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Review

Chitinases—Potential Candidates for Enhanced Plant Resistance towards Fungal Pathogens

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Received: 30 April 2018; Accepted: 20 June 2018; Published: 22 June 2018

Abstract: Crop cultivation is crucial for the existence of human beings, as it fulfills our nutritional requirements. Crops and other plants are always at a high risk of being attacked by phytopathogens, especially pathogenic fungi. Although plants have a well-developed defense system, it can be compromised during pathogen attack. Chitinases can enhance the plant's defense system as they act on chitin, a major component of the cell wall of pathogenic fungi, and render the fungi inactive without any negative impact on the plants. Along with strengthening plant defense mechanisms, chitinases also improve plant growth and yield. Chitinases in combination with recombinant technology can be a promising tool for improving plant resistance to fungal diseases. The applicability of chitinase-derived oligomeric products of chitin further augment chitinase prospecting to enhance plant defense and growth.

Keywords: chitinases; phytopathogenesis; fungi; plant defense

1. Introduction

The demand for agricultural land for crop cultivation has increased to a great extent in order to serve food to the world's rapidly increasing population. Approximately 1.5 billion hectares of land are utilized for crop cultivation out of the 13.4 billion hectares of the world's total available land [1]. The swift increase in population requires additional land for crop cultivation and existence. The limitation of cultivable natural land resources has created the need for the improvement of the management of crop diseases and yield. Plant growth and productivity is affected by various biotic and abiotic factors. Plant diseases are mainly caused by pathogens including bacteria, fungi, viruses, and nematodes. Among these, fungal pathogens are considered to be the predominant pathogens responsible for a drastic decrease in crop yields. Fungi are the causative agents of a range of plant diseases viz. basal stem rot, fusarium wilt, leaf mold, crown rot, rust, white rot, black mold, southern leaf blight, etc. [2]. Fungal species such as *Fusarium, Botrytis*, and *Magnaporthe* are the most common pathogens of crop plants worldwide [3]. Fungicides are being developed to overcome these phytopathogens and have succeeded to some extent, but improving plant resistance towards these pathogens is still necessary.

In the context of fungal disease management, the chitinolytic enzymes, mainly chitinases, have been in the limelight for a few decades due to their catalytic ability to degrade chitin, a key

component of the fungal cell wall, with no harm to the host plant. Chitinases can be isolated from a repertoire of organisms viz. plants, fungi, bacteria, insects, and marine resources [4]. Due to the significant affinity of chitinases towards the polysaccharide chitin, biotech companies are exploring its potential for the development of transgenic plants, disease-resistant seeds, as well as fungicides and insecticides. This review summarizes the potential and applicability of chitinases for controlling phytopathogenic fungi in order to enhance plant defense, growth, and yield.

2. Chitinases

Chitinases belong to the glycosyl hydrolase family, which catalyzes the hydrolysis of glycosidic bonds in chitin. The molecular size of chitinases varies from 20 to 90 kDa [5]. Chitinases mainly belong to families 18 and 19 of glycosyl hydrolases [6] on the basis of characteristics viz. N-terminal sequence, enzyme localization, isoelectric pH, signal peptides, and inducers. Family 18 contains chitinases of classes III and V, whereas family 19 includes chitinases of classes I, II, and IV. Chitinases are produced naturally by a wide range of organisms i.e., fungi, bacteria, yeasts, plants, actinomycetes, arthropods, and humans [7]. The molecular structure and the mode of action of chitinases cannot be easily determined due to the rapid hydrolysis rate of chitin by chitinases [8]. The molecular structure of plant chitinases largely consists of only one catalytic domain, whereas extracellular chitinase from yeast contain four domains—a signal sequence, a catalytic domain, a serine/threonine rich region, and a C-terminal chitin-binding domain [9,10]. Likewise, the fungal chitinases have been observed to consist of five different domains: (a) N-terminal signal peptide region, (b) catalytic domain, (c) chitin-binding domain, (d) serine/theonine rich-region, and (e) C-terminal extension region [4].

Chitinases possess specific affinity towards polymer chitin to degrade it into low-molecular-weight COS (chitooligosaccharides) and GlcNAc (*N*-acetylglucosamine) [4]. On the basis of the mode of action, chitinases can be endo- or exo-acting. Endochitinases randomly act on the chitin chain at an internal site, whereas exochitinases (chitobiodidases and 1,4 β-glucosaminidases) show progressive catalytic action starting from the non-reducing end of chitin [4]. Chitinases have been reported to exhibit diverse functions i.e., morphogenesis, pathogenesis, parasitism, nutrition, growth regulation, immunity, and defense [11]. Nowadays, chitinase-induced degraded products of chitin i.e., COS and GlcNAc, are gaining attention due to their enormous applicability for plant protection and growth enhancement [12].

2.1. Major Groups of Chitinases

Chitinases are found in a wide range of organisms to serve specific functions. Subsequently, on the basis of natural occurrence/source of production, chitinases can be clustered into the following major groups [4].

2.1.1. Chitinases from Plants

Plants possess endochitinases in their stems, seeds, flowers, and tubers that randomly hydrolyze internal β-1,4-linkages of chitin, resulting in the production of COS and GlcNAc [13]. Plant chitinases (majorly grouped in family 19 of glycosyl hydrolases) play a significant role in embryogenesis, ethylene synthesis, and in combating environmental stresses i.e., cold, drought, and high salt concentration [13]. Moreover, plants produce chitinases in response to phytopathogen attack [14]. Plant chitinases are mainly found in monocotyledonous and dicotyledonous crop species viz. bean, barley, cabbage, carrot, corn, cucumber, garlic, oat, onion, pea, peanut, potato, rice, tomato, etc. [14]. Many plant chitinases have been demonstrated to possess potential antifungal activity. In one study, Kabir et al. [15] reported the antifungal activity of a 39 kDa chitinase from *Trichosanthes dioica* seeds against *Aspergillus niger* and *Trichoderma* sp. Similarly, a 29 kDa chitinase with antifungal activity against *T. viride* was isolated from *Diospyros kaki* fruits [16] and showed an ability to hydrolyze colloidal chitin into chitotriose, chitobiose, and *N*-acetylglucosamine. Recently, a 32 kDa recombinant chitinase was isolated from barley, which showed better antifungal activity upon expression in *E. coli* [17]. The produced

recombinant chitinase was able to inhibit the growth of *Alternaria solani*, *Fusarium* sp., *Rhizoctonia solani*, and *Verticillium dahlia* [18]. Plant chitinases have also shown remarkable potential for tolerating abiotic stresses i.e., chitinase from *Hippophae rhamnoides* for cold stress [19] and chitinase from soybean for arsenic and cadmium stress [20].

2.1.2. Chitinases from Mammals

Mammalian chitinases belong to family 18 of glycosyl hydrolases. Mammalian chitinases can be divided into two sub-categories i.e., true chitinase and chitinase-like proteins based on their enzymatic activity and dormancy. Bussink et al. [21] reported the first mammalian chitinase, known as chitotriosidase. The molecular structure of mammalian chitinases revealed the presence of an N-terminal catalytic domain consisting of a triose-phosphate isomerase fold. In true mammalian chitinases, the glutamic acid donates a proton to hydrolyze the β (1-4) glycosidic bond in chitin, whereas in chitinase-like proteins, glutamic acid is exchanged for glutamine, leucine, and isoleucine as proton donor [22]. Mammalian chitinases have largely been explored for medicinal purposes viz. asthma [23], inflammation, cancer [24], tissue remodeling, and injury [25]. Still, there is a room for the exploration of mammalian chitinases in crop protection and yield enhancement.

2.1.3. Chitinases from Insects

Chitinases in insects are usually found in the ectodermal epithelial tissues such as the foregut, cuticles, trachea, and hindgut, as well in the intestinal peritrophic matrices in insects [26]. The digestion of old cuticle prior to ecdysis takes place by the combined action of hydrolytic enzymes viz., chitinases, proteinase, lipase, and β-*N*-acetylglucosaminidases [27]. Insect chitinases exhibit endo-hydrolyzing activity and mostly belong to family 18 of glycoside hydrolases [28]. Chitinases from *Manduca sexta* and *Bombyx mori* are those most widely studied [4]. Insect chitinases are beta/alpha-barrel proteins; the beta sheets are mostly arranged in parallel fashion [29]. The modular structure of insect chitinases reveals the presence of catalytic, cysteine-rich chitin-binding, and serine/threonine-rich linker domains [30]. Due to their nematocidal, fungicidal, and insecticidal properties, the enzymes have shown enormous applicability in agriculture. Reddy and Rajam [31] developed *Helicoverpa armigera*-resistant tobacco and tomato plants through the host-induced RNA interference. Insect-resistant transgenic maize plants were developed by the expression of a chitinase gene from *Spodoptera littoralis* [32]. The developed transgenic maize plants showed a significant increase (50%) in resistance against *Sesamia cretica* (50%). Agrawal et al. [33] designed a vector to produce artificial microRNA (amiR-24) to target the chitinases gene of one of the most damaging polyphagous pests, *H. armigera*. Insect chitinases have proved to be a significant biocontrol agent but their effectiveness can be further augmented by enhancing the gene expression levels in combination with other insecticidal agents.

2.1.4. Chitinases from Microorganisms

Most of the chitinases from microbial sources have been grouped into family 18 of glycosyl hydrolases [6], with the exception of some of the Gram-positive bacteria that are included in family 19 [34]. Microorganisms are considered as the preferred source of chitinases due to their vast abundance in nature and easy availability of raw material for cultivation that results in the lower production cost of chitinases [11]. Bacteria such as *Serratia marcescens* [35], *Aeromonas punctata* and *A. hydrophila* [36], *Bacillus pumlius* [37], *B. thuringiensis*, *B. licheniformis* [38], etc. have shown the potential to produce chitinases. Also, the fungi *Humicola grisea* [39], *Rhizomucor miehei* [40], and *A. flavus* [41] have proved to be potential candidates for the production of high chitinase titres. Actinomycetes viz. *Thermobifida fusca* [42], *Streptomyces pratensis* [43], and *Saccharothrix yanglingensis* [44] have also been reported to produce notable levels of chitinase. Bacteria primarily produce chitinases in order to degrade chitin for its utilization as an energy source, whereas some bacterial chitinases have shown potentiality as biological control agents against a variety of plant diseases caused by phytopathogenic fungi [45–47]. Fungal chitinases have also been observed to play a key role in

the nutrition, morphogenesis, and developmental processes in fungi [48]. The later sections of this review describe the role of fungi in plant pathogenicity and their mode of action, followed by the state-of-the-art information of the research conducted related to the application of chitinases in agriculture.

3. Fungi as Phytopathogens

Fungi, well-known organic matter decomposers and recyclers, interact with plants in both positive and negative manners [49]. In plant pathogenesis, fungi are known for their ability to be the prime causative agents for >70% of major diseases in agricultural crops, trees, and landscapes. Most of the species of flowering plants around the world are affected by pathogenic fungi. Some of the common fungal diseases in crops include rice blast, rice sheath blight, wheat rust, powdery mildew of pulses, etc. In molecular plant pathology, fungal pathologists have nominated some fungal pathogens as the prominent agents for the most frequently occurring plant diseases. The top 10 fungal pathogens on the basis of scientific/economic importance are *Magnaporthe oryzae*, *Botrytis cincerea*, *Puccinia* spp., *F. graminearum*, *F. oxysporum*, *Blumeria graminis*, *Mycosphaerella graminicola*, *Colletotrichum* spp., *Ustilago maydis*, and *Melampsora lini* [3]. Generally, opportunistic fungal parasites use natural openings or wounds in order to invade plants, but true phytopathogenic fungi transverse the plant's outer structural defense (the cuticle and epidermal cell wall) through a secreted hydrolytic enzyme cocktail consisting of chitinases, pectinases, cellulases, and proteases. For successful invasion into plants, fungi have to compete with the physical and chemical defense barriers of plants [50]. A plant's structural components i.e., cuticle, not only prevents the direct penetration of fungal pathogens but also averts fungal spore germination by wax secretion [51]. Fungi overcome plant defense by employing several strategies. In some cases, fungi secrete effector molecules to interact with the basal plant defense response [52]. Fungi also secrete chemical messengers to interact with the signaling process of the host plant to compete with the plant's chemical barriers [53]. Symbiotic fungi suppress plant defense by interacting with host defense signaling as well as other soil microorganisms [54]. Some fungi can also hide their identity by altering the physicochemical properties of proteins usually recognized by plant receptors [55].

A range of phytopathogenic microorganisms circumvent plant defenses; however, very few succeed. Plants induce a rapid defense response in localized cells and tissues, called hypersensitive response [56]. In the process of microorganism-assisted plant pathogenesis, following the invasion of pathogens into the plant interior, they have to conquer the rigid cell wall, followed by the interface with the plasma membrane where microbes encounter extracellular surface receptors that recognize pathogen-associated molecular patterns (PAMP). This results in the initiation of PAMP-triggered immunity that is suppressed by the pathogenic microorganisms either by secreting effector molecules into the cell cytosol to alter the resistance signaling or by interfering with recognition at the plasma membrane [57]. Apart from this, a panoply of defense responses viz. pH changes, the production of reactive oxygen species, swift ion fluxes, the production of local and systemic signaling molecules and antimicrobials, etc. are generated by plants to impede the invasion of pathogens.

Pathogenic fungi usually exploit conserved proteins during the infection process, in spite of their differences in lifestyle. [58]. One of the common phenomena among pathogenic fungi is to invade the host through appressoria i.e., a specialized infection structure required to penetrate the cuticle of the host cells. An extensive study was conducted on the mode of fungal infection through appressoria (Figure 1) on the rice blast fungus *M. oryzae* [59]. The development and infection caused by appressorium in *M. oryzae* can be divided into four different stages: pre-appressorium development, appressorium turgor generation, appressorium maturation, and the penetration of the peg [60]. The pre-appressorium development stage consists of a spore landing on the host surface, followed by the germination of conidium and attachment to the leaf surface. This attachment leads to the germination of spores as well as the development of germ tubes from the conidium [61]. Finally, the extension of the developed germ tubes takes place, followed by their differentiation into a

unicellular appressorium. During the appressorium turgor generation, along with the appressorium maturation, there is a rapid synthesis of glycerol and other polyols that results in the generation of turgor and a melanin layer on the inner side of the appressorium cell wall to provide support to the infected cell [62]. The turgor generation is followed by the maturation of appressorium, in which the development of an appressorium pore occurs that is the base of the infection cell. From these appressorium pores, the penetrating hypha emerges. These pores are also the site of the remodeling of the actin cytoskeleton and, during the formation of the penetration peg, rapid F-actin polymerization occurs, leading to the rapid polarized growth of the hypha. The formation of the penetration peg leads to the production of effector proteins, which suppress plant immunity responses and smooth the proliferation of the pathogens within the plant tissue [63].

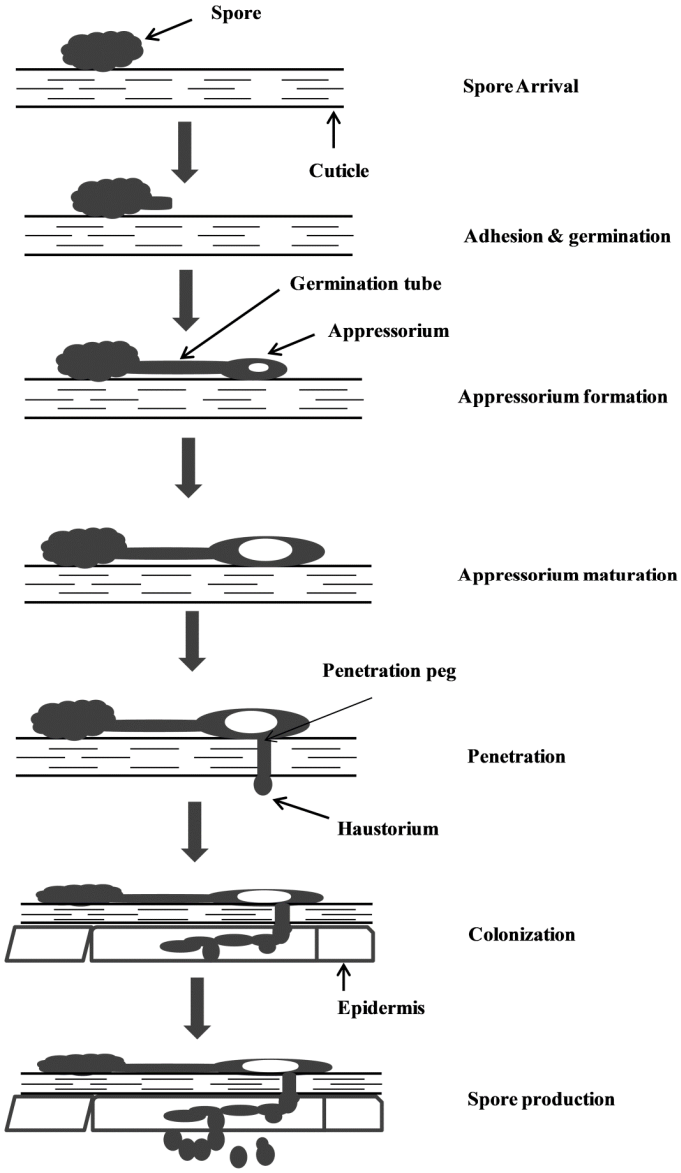

Figure 1. Process of fungal pathogenesis in plants. The figure illustrates the infection caused through am appressorium in *M. oryzae*. The whole process is sub-divided into four steps: formation of a pre-appressorium (spore arrival, attachment, germination, germ tube development, and differentiation into unicellular appressorium), appressorium turgor generation (appressorium maturation and synthesis of polyols), maturation (development of the appressorium pore and emergence of the penetration hypha), and penetration of the peg (effector proteins aid in plant infection).

4. Application of Chitinases in Agriculture

Plant diseases caused by pathogens play a major role in the reduction of crop yield. There are many chemical and biological means available for fighting against these pathogens, including the utilization of chitinolytic enzymes [64]. Chitinolytic enzymes like chitinases have been proven to be among the most promising candidates in plant disease maintenance, as the enzymes are able to hydrolyze chitin which is commonly present in plant fungal pathogens [9]. Chitinases not only provide assets to enhance plant immunity, but also take part in plant growth and development. The current scenario of plant pathogenesis focuses on the development of disease-resistant transgenic plants by the incorporation of chitinases encoding genes from any species to any plant in order to boost the disease resistance in plants (Table 1).

Table 1. Chitinase gene expression and application.

Gene	Origin	Application	Reference
Maize chitinase 2 gene	*Zea mays*	Effective against rot pathogen *F. graminearium*	[65]
Tobacco osmotin (*ap24*) and rice chitinase (*chi 11*) gene	*Nicotiana* sp. (Tobacco) and *Oryza sativa* (Rice)	Reduce sheath blight disease caused by *R. solani*	[66]
Chitinase I gene	*Hordeum vulgare* cultivar, Haider-93	Inhibits phytopathogenic fungi *A. solani*, *R. solani*, *F.* spp., *V. dahliae*	[18]
Class II endochitinase gene	*Hordeum vulgare*	Inhibit growth of *A. solani*	[67]
Chitinase (*Chit* 33)	*Trichoderma atroviride*	Resistance against *Sclerotinia sclerotiorum*-mediated stem rot disease	[68]
Endochitinase gene IIHR-JBM*ch*	*Trichoderma harzianum*	Resistance against wilt disease caused by *F. oxysporium*	[69]
Rice class I chitinase gene (*Rchit*)	Rice	Resistance against late leaf spot, rust disease, and *A. flavus* infection	[70]
Rice chitinase-3 gene	Rice	Resistance against leaf spot in peanut by *Cercospora arachidicola*	[71]

The transformation of canola by an endochitinase gene, *chit33* from *Trichoderma atroviride*, had led to increased resistance towards *Sclerotinia sclerotiorum* [68]. A detached leaf assay following *chit33* expression illustrated decreased lesion sizes as compared to non-transgenic canola. Transgenic varieties of peanuts expressing the *Rchit* gene from rice showed increased resistance towards major soil borne and foliar fungal pathogens. Two to 14-fold higher chitinase activity was detected in the leaves of transgenic peanut lines along with increased resistance against leaf rust spot and rust disease as well as *A. flavus* infections.

4.1. In Plant Defense

Plants are known to produce pathogenesis-related enzymes in response to phytopathogens and this naturally acquired defense mechanism can be implicated in strengthening the defense [72,73]. Nowadays, chitinase genes have been cloned and expressed into various plant species, resulting in improved disease resistance in the developed transgenic plants [74,75]. A class I chitinase gene

(*AF153195*) from potato was introduced into the tea genome and its overexpression resulted in an enhanced resistance against blister blight disease [76]. Zarinpanjeh et al. [77] reported improved resistance against *Sclerotinia* stem rot in *Brassica napus* by the co-expression of defensin and the chimeric chitinase gene. The study utilized the developed transgene for constitutive expression in transgenic lines that suppressed the growth of *Sclerotinia sclerotiorum* [77]. Resistance against the stripe rust disease in transgenic wheat (*Triticum aestivum* L.) was achieved to a greater extent by the expression of the rice chitinase gene *RC24* [78]. Shin et al. [79] reported increased resistance against the fusarium head blight disease by expressing a barley class II chitinase gene in wheat. The co-expression of tobacco osmotin (*ap24*) and rice chitinase (*chi11*) genes resulted in enhanced resistance in rice plants against the sheath blight disease caused by *Rhizoctonia solani* [66]. Karmaka et al. [80] showed improved resistance of rice plants against sheath blight disease by the co-expression of chitinase and oxalate oxidase 4 genes. Similarly, the chitinase gene *LOC_Os11g47510* overexpression in rice plants also showed improvement in the resistance against sheath blight disease [81]. Apart from utilization in the enhancement of plant defense system, chitinases also play a significant role in the enhancement of plant growth and yield.

4.2. In Plant Development and Yield

Chitinases support plant growth by improving their endurance towards various biotic and abiotic stresses that diminish crop productivity [82]. The endochitinase and chitobiosidase genes from *S. albidoflavus* were expressed in tomato plants and a significant decrease in plant height along with reduced flowering time was observed [83]. The study also reported an increased number of flowers and fruits on the transgenic tomato plants, leading to an enhanced yield [83]. Guo et al. [84] investigated a drought-induced gene (*DIP3*) encoding a chitinase III protein as a stress-induced protein that can regulate the plant stress response against abiotic stress viz. drought, salt, and low temperature. The overexpression of *CHIT33* and *CHIT42* genes from *T. harzianum* in transgenic tobacco resulted in enhanced forbearance against phytopathogens, salinity, and heavy metals stress [85]. Jeong et al. [86] developed transgenic rice plants by overexpressing the *OsNACS* gene under the control of root-specific (RCc3) or constitutive (GOS2) promoters and obtained 9–23% and 9–26% increments in yield under normal environmental conditions. Moreover, the study also suggested a higher grain yield of 22–63% in RCc3:OsNAC5 under drought conditions. Kumar et al. [87] developed transgenic tomato showing enhanced tolerance to salt and drought stress by the expression of Osmotin-like protein and *chi11* genes. The research also reported the significant role of phosphofructokinase2 in the enhancement of root biomass [87].

5. Chitooligosaccharides in Agriculture

In recent decades, the utilization of catabolic products of chitin, i.e., COS, has been studied for strengthening plant protection and growth [88]. COS is accepted as a potential bactericidal and fungicidal agent for plants. COS not only protects plants from pathogens, but also serves as a plant growth regulator [89]. The application of COS with different degrees of polymerization in synergy was also investigated by the researchers [89]. The utilization of a low-molecular-weight chitin mix consisting of dimers (92%), trimers, and tetramers resulted in a notable enhancement in the in vitro fresh weight (10%), radical weight (25%), total carbon (6%), and nitrogen content (8%) [89]. The application of COS for the improvement of plant growth has been extensively researched and patented [90,91]. Zong et al. [92] reported improved levels of cadmium tolerance and plant growth in *B. rapa* plants when COS was sprayed on the leaves. COS (50–100 mg L^{-1}) resulted in significant tolerance. Zou et al. [93] reported the use of sulfated COS on wheat to overcome salt stress. Sulfated COS treatment was able to decrease the content of malondialdehyde, increase the chlorophyll contents, and modulate fluorescence characters in wheat seedlings under salt stress. The ease of utilization along with a vast range of applicability of the chitin-based bioactive derivatives

and chitinolytic enzymes could, in the near future, contribute significantly to the enhancement of plant growth and yield via boosting plant defense systems against phytopathogens.

6. Conclusions

Chitinases are among the most effective biocontrol agents in controlling phytopathogenic fungi. Chitinases can be implicated in strengthening plant immunity by the expression of the desired chitinase, resulting in enhanced activity and sensitivity against pathogens. Chitinase-treated seeds and transgenic plants are able to provide better protection from the infection of pathogenic fungi. Directed evolution and site-directed mutagenesis could be explored to develop chitinases with broad activity and specificity. Ease of applicability makes COS a potential leading candidate of the near future in controlling plant diseases. However, not much is known about the role of fungal chitinases in their interaction with plants. Thus, intensive research is required to understand the mechanism and role of fungal chitinase during plant pathogenesis.

Funding: The research has been financially supported by Department of Biotechnology (BT/Bio-CARe/03/9840/2013-14) and the Department of Science and Technology (IFA14-LSPA-27), Government of India.

Acknowledgments: Authors acknowledge the Department of Biotechnology and the Department of Science and Technology, Government of India for financially supporting the work. M.K. and A.B. gratefully acknowledge University Grants Commission, Government of India for providing fellowship.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Bruinsma, J. *World Agriculture: Towards 2015/2030: An FAO Study*; Routledge: Abingdon, UK, 2017.
2. Chawade, A.; Armoniené, R.; Berg, G.; Brazauskas, G.; Frostgård, G.; Geleta, M.; Weih, M. A transnational and holistic breeding approach is needed for sustainable wheat production in the Baltic Sea region. *Physiol. Plant.* **2018**. [CrossRef] [PubMed]
3. Burgess, L.; Knight, T.; Tesoriero, L.; VaPhan, H. *Common Diseases of Some Economically Important Crops*; Australian Centre for International Agricultural Research: Canberra, Australia, 2009; Volume 11, pp. 166–167.
4. Dean, R.; Van Kan, J.A.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J. The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [CrossRef] [PubMed]
5. Hamid, R.; Khan, M.A.; Ahmad, M.; Ahmad, M.M.; Abdin, M.Z.; Musarrat, J.; Javed, S. Chitinases: An update. *J. Pharm. Bioallied Sci.* **2013**, *5*, 21–29. [PubMed]
6. Bhattacharya, D.; Nagpure, A.; Gupta, R.K. Bacterial chitinases: Properties and potential. *Crit. Rev. Biotechnol.* **2007**, *27*, 21–28. [CrossRef] [PubMed]
7. Henrissat, B.; Davies, G. Structural and sequence-based classification of glycoside hydrolases. *Curr. Opin. Struct. Biol.* **1997**, *7*, 637–644. [CrossRef]
8. Patil, R.S.; Ghormade, V.; Deshpande, M.V. Chitinolytic enzymes: an exploration. *Enzym. Microb. Technol.* **2000**, *26*, 473–483. [CrossRef]
9. Knowles, J.; Lehtovaara, P.; Teeri, T. Cellulase families and their genes. *Trends Biotechnol.* **1987**, *5*, 255–261. [CrossRef]
10. Blaak, H.; Schnellmann, J.; Walter, S.; Henrissat, B.; Schrempf, H. Characteristics of an exochitinase from *Streptomyces olivaceoviridis*, its corresponding gene, putative protein domains and relationship to other chitinases. *FEBS J.* **1993**, *214*, 659–669. [CrossRef]
11. Kuranda, M.J.; Robbins, P.W. Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1991**, *266*, 19758–19767. [PubMed]
12. Stoykov, Y.M.; Pavlov, A.I.; Krastanov, A.I. Chitinase biotechnology: production, purification, and application. *Eng. Life Sci.* **2015**, *15*, 30–38. [CrossRef]
13. Smith, R.S.; Osburn, R.M. Combined Used of Lipo-Chitooligosaccharides and Chitinous Compounds for Enhanced Plant Growth and Yield. U.S. Patent 9,253,989, 9 February 2016.

14. Collinge, D.B.; Kragh, K.M.; Mikkelsen, J.D.; Nielsen, K.K.; Rasmussen, U.; Vad, K. Plant chitinases. *Plant J.* **1993**, *3*, 31–40. [CrossRef] [PubMed]
15. Schlumbaum, A.; Mauch, F.; Vögeli, U.; Boller, T. Plant chitinases are potent inhibitors of fungal growth. *Nature* **1986**, *324*, 365–367. [CrossRef]
16. Kabir, S.R.; Rahman, M.M.; Tasnim, S.; Karim, M.R.; Khatun, N.; Hasan, I.; Amin, R.; Islam, S.S.; Nurujjaman, M.; Kabir, A.H. Purification and characterization of a novel chitinase from *Trichosanthes dioica* seed with antifungal activity. *Int. J. Biol. Macromol.* **2016**, *84*, 62–68. [CrossRef] [PubMed]
17. Zhang, J.; Kopparapu, N.K.; Yan, Q.; Yang, S.; Jiang, Z. Purification and characterization of a novel chitinase from persimmon (*Diospyros kaki*) with antifungal activity. *Food Chem.* **2013**, *138*, 1225–1232. [CrossRef] [PubMed]
18. Toufiq, N.; Tabassum, B.; Bhatti, M.U.; Khan, A.; Tariq, M.; Shahid, N.; Nasir, I.A.; Husnain, T. Improved antifungal activity of barley derived chitinase i gene that overexpress a 32 kDa recombinant chitinase in *Escherichia coli* host. *Braz. J. Microbiol.* **2017**, *42*, 414–421. [CrossRef] [PubMed]
19. Kashyap, P.; Deswal, R. A novel class I chitinase from *Hippophae rhamnoides*: Indications for participating in ice-cbf cold stress signaling pathway. *Plant Sci.* **2017**, *259*, 62–70. [CrossRef] [PubMed]
20. Gálusová, T.; Rybanský, Ľ.; Mészáros, P.; Spieß, N.; Piršelová, B.; Kuna, R.; Libantová, J.; Moravčíková, J.; Hauptvogel, P.; Matuíková, I. Variable responses of soybean chitinases to arsenic and cadmium stress at the whole plant level. *Plant Growth Regul.* **2015**, *76*, 147–155. [CrossRef]
21. Bussink, A.P.; Speijer, D.; Aerts, J.M.; Boot, R.G. Evolution of mammalian chitinase (-like) members of family 18 glycosyl hydrolases. *Genetics* **2007**, *177*, 959–970. [CrossRef] [PubMed]
22. Bussink, A.P.; van Eijk, M.; Renkema, G.H.; Aerts, J.M.; Boot, R.G. The biology of the gaucher cell: The cradle of human chitinases. *Int. Rev. Cytol.* **2006**, *252*, 71–128. [PubMed]
23. Chou, Y.-T.; Yao, S.; Czerwinski, R.; Fleming, M.; Krykbaev, R.; Xuan, D.; Zhou, H.; Brooks, J.; Fitz, L.; Strand, J. Kinetic characterization of recombinant human acidic mammalian chitinase. *Biochemistry* **2006**, *45*, 4444–4454. [CrossRef] [PubMed]
24. Bierbaum, S.; Nickel, R.; Koch, A.; Lau, S.; Deichmann, K.A.; Wahn, U.; Superti-Furga, A.; Heinzmann, A. Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. *Am. J. Respir. Crit. Care Med.* **2005**, *172*, 1505–1509. [CrossRef] [PubMed]
25. Kzhyshkowska, J.; Gratchev, A.; Goerdt, S. Human chitinases and chitinase-like proteins as indicators for inflammation and cancer. *Biomarker Insights* **2007**, 2. [CrossRef]
26. Lee, C.G.; Da Silva, C.A.; Dela Cruz, C.S.; Ahangari, F.; Ma, B.; Kang, M.-J.; He, C.-H.; Takyar, S.; Elias, J.A. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annu. Rev. Physiol.* **2011**, *73*, 479–501. [CrossRef] [PubMed]
27. Kumar, M.; Vivekanand, V.; Pareek, N. Structure, regulation, and potential applications of insect chitin-metabolizing enzymes. In *Trends in Insect Molecular Biology and Biotechnology*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 295–316.
28. Kramer, K.J.; Corpuz, L.; Choi, H.K.; Muthukrishnan, S. Sequence of a cDNA and expression of the gene encoding epidermal and gut chitinases of *Manduca sexta*. *Insect Biochem. Mol. Biol.* **1993**, *23*, 691–701. [CrossRef]
29. Kramer, K.; Muthukrishnan, S. Chitin metabolism in insects. In *Insect Development: Morphogenesis, Molting and Metamorphosis*; Gilbert, L.I., Ed.; FAO: Rome, Italy, 2009.
30. Kramer, K.J.; Muthukrishnan, S. Insect chitinases: Molecular biology and potential use as biopesticides. *Insect Biochem. Mol. Biol.* **1997**, *27*, 887–900. [CrossRef]
31. Arakane, Y.; Zhu, Q.; Matsumiya, M.; Muthukrishnan, S.; Kramer, K.J. Properties of catalytic, linker and chitin-binding domains of insect chitinase. *Insect Biochem. Mol. Biol.* **2003**, *33*, 631–648. [CrossRef]
32. Reddy, K.; Rajam, M. Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. *Plant Mol. Biol.* **2016**, *90*, 281–292.
33. Osman, G.H.; Assem, S.K.; Alreedy, R.M.; El-Ghareeb, D.K.; Basry, M.A.; Rastogi, A.; Kalaji, H.M. Development of insect resistant maize plants expressing a chitinase gene from the cotton leaf worm, *Spodoptera littoralis*. *Sci. Rep.* **2015**, *5*, 18067. [CrossRef] [PubMed]
34. Agrawal, A.; Rajamani, V.; Reddy, V.S.; Mukherjee, S.K.; Bhatnagar, R.K. Transgenic plants over-expressing insect-specific microrna acquire insecticidal activity against *Helicoverpa armigera*: An alternative to Bt-toxin technology. *Transgen. Res.* **2015**, *24*, 791–801. [CrossRef] [PubMed]

35. Cohen-Kupiec, R.; Chet, I. The molecular biology of chitin digestion. *Curr. Opin. Biotechnol.* **1998**, *9*, 270–277. [CrossRef]
36. Synstad, B.; Vaaje-Kolstad, G.; Cederkvist, F.H.; Saua, S.F.; Horn, S.J.; Eijsink, V.G.; SØRLIE, M. Expression and characterization of endochitinase C from *Serratia marcescens* BJL200 and its purification by a one-step general chitinase purification method. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 715–723. [CrossRef] [PubMed]
37. Kuddus, M.; Ahmad, I. Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. *J. Genet. Eng. Biotechnol.* **2013**, *11*, 39–46.
38. Rishad, K.; Rebello, S.; Nathan, V.K.; Shabanamol, S.; Jisha, M. Optimised production of chitinase from a novel mangrove isolate, *Bacillus pumilus* MCB-7 using response surface methodology. *Biocatal. Agric. Biotechnol.* **2016**, *5*, 143–149. [CrossRef]
39. Gomaa, E.Z. Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: their potential in antifungal biocontrol. *J. Microbiol.* **2012**, *50*, 103–111. [CrossRef] [PubMed]
40. Kumar, M.; Brar, A.; Vivekanand, V.; Pareek, N. Production of chitinase from thermophilic *Humicola grisea* and its application in production of bioactive chitooligosaccharides. *Int. J. Biol. Macromol.* **2017**, *104*, 1641–1647. [CrossRef] [PubMed]
41. Yang, S.; Fu, X.; Yan, Q.; Jiang, Z.; Wang, J. Biochemical characterization of a novel acidic exochitinase from *Rhizomucor miehei* with antifungal activity. *J. Agric. Food Chem.* **2016**, *64*, 461–469. [CrossRef] [PubMed]
42. Rawway, M.; Beltagy, E.A.; Abdul-Raouf, U.M.; Elshenawy, M.A.; Kelany, M.S. Optimization of process parameters for chitinase production by a marine *Aspergillus flavus* MK20. *J. Ecol. Health Environ.* **2018**, *6*, 1–8. [CrossRef]
43. Gaber, Y.; Mekasha, S.; Vaaje-Kolstad, G.; Eijsink, V.G.; Fraaije, M.W. Characterization of a chitinase from the cellulolytic actinomycete *Thermobifida fusca*. *Biochim. Biophys. Acta (BBA) Proteins Proteom.* **2016**, *1864*, 1253–1259. [CrossRef] [PubMed]
44. Shivalee, A.; Lingappa, K.; Mahesh, D. Influence of bioprocess variables on the production of extracellular chitinase under submerged fermentation by *Streptomyces pratensis* strain KLSL55. *J. Genet. Eng. Biotechnol.* **2018**, in press. [CrossRef]
45. Lu, Y.; Wang, N.; He, J.; Li, Y.; Gao, X.; Huang, L.; Yan, X. Expression and characterization of a novel chitinase with antifungal activity from a rare actinomycete, *Saccharothrix yanglingensis* Hhs. 015. *Protein Expr. Purif.* **2018**, *143*, 45–51. [CrossRef] [PubMed]
46. Kavroulakis, N.; Ntougias, S.; Besi, M.I.; Katsou, P.; Damaskinou, A.; Ehaliotis, C.; Zervakis, G.I.; Papadopoulou, K.K. Antagonistic bacteria of composted agro-industrial residues exhibit antibiosis against soil-borne fungal plant pathogens and protection of tomato plants from *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Plant Soil* **2010**, *333*, 233–247. [CrossRef]
47. Babashpour, S.; Aminzadeh, S.; Farrokhi, N.; Karkhane, A.; Haghbeen, K. Characterization of a chitinase (chit62) from *Serratia marcescens* b4a and its efficacy as a bioshield against plant fungal pathogens. *Biochem. Genet.* **2012**, *50*, 722–735. [CrossRef] [PubMed]
48. Sharma, C.K.; Vishnoi, V.K.; Dubey, R.; Maheshwari, D. A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen *Macrophomina phaseolina* in mung bean. *Rhizosphere* **2018**, *5*, 71–75. [CrossRef]
49. Sahai, A.; Manocha, M. Chitinases of fungi and plants: Their involvement in morphogenesis and host-parasite interaction. *FEMS Microbiol. Rev.* **1993**, *11*, 317–338. [CrossRef]
50. Altieri, M.A. The ecological role of biodiversity in agroecosystems. In *Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes*; Elsevier: New York City, NY, USA, 1999; pp. 19–31.
51. Jones, J.D.; Dangl, J.L. The plant immune system. *Nature* **2006**, *444*, 323–329. [CrossRef] [PubMed]
52. Chawade, A.; Alexandersson, E.; Bengtsson, T.; Andreasson, E.; Levander, F. Targeted proteomics approach for precision plant breeding. *J. Proteome Res.* **2016**, *15*, 638–646. [CrossRef] [PubMed]
53. Cui, P.; Zhang, S.; Ding, F.; Ali, S.; Xiong, L. Dynamic regulation of genome-wide pre-mRNA splicing and stress tolerance by the Sm-like protein LSm5 in *Arabidopsis*. *Genome Biol.* **2014**, *15*, R1. [CrossRef] [PubMed]
54. Zeilinger, S.; Gupta, V.K.; Dahms, T.E.; Silva, R.N.; Singh, H.B.; Upadhyay, R.S.; Gomes, E.V.; Tsui, C.K.-M.; Nayak S, C. Friends or foes? Emerging insights from fungal interactions with plants. *FEMS Microbiol. Rev.* **2015**, *40*, 182–207. [CrossRef] [PubMed]
55. Puga-Freitas, R.; Blouin, M. A review of the effects of soil organisms on plant hormone signalling pathways. *Environ. Exp. Bot.* **2015**, *114*, 104–116. [CrossRef]

56. Underwood, W. The plant cell wall: A dynamic barrier against pathogen invasion. *Front. Plant Sci.* **2012**, *3*, 85. [CrossRef] [PubMed]
57. Baker, B.; Zambryski, P.; Staskawicz, B.; Dinesh-Kumar, S. Signaling in plant-microbe interactions. *Science* **1997**, *276*, 726–733. [CrossRef] [PubMed]
58. Chisholm, S.T.; Coaker, G.; Day, B.; Staskawicz, B.J. Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell* **2006**, *124*, 803–814. [CrossRef] [PubMed]
59. Yang, J.; Hsiang, T.; Bhadauria, V.; Chen, X.-L.; Li, G. Plant fungal pathogenesis. *BioMed Res. Int.* **2017**, *2017*, 1–2. [CrossRef] [PubMed]
60. Wilson, R.A.; Talbot, N.J. Under pressure: Investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev. Microbiol.* **2009**, *7*, 185–195. [CrossRef] [PubMed]
61. Ryder, L.S.; Talbot, N.J. Regulation of appressorium development in pathogenic fungi. *Curr. Opin. Plant Biol.* **2015**, *26*, 8–13. [CrossRef] [PubMed]
62. Veneault-Fourrey, C.; Barooah, M.; Egan, M.; Wakley, G.; Talbot, N.J. Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* **2006**, *312*, 580–583. [CrossRef] [PubMed]
63. Dagdas, Y.F.; Yoshino, K.; Dagdas, G.; Ryder, L.S.; Bielska, E.; Steinberg, G.; Talbot, N.J. Septin-mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae*. *Science* **2012**, *336*, 1590–1595. [CrossRef] [PubMed]
64. Giraldo, M.C.; Valent, B. Filamentous plant pathogen effectors in action. *Nat. Rev. Microbiol.* **2013**, *11*, 800–814. [CrossRef] [PubMed]
65. Dowd, P.F.; Naumann, T.A.; Price, N.P.; Johnson, E.T. Identification of a maize (*Zea mays*) chitinase allele sequence suitable for a role in ear rot fungal resistance. *Agri Gene* **2018**, *7*, 15–22. [CrossRef]
66. Sripriya, R.; Parameswari, C.; Veluthambi, K. Enhancement of sheath blight tolerance in transgenic rice by combined expression of tobacco osmotin (ap24) and rice chitinase (chi11) genes. *In Vitro Cell. Dev. Biol. Plant* **2017**, *53*, 12–21. [CrossRef]
67. Khan, A.; Nasir, I.A.; Tabassum, B.; Aaliya, K.; Tariq, M.; Rao, A.Q. Expression studies of chitinase gene in transgenic potato against *Alternaria solani*. *Plant Cell Tissue Organ Cult.* **2017**, *128*, 563–576. [CrossRef]
68. Solgi, T.; Moradyar, M.; Zamani, M.R.; Motallebi, M. Transformation of canola by chit33 gene towards improving resistance to *Sclerotinia sclerotiorum*. *Plant Prot. Sci.* **2015**, *51*, 6–12. [CrossRef]
69. Mishra, M.; Jalil, S.U.; Sharma, N.; Hudedamani, U. An agrobacterium mediated transformation system of guava (*Psidium guajava* L.) with endochitinase gene. *Crop Breed. Appl. Biotechnol.* **2014**, *14*, 232–237. [CrossRef]
70. Prasad, K.; Bhatnagar-Mathur, P.; Waliyar, F.; Sharma, K.K. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. *J. Plant Biochem. Biotechnol.* **2013**, *22*, 222–233. [CrossRef]
71. Iqbal, M.M.; Nazir, F.; Ali, S.; Asif, M.A.; Zafar, Y.; Iqbal, J.; Ali, G.M. Over expression of rice chitinase gene in transgenic peanut (*Arachis hypogaea* L.) improves resistance against leaf spot. *Mol. Biotechnol.* **2012**, *50*, 129–136. [CrossRef] [PubMed]
72. Daizo, K. Application of chitinase in agriculture. *J. Met. Mater. Miner.* **2005**, *15*, 33–36.
73. Rathore, A.S.; Gupta, R.D. Chitinases from bacteria to human: Properties, applications and future perspectives. *Enzym. Res.* **2015**, *2015*, 791907. [CrossRef] [PubMed]
74. Fahmy, A.; Hassanein, R.; Hashem, H.; Ibrahim, A.; El Shihy, O.; Qaid, E. Developing of transgenic wheat cultivars for improved disease resistance. *J. Appl. Biol. Biotechnol.* **2018**, *6*, 31–40.
75. Jalil, S.U.; Mishra, M.; Ansari, M.I. Current view on chitinase for plant defence. *Trends Biosci.* **2015**, *8*, 6733–6743.
76. Singh, H.R.; Deka, M.; Das, S. Enhanced resistance to blister blight in transgenic tea (*Camellia sinensis* [L.] o. Kuntze) by overexpression of class I chitinase gene from potato (*Solanum tuberosum*). *Funct. Integr. Genom.* **2015**, *15*, 461–480. [CrossRef] [PubMed]
77. Zarinpanjeh, N.; Motallebi, M.; Zamani, M.R.; Ziaei, M. Enhanced resistance to *Sclerotinia sclerotiorum* in brassica napus by co-expression of defensin and chimeric chitinase genes. *J. Appl. Genet.* **2016**, *57*, 417–425. [CrossRef] [PubMed]
78. Huang, X.; Wang, J.; Du, Z.; Zhang, C.; Li, L.; Xu, Z. Enhanced resistance to stripe rust disease in transgenic wheat expressing the rice chitinase gene RC24. *Transgen. Res.* **2013**, *22*, 939–947. [CrossRef] [PubMed]

79. Shin, S.; Mackintosh, C.A.; Lewis, J.; Heinen, S.J.; Radmer, L.; Dill-Macky, R.; Baldridge, G.D.; Zeyen, R.J.; Muehlbauer, G.J. Transgenic wheat expressing a barley class ii chitinase gene has enhanced resistance against *Fusarium graminearum*. *J. Exp. Bot.* **2008**, *59*, 2371–2378. [CrossRef] [PubMed]
80. Karmakar, S.; Molla, K.A.; Chanda, P.K.; Sarkar, S.N.; Datta, S.K.; Datta, K. Green tissue-specific co-expression of chitinase and oxalate oxidase 4 genes in rice for enhanced resistance against sheath blight. *Planta* **2016**, *243*, 115–130. [CrossRef] [PubMed]
81. Richa, K.; Tiwari, I.M.; Devanna, B.; Botella, J.R.; Sharma, V.; Sharma, T.R. Novel chitinase gene LOC_Os11g47510 from Indica Rice Tetep provides enhanced resistance against sheath blight pathogen rhizoctonia solani in rice. *Front. Plant Sci.* **2017**, *8*, 596. [CrossRef] [PubMed]
82. Nagpure, A.; Choudhary, B.; Gupta, R.K. Chitinases: In agriculture and human healthcare. *Crit. Rev. Biotechnol.* **2014**, *34*, 215–232. [CrossRef] [PubMed]
83. Gongora, C.E.; Broadway, R.M. Plant growth and development influenced by transgenic insertion of bacterial chitinolytic enzymes. *Mol. Breed.* **2002**, *9*, 123–135. [CrossRef]
84. Guo, X.; Bai, L.; Su, C.; Shi, L.; Wang, D. Molecular cloning and expression of drought-induced protein 3 (dip3) encoding a class III chitinase in upland rice. *Genet. Mol. Res.* **2013**, *12*, 6860–6870. [CrossRef] [PubMed]
85. De las Mercedes Dana, M.; Pintor-Toro, J.A.; Cubero, B. Transgenic tobacco plants overexpressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. *Plant Physiol.* **2006**, *142*, 722–730. [CrossRef] [PubMed]
86. Jeong, J.S.; Kim, Y.S.; Redillas, M.C.; Jang, G.; Jung, H.; Bang, S.W.; Choi, Y.D.; Ha, S.H.; Reuzeau, C.; Kim, J.K. Osnac5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. *Plant Biotechnol. J.* **2013**, *11*, 101–114. [CrossRef] [PubMed]
87. Kumar, S.A.; Kumari, P.H.; Jawahar, G.; Prashanth, S.; Suravajhala, P.; Katam, R.; Sivan, P.; Rao, K.; Kirti, P.; Kishor, P.K. Beyond just being foot soldiers–osmotin like protein (OLP) and chitinase (Chi11) genes act as sentinels to confront salt, drought, and fungal stress tolerance in tomato. *Environ. Exp. Bot.* **2016**, *132*, 53–65. [CrossRef]
88. Kumar, M.; Brar, A.; Vivekanand, V.; Pareek, N. Bioconversion of chitin to bioactive chitooligosaccharides: Amelioration and coastal pollution reduction by microbial resources. *Mar. Biotechnol.* **2018**, 1–13. [CrossRef] [PubMed]
89. Winkler, A.J.; Dominguez-Nuñez, J.A.; Aranaz, I.; Poza-Carrión, C.; Ramonell, K.; Somerville, S.; Berrocal-Lobo, M. Short-chain chitin oligomers: promoters of plant growth. *Mar. Drugs* **2017**, *15*, 40. [CrossRef] [PubMed]
90. Smith, R.S.; Habib, A. Chitooligosaccharides and Methods for Use in Enhancing Plant Growth. U.S. Patent Application 15/206,507, 3 November 2016.
91. Ané, J.-M.; Kalil, A.; Maeda, J. Chitin Oligomers for Use in Promoting Non-Leguminous Plant Growth and Development. U.S. Patent Application 15/185,587, 22 December 2016.
92. Zong, H.; Li, K.; Liu, S.; Song, L.; Xing, R.; Chen, X.; Li, P. Improvement in cadmium tolerance of edible rape (*Brassica rapa* L.) with exogenous application of chitooligosaccharide. *Chemosphere* **2017**, *181*, 92–100. [CrossRef] [PubMed]
93. Zou, P.; Li, K.; Liu, S.; He, X.; Zhang, X.; Xing, R.; Li, P. Effect of sulfated chitooligosaccharides on wheat seedlings (*Triticum aestivum* L.) under salt stress. *J. Agric. Food Chem.* **2016**, *64*, 2815–2821. [CrossRef] [PubMed]