Review Article

Application of Chitosan in Plant Defense Responses to Biotic and Abiotic Stresses

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Abstract
Chitosan, a copolymer of N-acetyl-D-glucosamine and D-glucosamine, which possesses properties that make it useful in various fields, is produced by the deacetylation of chitin derivatives. It is used in agriculture as a biostimulant for plant growth and protection, it also induces several responsive genes, proteins, and secondary metabolites in plants. Chitosan elicits a signal transduction pathway and transduces secondary molecules such as hydrogen peroxide and nitric oxide. Under biotic stress, chitosan can stimulate phytoalexins, pathogenesis-related proteins, and proteinase inhibitors. Pretreatment of chitosan before exposure to abiotic stresses (drought, salt, and heat) induces plant growth, production of antioxidant enzymes, secondary metabolites, and abscisic acid (ABA). It also causes changes in physiology, biochemistry, and molecular biology of the plant cells. However, plant responses depend on different chitosan-based structures, concentrations, species, and developmental stages. This review collects updated information on chitosan applications, particularly in plant defense responses to biotic and abiotic stress conditions.

Keywords: Abiotic stress, Biotic stress, Chitosan, Plant response

1 Introduction

Chitin was the first polysaccharide isolated from mushrooms by Henry Braconnot in 1811 (1780–1855). Later, Prof. C. Rouget, who found that the treatment of chitin with alkali solution resulting in a substance that could be dissolved in acids in 1859. The term "chitosan" was obtained by deacetylation of chitin, a study by Hoppe-Seiler in 1894 [1]. Chitosan is a major component of fungal cell walls, arthropods exoskeletons, and crustacean shells. The physicochemical properties of chitosan gives rise to its several biological applications, in food and nutrition, biotechnology, drug and pharmaceuticals, agriculture,

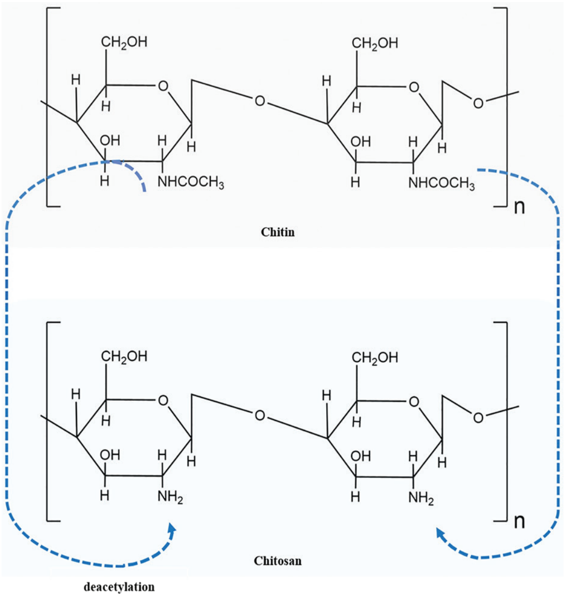

Figure 1: Structures of chitin and chitosan [4]. Dashed lines indicated by transforming the acetamide groups (-$NHCOCH_3$) into amino groups (-NH_2).

and environmental protection. In agriculture, chitosan is applied to many plant species, including food crops, economic crops, ornamental, fruit, and medicinal crops. Effects of chitosan on plant responses depend on the structure and concentration, species, and stage of development of the plant [2], [3]. This review focuses on chitosan as an elicitor molecule and its involvement in signal transduction pathways under biotic and abiotic stresses in the plant defense response.

2 Chitosan Structure and Production

2.1 *Chemical structure of chitosan*

Chitosan a heteropolysaccharide, is derived by the partial deacetylation of chitin [4] (Figure 1). It is as a copolymer of 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine) and 2-amino-2-deoxy-β-D-glucose (glucosamine) [5]. Chitosan consists of reactive functional groups, namely, the amino group (C-2), primary and secondary hydroxyl groups (C-3 and C-6, respectively), which affect its mechanical and physical properties, thereby resulting in flexibility in its application in various fields [6].

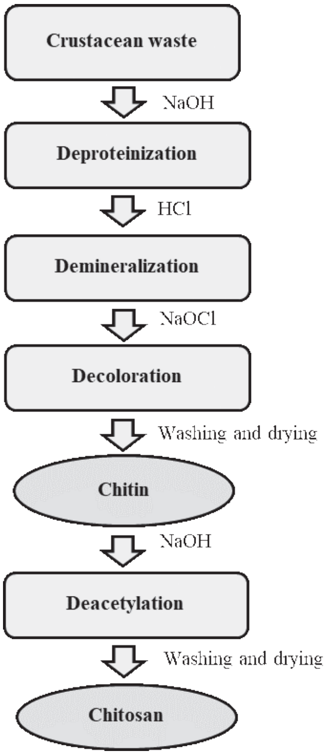

Figure 2: Preparation of chitosan from chitin [8]. NaOH (sodium hydroxide), HCl (hydrochloric acid), NaOCl (sodium hypochlorite).

2.2 *Chitosan production*

A schematic of the preparation of chitosan from chitin is shown in Figure 2. Chitosan is prepared by the hydrolysis of the acetamide groups (-$NHCOCH_3$) of chitin, which is found in several organisms such as crustaceans (crab, shrimp, and prawn) and fungi (mushroom) [7]. Commercially, the following steps are used for the production of chitosan from marine crustacean waste: 1) deproteinization, 2) demineralization, 3) decoloration, and 4) deacetylation. Typically, the crustacean shell is composed of proteins (30–40%), calcium carbonate (30–50%), and pigments (carotenoids; 20–30%) [8]. The deacetylation step is generally conducted by 40% (w/v) sodium hydroxide (NaOH) at 120°C for 1–3 h, thereby resulting in 70% deacetylated chitosan [5]. Alkaline at a concentration of 30–50% (w/v), and a temperature of 100°C leads to partial deacetylated chitin (less than 30%), resulting in chitosan [8].

3 Mechanism of Chitosan Action in Plant Responses

To date, the mode of action of chitosan in plants remains unclear. However, several reports suggest that chitosan elicits several defense response in plants [9], [10].

3.1 *Signal perception and transduction*

3.1.1 Signal perception by chitosan induction

A chitosan-binding glycoprotein in the lectin family has been identified from mustard leaves (*Brassica campestris*) [11]. Consequently, the isolation vesicle from *Mimosa pudica* and *Cassia fasciculate* indicated rapid activation of the H^+-ATPase plasma membrane, thereby revealing chitosan receptor molecules [12]. Also, a knockout mutant in *Arabidopsis thaliana* showed that chitosan could induce a receptor-like kinase gene, the mitogen-activated protein kinase pathway, and lysin motif receptor-like kinase as a chitin elicitor receptor kinase 1 (CERK1), which can bind with chitin and chitosan [13]. However, a report involving *A. thaliana* seedlings stated that the chitosan receptor did not involve CERK1, and it also reacted through a CERK1-independent pathway [14]. Therefore, signal perception induced by chitosan remains clarified.

3.1.2 Signal transduction

Chitosan application could stimulate defense responses of hydrogen peroxide (H_2O_2) via the octadecanoid pathway and nitric oxide (NO) in the chloroplast, MAP-kinase activation, oxidative production, and hypersensitive responses [15]. In chitosan-treated plants, these signal molecules affect adaptive mechanism in response to biotic and abiotic stresses.

The interaction between chitosan and plant cell is initiated when chitosan binds to specific receptors, it then elicits secondary messengers such as H_2O_2, calcium ion (Ca^{2+}), NO, and phytohormones inside the cell to induce physiological responses [10], [15]. Hydrogen peroxide acts as a signal molecule to induce resistance to osmotic stress in the 'Leung Pratew123' ('LPT123') rice (*Oryza sativa*) and mutated line, LPT123-TC171, by enhancing plant growth and maintaining photosynthetic pigments under osmotic stress [16].

Chitosan induces Ca^{2+} in plant species by regulating callose synthase activity [17], [18], which results in Ca^{2+}-mediated programmed cell death in soybean (*Glycine max*) cells [19]. However, NO-signaling has been found in pearl millet (Pennisetum glaucum) seedlings treated with chitosan [20].

Chitosan also elicits the accumulation of jasmonic acid (JA) in several plants such as tomato (*Solanum lycopersicum*) [21], French bean (*Phaseolus vulgaris*) [22], and rapeseed (*Brassica napus*) [23]. In rice (*O. sativa*) seedlings, chitosan induced an increase in JA and the accumulation of 12-oxo-phytodieonic acid *via* the octadecanoid pathway [24]. Additionally, abscisic acid (ABA) also increased via H_2O_2 signaling, thereby leading to stomatal closure and reduced water usage in plants under abiotic stress [15]. Chitosan application induced ABA accumulation in leaf tissues and elicited resistance to tobacco necrosis virus (TNV) [25]. Moreover, JA and salicylic acid (SA) are plant hormones required for signal transduction leading to plant resistance to pathogens and insects [26], JA mediates induced systemic resistance, while SA mediates systemic acquired resistance (SAR) [27].

3.2 *Chitosan response genes, proteins and metabolites*

In the transcriptional level, oligochitosan-treated plants induced gene expression in 2-fold compared to the control plants. These genes were involved in primary metabolism, transcription, defense, and signal transduction [23].

Nowadays, proteomics has become a powerful tool for identifying protein responses in plants. Several literatures revealed that chitosan induced some defense-related proteins. Defense responses in chitosan-induced rice revealed 14 up-or down-regulated proteins which were detected and related to signal transduction [28]. Chitosan application sharply induced 11 proteins of the pathogenesis-related protein-10 (PR-10) family using two-dimensional gel electrophoresis (2D-PAGE) [29]. The one-dimensional (1D)-polyacrylamide gel electrophoresis (PAGE) proteomics analysis revealed that chitosan-induced expression levels in rice leaves changes significantly in 352 proteins and co-expressed proteins were observed in the chloroplasts [30].

Moreover, metabolomics study showed that chitosan-induced the accumulation of stress

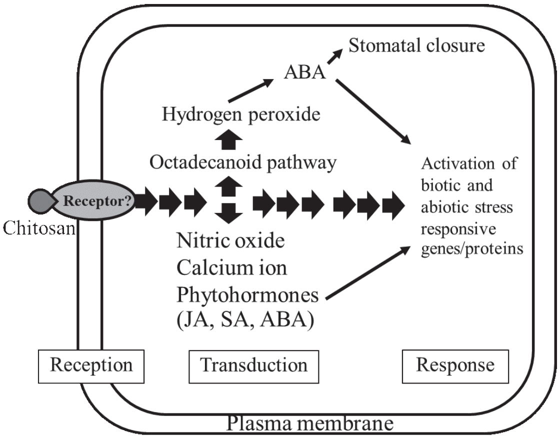

Figure 3: Signal transduction by chitosan in plant cell subjected to stresses. JA (jasmonic acid), SA (salicylic acid), ABA (abscisic acid).

protective metabolites, the enhancement of ascorbate-glutathione, tricarboxylic acid cycle, increasing in the γ-aminobutyric acid shunt, polyamine synthesis and flavonoids metabolism to improve osmotic adjustment, antioxidant capacity, stress signaling and energy production for stress defense under drought stress in white clover [31]. Below is the summarized view of signal transduction by chitosan in plants under stress conditions (Figure 3).

3.3 *Secondary metabolites and production*

The effect of chitosan on plant defense-related secondary metabolites accumulation was studied in many plant species. In 24 h, chitosan induced a phytoalexin (pisatin) in soybean (*Glycine max*) pod [32]. Similar results were found in parsley (*Petroselinum crispum*) [33] and bright eyes (*Catharantus roseus*) [34]. Also, oligochitosan induced the accumulation of chlorogenic acid and rutin in tobacco (*Nicotiana tabacum*) leaves [35]. In suspension-cultured cells of soybean (*G. max*), the synthesis of callose started within 20 min of treatment with chitosan and persisted for hours [36]. Recent works indicated that callose apposition caused by chitosan is related to ABA accumulation [25]. Oligochitosan at a different molecular weight and deacetylation degree also elicited lignin deposition in wheat (*Triticum aestivum*) leaves [37].

Moreover, chitosan induced the accumulation of phenolic compounds in many plant species such as apricot (*Prunus armeniaca*) [38], cherries (*Prunus avium*) [39], and dragon fruit (Cereus enneacanthus) [40].

4 Chitosan Application During Biotic and Abiotic Stresses

In plants, chitosan is proposed to be an elicitor in defense responses involving biotic and abiotic stresses (Table 1). The effect of chitosan was initially studied in the different cell wall compositions of fungi. Chitosan has antifungal, anti-bacterial, and anti-viral activities against invading pathogens, it also contributes to strengthening the plant immune system [41].

4.1 *Biotic stress*

Chitosan reveals a wide range of eliciting compounds in many plants [42]. Under biotic stress, chitosan-treated plants can induce defense responses, including the assembly of phytoalexin, pathogenesis-related proteins (chitinase and β-glucanase), and proteinase inhibitors [15]. Mainly, Oligomeric chitosan molecules were found to increase the defense-related compounds and played the role of antimicrobial compounds, thereby stimulating plant defense [43].

Chitosan was seen to have induced the production of phytoalexin in tomato (*Lycopersicon esculentum*) [44]. Furthermore, chitosan elicited an increase of chitinase and glucanase in several plants, including grape (*Vitis vinifera*) [45], coconut (*Cocos nucifera*) [46] peach (*Prunus persica*) [47], and dragon fruit (*C. enneacanthus*) [48]. However, the different molecular weight of chitosan affected the level of induction of pathogenesis-related protein in rice, suggesting that different types of chitosan possess varying functionalities in plant species [49]. In addition, the application of chitosan was induced protein inhibition in pea (*G. max*) [50] and tomato (*S. lycopersicum*) [51].

4.2 *Abiotic stress*

Chitosan has the potential to elicit beneficial responses in plant species, especially chitosan-treated plants against various abiotic stresses. However, chitosan function is dependent on the structure, concentration, species, and developmental stage of plants

Table 1: Chitosan effects in different plants under biotic and abiotic stresses

Plants	Stress	Method of Application	Effects	References
Tomato (*Lycopersicon esculentum*)	Biotic	Fruit dipping in post-harvest	Induced production of rishitin (a phytoalexin)	[44]
Grape (*Vitis vinifera*)	Biotic	Excised leaf incubation	Induced chitinase activity	[45]
Coconut (*Cocos nucifera*)	Biotic	Adding to cell culture	Increased glucanase activity	[46]
Peach (*Prunus persica*)	Biotic	Fruit dipping in post-harvest	Increased glucanase activity	[47]
Dragon fruit (*C. enneacanthus*)	Biotic	Fruit dipping in post-harvest	Increased glucanase and chitinase activities	[48]
Pea (*Glycine max*)	Biotic	Application on the surface of pea pods	Induced proteinase inhibitor (pisatin)	[50]
Grapevine (*V. vinifera*)	Drought	Dipping of stem cutting before planting	Maintained chlorophyll content	[54]
Rice (*Oryza sativa*)	Drought	Seed soaking and foliar application on seedlings	Induced H_2O_2 production	[55]
Pepper (*Capsicum* sp.)	Drought	Foliar application on seedlings	Reduced the water usage	[56]
Cowpea (*Vigna ungiculata*)	Drought	Foliar application on seedlings	Improved growth and yield	[57]
White clover (*Trifolium repens*)	Drought	Foliar application on seedlings	Increased the production of stress-responsive metabolites	[31]
Thymus daenensis Celak	Drought	Spraying before the flowering stage	Increased flowering and full bloom	[58]
Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.)	Salt	Seed soaking	Reduced oxidative stress	[60]
Ajowan (*Carum copticum*)	Salt	Seed soaking	Increased shoot and root length	[61]
Plantago ovata	Salt	Seed soaking	Increased roots hoot	[62]
O. sativa	Salt	Seed soaking	Enhanced catalase and peroxidase enzymes	[63]
Vigna radiata	Salt	Seed soaking	Stimulated morphological parameters	[64]
Zea mays	Salt	Foliar application	Enhanced growth	[65]
Triticum aestivum	Salt	Adding to the nutrient solution	Alleviated adverse effect of salt stress	[66]

4.2.1 Drought stress

Drought stress or water deficit reduces plant growth and yield [52]. However, the application of chitosan prior to drought stress can stimulate plant growth and increase water and essential nutrients uptake by enhancing antioxidants to scavenge reactive oxygen species (ROS) [53].

In the grapevine (*V. vinifera*) stem, chitosan concentration of 1.0% (w/v) induced drought tolerance by the maintenance of the chlorophyll content under drought stress [54]. Chitosan-induced drought resistance was also recorded in rice (*O. sativa*) [55]. Also, Chitosan-induced drought tolerance was found in the 'LPT123' rice line, which functioned via H_2O_2 production following chitosan treatment, suggesting that it was essential for the induction of drought tolerance [16]. In other plants, chitosan application by foliar treatment decreased transpiration rate. It reduced the water usage of pepper (*Capsicum* sp.) leaves by 26–43%, whereas the biomass and yield did not change [56]. In cowpea (*Vigna ungiculata*), foliar application of chitosan improved growth and yield parameters in both drought stress and non-stress conditions [57]. Pretreatment with chitosan containing Hoagland's solution before drought stress increased the production of stress-responsive metabolites in white clover (*Trifolium repens*) [31]. The application of chitosan three times by spraying before the flowering stage induced an increased flowering by 50% and full bloom, also reduced the negative effect of drought stress in *Thymus daenensis* Celak [58]. Although, spraying of the foliar three times with chitosan before flowering enhanced plant growth in sweet basil (*Ocimum ciliatum* and *O. basilicum*) [59].

4.2.2 Salt stress

Salinity affected the changes in physiology and biochemistry of plant cells. It can inhibit water and nutrient uptake. Salt stress-induced ROS leads to oxidative stress. Salt stress-induced lipid peroxidation determined by malondialdehyde (MDA) accumulation also leads to ion toxicity. Interestingly, chitosan treatment at low concentrations could relieve the adverse effects of salt stress. Chitosan-treated plants were able to reduce oxidative stress by producing enzyme activities in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) [60]. Chitosan pretreatment under salt stress also increased the activities of antioxidant enzymes. It revealed a lower MDA content in *Carum copticum* [61], *Plantago ovata* [62], *O. sativa* [63], *Vigna radiata* [64], and *Zea mays* [65]. Oligomeric chitosan was applied to seeds that showed a significant increase in antioxidant enzymes under salt stress in *T. aestivum* [66].

4.2.3 Heat stress

Heat stress typically occurs along with drought stress [67]. A combination of zinc and humic acid with chitosan sprayed on dry bean leaves could result to resistance to heat stress [68]. ABA can also trigger heat shock-responsive genes such as abscisic acid responsive-element-binding factor 3 (*ABF3*), thereby reducing tolerance to heat stress [69]. In correlation with previous reports, chitosan can induce stomatal closure by inducing ABA synthesis [56].

5 Conclusions

Chitosan is a co-polymer, which stimulates various plant responses, including the induction of plant defense response to biotic and abiotic stresses. It has the potential of anti-pathogen invasion in plant immune systems and induces the elicitation of plant compounds. Under abiotic stress, chitosan induces several antioxidants and ABA to improve plant tolerance. However, the complexity of plant perception and transduction of chitosan has been investigated but still remains unclear. Further, more information on how plants responding to chitosan through these processes is needed. The transcriptome and proteome analyses following the application of chitosan are required to provide methods for improving plant tolerance under stress conditions.

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