV	<i>Vitis vinifera</i>	[38]
nodulation	acidic, class III	<i>Sesbania rostrata</i>	[7]
mycorrhiza	basic, extracellular, class I	<i>Picea abies</i>	[60]
growth process	chitinase-like (related to class II)	<i>Arabidopsis thaliana</i>	[61]
embryogenesis	acidic, extracellular, class IV	<i>Daucus carota</i>	[6,45]
	extracellular	<i>Cichorium</i>	[8]
	basic, class IV	<i>Picea glauca</i>	[63]
chilling/frost resistance	extracellular, class I and class II intra- and extracellular class II	<i>Secale cereale</i>	[28]
		<i>Lycopersicon esculentum</i>	[32]
		<i>Cynodon</i> sp.	[56]
PCD	class IV	<i>Brassica napus</i>	[26]
	class IV	<i>Arabidopsis thaliana</i>	[47]
storage protein	chitinase-like (class III homolog)	<i>Musa</i> spp.	[44]
inhibitor	basic, extracellular endochitinase	<i>Solanum tuberosum</i>	[58]
		<i>Coix lachrymajobi</i>	[5]

similarly to *Daucus carota* EP3, is involved in regulation of processes leading to PCD [47]. Chitinase may also have other functions. In *Musa* spp., a protein homologous to class III chitinase behaves as a fruit-specific vegetative storage protein that accumulates during early fruit formation and serves as a source of amino acids for the synthesis of ripening-associated proteins [44]. Moreover, in

Solanum tuberosum tubers, a basic chitinase which inhibits aspartic protease activity was found [58]. An endochitinase with α -amylase inhibitor activity was purified from *Coix lochrymajobi* [5].

The involvement of chitinases in pathogenesis

It seems that chitinases have a double function in protection against pathogenic fungus colonisation. The apoplastic chitinases play a role in the early stage of pathogenesis. They release elicitor molecules, involved in the transfer of information about the infection, from hyphae that penetrate the intercellular space [5, 31]. Subsequently, these particles bind to particular receptors switching on the active defence mechanisms, e.g. a higher rate of apoplastic chitinase synthesis and secretion, as well as synthesis of vacuolar chitinases. The increase in apoplastic chitinase content intensifies the production of elicitor molecules and indirectly enhances the infection signalling [31]. During the following phase of pathogenesis, when hyphae penetrate and destroy the cell, thereby causing the protoplast to burst, the vacuolar chitinases swing into action. They degrade the newly-synthesised chains of chitin, repressing fungal growth [5]. It seems that apoplastic chitinases take part in the generation of signalling that informs the plant about the attack, whereas vacuolar chitinases inhibit pathogen growth [5]. This hypothesis is supported by the substrate specificity of cell wall and vacuolar enzymes. Vacuolar chitinases are more active than apoplastic enzymes against crystalline chitin, whereas apoplastic forms better hydrolyse soluble chitin [5]. The role of vacuolar chitinases (especially class I) in plants' defence mechanism is confirmed by evidence about the co-evolution of these enzymes and fungal proteins [48]. This co-evolution involved changes in the amino acid protein composition, mainly occurring in the active site of class I chitinases. This suggests that fungi directly counteracted the plant resistance mechanism through enzymatic or chemical inhibition of the chitinolytic activity, whereas a rapid adaptive evolution of the chitinase active site occurred in plants [48]. By contrast, the active site of class III chitinases is strongly conserved and the majority of the evolutionary changes rather concern their non-catalytic region [48]. Moreover, the importance of class I chitinases in plant defence mechanisms is supported by their antifungal activity under *in vitro* conditions. This activity is strongly influenced by the presence of the N-terminal chitin-binding domain, known also as the hevein domain. This domain is responsible for substrate binding and has no influence on the catalytic activity of the enzyme. However, its presence may be a crucial or enhancing factor that determines the antifungal properties of chitinases [31, 59].

The role of chitinases in symbiosis

Plant chitinases also take part in legume nodulation. The increase in the chitinolytic activity and the types of induced chitinase isoforms are related to interaction between the host plant and symbiotic bacteria [19]. In the case of root infection by bacteria which are specific for particular plant species, chitinases

degrade and deactivate a part of the bacterial chitoooligosaccharides (Nod factors), thus repressing the intensity of root nodule formation [7, 18, 45]. When the bacteria that infect the roots are not compatible with the plant, the Nod factors released by them are recognised as elicitor molecules and trigger the plant defence mechanisms [12]. The chitinase isoforms emerging during this process are the same as the isoforms induced by pathogens. Similar relations have been observed during symbiosis formation between plants and mycorrhizal fungi [19]. By contrast, when the fungus that infects the roots is compatible, its chitoooligosaccharides are hydrolysed by plant chitinases, and the plant defence reaction is reduced [60]. However, if the fungus is not symbiotic, then the chitinases present in the roots do not cleave the fungal elicitors, which subsequently bind to plasmalemma receptors and trigger a hypersensitive reaction [60].

The role of chitinases in plant growth processes

The function of chitinases in plant defence mechanisms is well-known and experimentally proved, but the suggested role of these enzymes in plant growth and development processes requires more detailed studies. So far, it is only known that in the *Arabidopsis thaliana* plant, mutation in the gene encoding a hypothetical class II chitinase protein causes ethylene overproduction and evident phenotypic changes [61]. This mutant exhibits aberrant cell shapes, ectopic deposition of lignin in the secondary cell wall and several growth alterations. Light-grown seedlings of mutant plants have shorter internodes and roots, whereas dark-grown seedlings are significantly smaller than the wild type plants. The extremely interesting point is the connection between chitinase activity and ethylene production. There is nothing surprising in the fact that ethylene induces some chitinase isoforms, especially class I. Ethylene is a stress hormone, and great number of proteins are regulated by its increased level. However, it is surprising that mutation in a chitinase-like gene caused overproduction of this growth regulator, because it is usual that enhanced chitinase activity leading to signal molecule generation increases ethylene production [61]. Most probably, the chitinases here play a role in the degradation of an endogenous activator or in the formation of an ethylene synthesis inhibitor. Nevertheless, the phenotype of the studied mutant is only partially due to ethylene overproduction. Plant treatment with an ethylene biosynthesis inhibitor (AVG) or a competitive inhibitor of ethylene action (Ag^+) only incompletely rescued the wild type phenotype. So, the possible substrate or product of the hypothetical class II chitinase activity is not only involved in the ethylene pathway but also participates in the cell elongation processes that are independent of this growth regulator [61].

Further support of the involvement of chitinases in developmental processes comes from experimental data concerning an embryogenic suspension culture of the thermosensitive *Daucus carota* (*ts11*) mutant [6]. The *ts11* mutant is arrested in the globular stage at a non-permissive temperature. The lack of extracellular

class IV chitinases in the medium is responsible for this phenotype. In wild type *D. carota* embryogenic culture, some nonembryogenic cells produce and secrete this enzyme into the culture medium [45]. However, in the *ts11* mutant, the chitinase secretion process is blocked. Medium conditioning via the addition of chitinases from the wild type *Daucus carota* culture restores the development of somatic embryos. *ts11*'s ability of further embryo development is also restored by bacterial lipochitooligosaccharides and chitinase pre-treated AGPs [20, 62]. These results suggest that chitinases from *Daucus carota* culture are involved in the generation of somatic embryogenesis-promoting factors [45]. Other data supporting the view that chitinases regulate embryo development come from experiments with embryogenic cultures of *Cichorium*, *Picea glauca* and *Picea abies* [8, 23, 63]. The induction of chitinases has also been observed in the germinating seeds and developing zygotic embryos of numerous plants [42, 43]. Earlier, it was thought that these enzymes only function in defence mechanisms; now it is expected that they may also be involved in the control of embryo cell division, or that in some other way they affect embryonal development [20, 31].

The role of chitinases in plant frost resistance

It was found that apoplastic chitinases which accumulate in monocotyledonous plants upon cold acclimation have an additional function to that of chitinolytic activity. They display so-called antifreeze activity –the ability to inhibit and modify the ice crystal growth in plant apoplasts [28]. It is supposed that proteins with antifreeze activity adsorb onto the surface of growing ice crystals and block their further growth. In monocotyledonous plants, β -1,3-glucanases and thaumatin-like proteins (TLPs) also have this ability. More detailed experiments showed that in *Secale cereale* leaves, the antifreeze activity of chitinases also occurred after ethylene and drought treatments [29]. Also, in *Cynodon* sp., the class II chitinase gene was induced during cold acclimation and dehydration [56]. However, it is not known whether these chitinases possess antifreeze activity. No chitinases revealing this activity were found in dicotyledonous plants.

CHITTOOLIGOSACCHARIDE RECEPTORS

Rhizobial Nod factors and chitooligosaccharides released by chitinases are signal molecules that trigger plant cell responses. Oligomers of *N*-acetylglucosamine are involved in oxidative burst, phosphorylation of specific proteins, phytoalexin biosynthesis, transcriptional activation of defence genes and cell division stimulation [64, 24, 65]. Very sensitive and selective receptors responsible for chitooligosaccharide perception are located in plant cell plasmalemma [66]. The affinity of these proteins to chitin fragments increases with the degree of chitooligomer polymerisation. It was shown that chitotetraose is a minimal length polymer; enough for plasmalemma receptor binding, although a more intense cellular response is induced by octamers of *N*-acetylglucosamine [65]. Transient desensitisation of the plasmalemma

perception system caused by the chitin fragment has also been observed [66]. However, the character and type of these receptors are unknown. It is suggested that lectins and lectin-like and chitin-like proteins are responsible for bacterial Nod factor perception [18]. Recently, another putative *N*-acetylglucosamine oligomer-binding membrane receptor was found [64]. This Chitinase-Related Receptor-Like Kinase (CHRK1) consists of an extracellular enzymatically inactive catalytic domain of family 18 chitinases linked *via* a hydrophobic transmembrane region to the serine/threonine kinase domain. Accumulation of CHRK1 transcript is strongly stimulated by fungal and viral infection [64]. Supposedly, chitoooligosaccharides released from pathogen cell walls by apoplastic chitinases bind to the extracellular CHRK1 domain, which activates an intracellular serine/threonine kinase domain, and thus triggers signal transduction [64].

SUMMARY

The role of chitinases in healthy plant and animal tissues is strongly connected with oligosaccharide signal molecules [22, 24, 67]. Plant chitinases that hydrolyse fungal cell wall chitin, thereby inhibit the growth of fungi and also generate chitin oligosaccharides acting as elicitors. These enzymes probably play a role in the generation of signal molecules; not only those involved in plant resistance to external environmental factors, but also in plant growth and development. It is supposed that chitinases detach these molecules from larger precursors, i.e. polysaccharides or glycoproteins [61]. Additionally, the regulatory role of chitinases may involve oligosaccharide degradation. This was proved in the case of regulation of the nodulation process in legume plants where chitinases hydrolysed bacterial lipochitoooligosaccharides [18]. Chitinase isoforms degrading lipooligosaccharides were also found in a *Picea abies* suspension culture [23]. To shed more light on the involvement of chitinases in plant growth and development further experiments are required.

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