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Exploiting Plant Innate Immunity to Protect Crops Against Biotic Stress: Chitosaccharides as Natural and Suitable Candidates for this Purpose

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1. Introduction

One of the most innovative approaches for controlling plant diseases is through the enhancement of the plant's own defence mechanisms (induced resistance), which would not involve the application of toxic compounds to plants. It has been well established for over 100 years, that plants can defend themselves; however, in the last 20 years, a significant progress in our knowledge on plant immunity, has provided the understanding required to allow induced resistance to be used in practice. In this chapter we will discuss the bioactivity of chitosaccharides as pathogen associated molecular patterns and their potentiality in crop protection.

2. General remarks on chitosaccharides structure and availability

The fungal kingdom is extremely varied in species and reproductive structures; however, the shape and integrity of a microorganism is determined by the mechanical strength of its cell wall. This complex cellular structure performs a broad range of crucial roles during the interaction with the environment. Despite the fact that its composition varies noticeably between species, it is composed typically of glucan, mannan, proteins and chitin (Dhume et al., 1993).

Chitin is a lineal polysaccharide composed of 2-acetamide-2-deoxy-D-glucopyranoside (N-acetyl-D-glucosamine) residues ß-(1-4) linked (Figure 1) that can be also found in insect exoskeletons and crustacean shells but not in plants. Chitosan is the name used for low acetyl substituted forms of chitin and consequently, is a linear heteropolysaccharide composed of ß-1,4-linked 2-amino-2-deoxy-D-glucopyranose (D-glucosamine) and N-acetyl-D-glucosamine in varying proportions (Figure 1). Chitosan is commercially produced by

alkaline deacetylation of chitin and also occurs naturally in some fungi but its occurrence is much less widespread than that of chitin. Interestingly, conversion of chitin to chitosan in surface-exposed cell wall of fungal infection structures growing in plant tissues has been reported (El Gueddari et al.,2002).

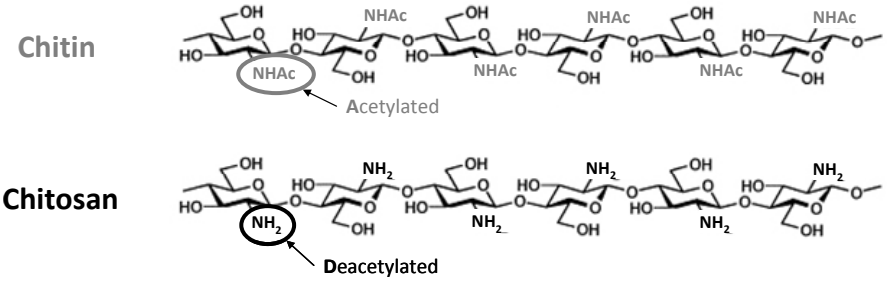

Fig. 1. Structure of chitin and chitosan molecules. Chitin is a ß-(1-4) polymer of N-acetyl-D-glucosamine and fully de-acetylated chitosan is a ß-(1-4) polymer of D-glucosamine. In chitosan, some N-acetyl-D-glucosamine residues could appears and the ratio between both structural units is defined as degree of acetylation (DA).

Chitin is estimated to be produced almost as much as cellulose. Crustacean shells, wastes from the processing marine food products, constitute the conventional and major current commercial source of chitin. Conversely, progress in the control of fungal fermentation processes to produce high quality chitin makes fungal mycelia an attractive alternative source. The total annual world production of purified chitin is estimated in 1600 tonnes. Japan and USA are the most important producers; however, chitin and chitosan are also manufactured in lower quantities in India, Italy, Poland, Brazil, Cuba, Ireland, Norway, Uruguay, Russia and Belgium.

Chitosaccharides have been proven to have a wide variety of applications in the biomedical, pharmacological, agricultural and biotechnological industries. Therefore, recent studies on chitosan have attracted interest in converting it to more soluble chitooligosaccharides, which possess a number of interesting biological activities, such as antibacterial, antifungal and antitumor properties as well as immune enhancing effects on animal health.

3. Chitosaccharides as inducers of plant defence responses

3.1 Chitosaccharides in plant innate immunity

Today, nobody is in doubt about the ability of plants to defend themselves against potential pathogens through a peculiar immune system (Iriti & Faoro, 2007). Consequently, considering the huge collection of potential phytopathogens surrounding, plant diseases can be seen as an exceptional event.

The plant defense system is composed by a many-sided arrangement of passive and active responses. Some plant structures constitute material barriers hindering access of the pathogen; prevent free nutrient movement and therefore helping to retain away the pathogen. The outer surfaces of plants have waxy cuticles and preformed

antimicrobials to avoid potentially infectious invaders access. Furthermore, the plant cell walls provide a major second barrier to any invaders who gains access to inner spaces (da Cunha et al., 2006).

The cell wall performs many of the most important functions of the cell. It provides the protoplast, or living cells, with mechanical protection and a chemically buffered environment. The cell wall allows for the circulation and distribution of water, minerals, and other small nutrient molecules inside and outside of the cell. It provides rigid building blocks from which stable structures such as leaves and stems can be produced. The cell walls are composed of polysaccharides, smaller proportions of glycoprotein and, in some specialized cell-types, various non-carbohydrate substances such as lignin, suberin, cutin, cutan or silica. Wall polysaccharides fall into three categories: pectins, hemicelluloses and cellulose (Fry, 2004). Pectins and hemicelluloses are components of the wall "matrix", within which are embedded the skeletal, cellulosic micro-fibrils.

These polysaccharides are cross-linked by both ionic and covalent bonds into a network that resists physical penetration. Perhaps, this is one reason why, early in the interaction, microbial pathogens excrete cell wall degrading enzymes (CWDE) in the apoplast to degrade this first barrier and to allow plant penetration and colonization (Figure 2). The degradation of the plant cell wall polysaccharides by the CWDE provides microbes with nutrients, but also releases oligosaccharides functioning as molecular signals in the regulation of growth, development and defense responses. In this sense, the plant cell wall could also be considered as storage site for regulatory molecules (Fry, 2004).

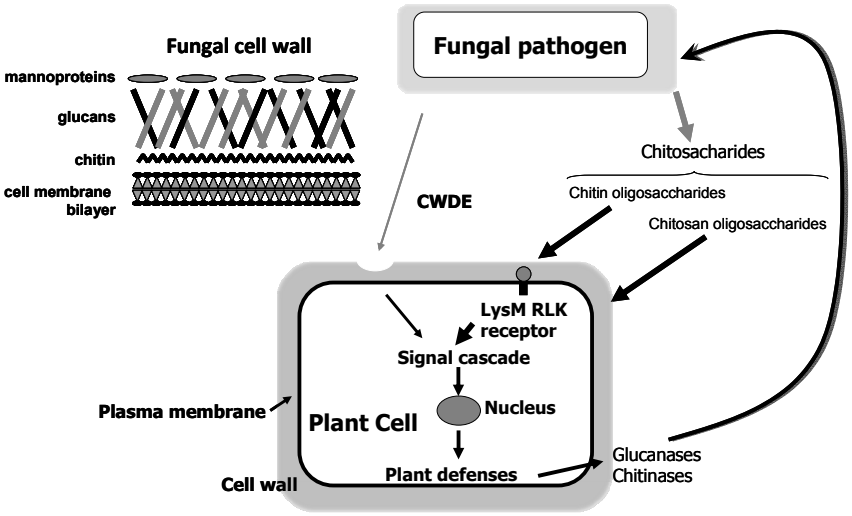

Fig. 2. Chitosaccharides are involved in different aspects related to plant innate immunity. Chitin is a major component of fungal cell wall, serving as a fibrous reinforcement constituent responsible for cell wall rigidity. Consequently, fungal cell wall chitin is a major target of defensives plant chitinases, and resulting partially degraded chitin structures (chitin and chitosan oligosaccharides) are PAMPs, which trigger plant immunity (PAMP-triggered immunity) responses.

A germ that overcomes these physical obstacles (passive defenses) is susceptible to molecular recognition by plant cells. During the evolution, plants have been provided with sophisticated defensive strategies to "perceive" these pathogens, and to transform that "perception" into a suitable resistance (active defenses).

Plant cells are able to recognize and respond to pathogens autonomously (Zipfel, 2008). In addition, systemic signaling can be triggered to prepare other tissue in the vicinity for imminent attack. Initially, microbes can be detected via perception of pathogen associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) located on the plant cell surface.

PAMPs are conserved, indispensable molecules that are characteristic of a whole class of microbes and therefore are difficult to mutate or delete (Shibuya and Minami, 2001). They are also referred to as microbe associated molecular patterns (MAMPs), as they are not restricted to pathogenic microbes. This first level of recognition is referred to as PAMP-triggered immunity (PTI). Intracellular responses associated with PTI include rapid ion fluxes across the plasma membrane, MAP kinase activation, production of reactive-oxygen species, rapid changes in gene expression and cell wall reinforcement (Chisholm et al., 2006).

Many PAMPs fulfill a critical function to the lifestyle of the organism or for penetration and invasion of a host cell and are therefore broadly conserved among diverse microbial pathogens, and are not normally present in the host (Krzeminski et al., 2006). These include lipopolysaccharides of Gram-negative bacteria, peptidoglycans from Gram-positive bacteria, flagellin, protein subunit of the flagellum, glucans, and proteins derived from fungal cell walls (Nurnberger et al., 2004).

Prominent PAMPs recognized by plant cells are chitin fragments released from fungal cell walls during pathogen attack, which in many plants elicit the plant defense response (oxidative burst, protein phosphorylation, transcriptional activation of defense-related genes, phytoalexin biosynthesis, etc (Shibuya and Minami, 2001).

During decades, the eliciting of plant defense responses by chitosaccharides have been broadly studied in a great number of plant species or plant-pathogen interactions (for recent reviewing see Bautista-Baños et al., 2006; Yin et al., 2010; El Haldrami et al., 2010). Upon application, chitosaccharides behave as a PAMP, meaning that they are recognized as a general pathogenic pattern by plant cells and thereby provoking the activation of unspecific or basal resistance that causes a general protection to different plant pathogens.

3.2 Perception of chitosaccharides by plant cell

Plant cell receptors to chitin and chitosan have been extensively searched for two decades. For chitin oligosaccharides, a high-affinity binding site was found in a microsomal plasma membrane preparation from suspension cells of several plant species as rice (Shibuya et al., 1996), tomato (Baureithel et al., 1994), soybean (Day et al., 2001), wheat, barley and carrots (Okada et al., 2002) by using the techniques of photo-affinity labelling and protein-carbohydrate affinity cross-linking.

Later on, the chitin rice cell receptor was purified and characterized as a glycoprotein of 328 aminoacids residues linked to glycan chains (Kaku et al., 2006). The function of this rice

membrane receptor as the chitin perception to unchain the defence signal transduction in rice was confirmed by suppression experiments of the elicitor-induced oxidative burst as well as the gene responses (Kaku et al., 2006). Moreover, by monitoring the production of reactive oxygen species and the expression of early-responsive genes in protoplasts, treated with chitin oligosaccharides and including inhibitors of signal transduction it was demonstrated that rice protoplasts conserve the machinery for the recognition of, and the initial defense signaling activation by chitosaccharides (Nishimura et al., 2001). Altogether support the induction of plant resistance starting from a chitin perception in plant cell membrane. Interestingly, structural studies of the rice glycoprotein receptor showed that this membrane protein bears two LysM motifs in the extracellular portion of the protein.

Recently, a LysM receptor-like kinase (RLK 1) was detected in Arabidopsis. The authors demonstrated its critical role in chitin-induced resistance since a mutation in RLK 1 blocked the induction of oligochitin responsive genes and caused enhanced plant susceptibility to fungal pathogens (Wan et al., 2008).The identified chitin-binding proteins, apparently, do not have specific binding interaction with oligochitosan. So, the acetyl groups have a role in this protein–carbohydrate interaction. Several authors speculated that chitosan derivatives are perceived by plant cell as result of their interaction with pectic homogalacturonan in the plant cell wall (Cabrera et al. 2010) and membrane negative charges, as for instance, those of the phospholipids exposed to the apoplast (Kauss et al., 1989).

The disruption of Ca^{2+} induced association of pectin molecules (pectic egg boxes) by degradation products of fungal chitosan would be perceived and interpreted by plant cells as a distress signal commanding the defense responses (Cabrera et al. 2010). This is also in agreement with an earlier report in which chitosan is shown to displace calcium ions from isolated cell walls of *Glycine max* suspension cultures (Young & Kauss, 1983). Deacetylated chitosans but not chitin are present on the surface of the cell walls of fungal infection structures growing *in planta* after pathogens such as *Puccinia graminis, Uromyces fabae or Colletotrichum lindemuthianum* have invaded their hosts (El Gueddari *et al*., 2002). *Colletotrichum lagenarium* (Siegrist & Kauss, 1990) and *Fusarium solani* (Hadwiger & Beckman, 1980) start producing chitin deacetylase when they establish intimate contact with the tissue of their host plants. In *Uromyces viciae-fabae*, chitin deacetylase activity massively increases when the fungus starts to penetrate through the stomata (Deising & Siegrist, 1995). Once deacetylated, chitosan depolymerization can be carried out, at least *in vitro*, by a plethora of enzymes including lipases, glucanases, cellulases, hemicellulases and pectinases (Cabrera & Van Cutsem, 2005).

Chitosan interaction with plant cell membrane can also occur by electrostatic interaction (Kauss et al., 1989). Distances between glucosamine units in chitosan polymers are at 0,52 nm being a not rigid structure but a floppy conformation that allow to reduce this distance and to match glucosamine units with polar heads of phospholipids exposed to the outer membrane in an area of 0,4 to 0,6 nm^2. Consequently, depending on the polymerization of the chitosan fragment and the distribution of the glucosamine monomers, there is going to be interactions at numerous membrane sites causing changes on membrane fluidity and ion flux alterations that could trigger the signal transduction cascade leading to plant resistance.

3.3 Plant signalling and defences induced by chitosaccharides

Upon chitosaccharides perception, membrane depolarization constitutes the first macroscopic incident detected at seconds or a few minutes after recognition. The depolarization is the result of an alteration of ionic flux across the membrane (Shibuya & Minami, 2001). There is a transient influx to the cytosol of an elevate amount of Ca^{2+} along with H^+ followed by the transient efflux of K^+ and Cl^- in order to equilibrate charges in both sites of the membrane. This process provokes an alkalinization of the apoplast and an acidification of the cytoplasm (Table 1).

Plant model	Chitosan /rate	Defense signal	References
Arabidopsis thaliana cell suspension	Chitin and chitosan oligomers (100- 500 mg/L)	H_2O_2 accumulation.	Cabrera et al., 2006
Brassica napus seedlings	Oligochitosans at 50 mg/ L	Nitric oxide and H_2O_2 production in plant leaves	Li et al., 2009
Cocus nucifera calli	Chitosan	MAP-Kinase activation	Lizama-Uc et al., 2007
Mimosa pudica cell suspension	Chitosan (10-100 mg/L)	Membrane depolarization , extracellular alkalinisation	Amborabé et al., 2008
Rice cell suspension	Chitosan of different molecular weight (5-1000 mg/L)	H_2O_2 accumulation. Best results found between 50-100 mg/L	Lin et al., 2005
Rice seedlings	Chitosan MW (3000-30000) at 1000 mg/L	Octadecanoid intermediates and jasmonic acid production	Rakwal et al., 2002
Soybean cell suspension	Chitosan (25-200 mg/ L)	Increased cytosolic Ca^{2+} concentration and accumulation of H_2O_2. Best results with 25 mg/L	Zuppini et al., 2003
Tobacco cell suspension	Chitin and chitosan oligomers. 25 mg/L	Increased cytosolic Ca^{2+} concentration and Oxydative burst	Kawano et al., 1999
	Oligochitosans DP 3-9 (25-100 mg/L)	Nitric oxide and H_2O_2 production	Zhao et al., 2007
Tobacco plants	Oligochitosans	MAP-Kinase activation	Yafei et al., 2010

Table 1. Plant defence signalling activated by chitosaccharide derivatives

Recently, Amborabé and coworkers (2008) demonstrated membrane depolarization and the rise of pH of culture media in *Mimosa pudica* cells elicited by chitosan. By using plasma membrane vesicles they detected an inhibition by chitosan on the proton pumping and in the catalytic activity of the H^+ATPase enzyme at least 30 minutes after the elicitor treatment that disturb the H+ fluxes and in consequence it modified the membrane transport of nutrients (Amborabé et al., 2008).

Alkalinization of the extracellular site of the membrane causes activation of the $NADH^+$ oxydase complex which is the main cause of the oxidative burst and ulterior formation of H_2O_2 catalyze by several oxidative enzymes (García-Brugger et al., 2006). Several studies, mainly in cell suspensions, demonstrated a typical PAMP-activated oxidative burst after chitosaccharides treatments (Table 1), sometimes showing its relation with other signals as Ca^{2+} and nitric oxide (Kawano et al., 1999; Zuppini et al., 2003; Zhao et al., 2007; Li et al., 2009). For instance, Ca^{2+}, a second messenger in defense reactions that enters in the cytoplasm upon PAMP activation, has been reported to accumulate H_2O_2 previous to gene activation and cell death in soybean cells (Zuppini et al., 2003).

Mitogen-activated protein kinases (MAPK) are one of the largest families of threonine kinases that transduce extracellular signals to inner responses in higher plants, including abiotic and biotic factors. Working with transgenic (oligochitosan induced protein kinase antisense gene) and wild type tobacco plants Yafei and coworkers (2010) demonstrated a tight relationship between oligochitosan induced MAPK activation and tobacco plant resistance against viral infection (TMV). When analyzing defense activation, these authors found a positive correlation between some oxidative enzymes activities and the oligochitosan induced MAPK. In addition, they demonstrated the up-regulation by this transduction protein of the PR-proteins mRNA transcripts (Yafei et al., 2010). Other secondary signals related to defense activation, as nitric oxide, salycilic acid, jasmonic acid and ethylene has also been directly or indirectly reported as activated after chitosaccharides treatments (Rakwal et al., 2002; Zhao et al., 2007; Iriti et al., 2010).

Below we review several results of chitosaccharides induced defenses in different plant systems (Table 2).

Callose and lignifications: Increments of appositions of β 1-3 glucan (callose) at, or around, penetration sites and the deposition of phenolic (lignin-like) compounds over the entire wall of the infected cells constitute one of the early plant defense responses activated by chitosaccharides. Chitin and chitosan have been reported as inducers of both histological barriers in several plant species, but especially in monocots (Table 2). Chitin oligomers, chitosan polymers and chitosaccharide of different physico-chemical features (Vander et al., 1998) are good inducers of lignin formation in wheat leaves (Barber et al., 1989). A high stimulation of phenolic acids synthesis and lignin precursors as ferulic, p-coumaric and sinapic acids were found to correlate to increasing of the oligochitosan concentrations (Bhaskara-Reddy et al., 1999).

Chitosan also induce callose and lignin formation in tomato (Mandal & Mitra, 2007), parsley (Conrath et al., 1989) and beans (Faoro & Iriti, 2007). Using parleys cell suspension cultures, the persistence of the chitosan signal was established (Conrath et al., 1989). When cells previously elicited were subcultivated, less chitosan concentration was required to elicit callose synthesis. Chitosan-induced cell callose synthesis is enhanced by external concentrations of Ca^{2+} probably by both, its action as a secondary defense messenger that enters the cell and also by stabilizing cell membranes (Kauss et al., 1989).

Phytoalexins: Many studies established a correlation between phytoalexin accumulation and resistance to disease. Theses plant compounds, chemical and structurally diverse, are toxic towards a wide range of organisms, including bacteria, fungi, nematodes and higher animals, and even plant themselves (for reviewing see Garcion et al., 2007). Phytoalexin accumulations induced by chitosaccharides have been studied in several plant species (Table 2).

Plant model	Chitosan /rate	Defense response	References
Arabidopsis thaliana cell suspension	Chitin and chitosan oligomers (5- 500 mg/L)	PAL activity and cell death	Cabrera et al., 2006
Bean (*P. vulgaris*) plants	Different MW chitosans (0.1-0.2 %)	Callose deposition. Correlation with antiviral activity.	Faoro & Iriti, 2007
Catharantus roseous cell suspension and protoplast	Chitosans of different MW and DA and chitin oligomers (40-500 µg/ 300 mg of cells)	Callose formation and accumulation	Kauss et al., 1989
Cocos nucifera cell suspension	Chitosan polymer	Phenyl-propanoid derivatives	Chakrabort y et al., 2008
Cucumber plants	Chitosan polymer (100, 400 µg/ L)	Structural barriers, antifungal hydrolases	El Gaouth et al., 1994
	Chitosan polymer and chitin oligomer sprayed at 0.1%	Peroxidase and chitosanase	Ben-Shalom et al., 2003
Grapevine plants	Chitosan of 10% DA and 5 Kda MW. Doses: 75-300 mg/L	Lipoxygenese, PAL and chitinase activities	Trotel-Aziz et al., 2006
	Chitosan of different MW and DA (200 µg/ mL)	Phytoalexyn, β 1-3 glucanase and chitinase activities	Aziz et al., 2006
Parsley cell suspension	Chitosans of different MW and DA	Callose apossition and coumarin	Conrath et al., 1989
Pea plant pods	Chitosan and chitosan oligomers (500-2000 µg/L)	Pisatin (phytoalexin) in pods, being heptamer and octamer oligochitosans the best elicitors	Kendra & Hadwiger, 1984 Hadwiger et al., 1994
Potato tubers	Chitosans of different MW (0,01-3000 µg/mL)	Phytoalexin , β 1-3 glucanase and chitinase activities	Vasyukova et al., 2001
Rice cell suspension	Chitosan of different molecular weight (5-150 µg/mL)	PAL and chitinase activity and transcripts of β-1,3 glucanase, chitinase and accumulation of PR1.	Lin et al., 2005
Rice seedlings	Chitosan (1000 mg/L)	PR proteins and phytoalexin accumulation in leaves	Agrawal et al., 2002
	Chitosan and chitosan partially hydrolized (100-1000 mg/L)	PAL, β 1-3 glucanase, chitinase and chitosanase	Rodríguez et al., 2004; 2006; 2007
Ruta graveolens shoots	Oligochitosan (0.1%)	Coumarins synthesis	Orlita et al., 2008

Plant model	Chitosan /rate	Defense response	References
Soybean cell suspension	Low MW chitosan (25-200 mg/L)	Programme cell death and chalcone synthase	Zuppini et al., 2003
Soybean seedlings	Chitin and chitosan oligomers: tetramer, pentamer, hexamer (100 µmol/L)	PAL and TAL activities	Khan et al., 2003
Taxus canadensis cell suspension	Chitin oligomer, colloidal chitin and oligochitosan plus Methyl jasmonate. (0,05 and 80 mg/L)	Paclitaxel (Phytoalexin)	Linden & Phisalaphong, 2000
Tobacco cell suspension	Oligochitosans	Programme cell death	Wang et al., 2008
	Oligochitosan mixture with DP of 3-9. (50 µg/mL)	PAL activity	Zhao et al., 2007
Tobacco cell suspension and whole plants	Chitosan (0,01-0,1%)	Callose accumulation and cell death	Iriti et al., 2006
Tobacco seedlings	Oligochitosans	PAL, peroxidase, POX, catalase and SOD	Yin et al., 2008 Yafei et al., 2009
	Chitosan of different DA, chitosan partially hydrolyzed and oligochitosans (0,1- 2,5 g/L)	PAL, peroxidase and β 1-3 glucanase activities in roots and leaves	Falcón-Rodríguez et al., 2008; 2009 & 2011
Tomato plants	Oligochitosans	Lignin, phenolic compounds and phenyl propanoids enzymes in roots	Mandal & Mitra, 2007
	Chitosan oligosaccharides	Proteinase-inhibitor and phytoalexin	Walker-Simmon et al., 1984
	Oligochitosans	Volatile secondary metabolites with antifungal activity	Zhang & Chen, 2009
Wheat plants	Chitin and chitosan of different MW and DA	Lignification	Barber et al., 1989
	Oligochitosans	Lignin-like and others phenolic compunds	Bhaskara-Reddy et al., 1999
	Chitosan of diferent DA, oligochitosans and oligochitins. (1-100 mg/L)	PAL and peroxidase activity and lignin accumulation	Vander et al., 1998

Table 2. Plant defence responses induced by chitosaccharide derivatives

Chitosan oligomers (above hexamers) were the best pisatin (Pea phytoalexin) inducers in pea endocarp tissues, duplicating the eliciting activity showed by chitosan (Hadwiger et al., 1994). The elicitation of coumarins has been reported in parsley cell suspensions (Conrath et al., 1989) and in *Ruta graveolens* shoots where, in addition, furanocoumarins and other alkaloids were also induced (Orlita et al., 2008). In experiments with wheat whole plants several phenylpropanoid intermediates with antimicrobial activity were stimulated in primary leaves (Bhaskara Reddy et al., 1999). In tomato plants, chitosan increase ferulic acid, 4-hydroxybenzoic acid and 4-coumaric acid content in root cell walls (Mandal & Mitra, 2007). Chitosan oligomers of different molecular weight and degree of acetylation triggered an accumulation of phytoalexins in grapevine leaves. Highest phytoalexin production was achieved in 48 h of incubation with chitosan at 200 µg/mL (Aziz et al., 2006).

Besides phenylpropanoids, terpenoids also form a structural family encompassing many phytoalexins. Agrawal and coworkers (2002) evaluated the accumulation in rice seedlings leaves of Momilactone A, a typical diterpene of Oryza genre, when rice leaves were treated with 0,1% of chitosan polymer. Its induction was also accompanied by an increased of sakuranetin, another rice phytoalexin that belongs to the flavonone group (Agrawal et al., 2002). Terpenoids activation by chitosan has been also reported in solanaceous. A former work showed sesquiterpenes induction by chitosan in tomato leaves (Walker-Simmon et al., 1984). Moreover, in potato, the sesquiterpene phytoalexin Rishitin was induced in tuber discs previously treated with low molecular weight chitosans (Vasyukova et al., 2001). Additional reports showed activation of some other phytoalexin like compounds induced by chitosaccharides. Linden and Phisalaphong (2000) studied the interaction of methyl jasmonate with chitin and chitosan-derived oligosaccharides to stimulate paclitaxel (a taxan) production in the cell suspension system of *Taxus Canadensis*.

Hypersensitive response: Plants often exhibit a form of programmed cell death, called the hypersensitive response (HR) following bacterial, viral or fungal challenge. The HR, which is also activated by several kinds of elicitors, is characterized by the rapid collapse and death of the plant cells in and around the site of attempted infection with deposition of chemical barriers, proteins and phytoalexyns that confine the pathogen to prevent spreading into healthy adjacent tissues. In soybean cells, Zuppini and co-workers (2003) demonstrated triggering of plant cell death, 24 hours after chitosan treatment. Oligochitosans caused tobacco cell death in a dose-dependent manner. About 40.6 % tobacco cells died when cultured for 72 h after 500 µg ml^{-1} oligochitosan treatment (Wang et al., 2008). Similarly, Cabrera and co-workers (2006) demonstrated that chitosan oligosaccharides, depending on their physic-chemical features and concentrations elicit cell death in *Arabidopsis thaliana* cell suspensions.

Pathogenesis related proteins (PRs), recognized plant defenses against pathogens (van Loon et al., 2006), have been reported as elicited by chitosaccharides in many plant species, in most cases, in coordination to other key enzymes from the secondary metabolism as peroxidases, PAL and lipoxygenases (LOX) covering different plant families (Table 2). Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) enzymatic activity and its gene expression is likely one of the defense proteins more studied in response to these elicitors, since it is a key enzyme in the phenylpropanoid pathway and it is also involved in the synthesis of salicylic acid a key signal in plant resistance against pathogens (Way et al., 2002; Ogawa et al., 2006). In this sense, several authors informed PAL activation by chitosan polymers, partially hydrolyzed

chitosan (Falcón et al., 2008) and oligochitosans (Khan et al., 2003) in a variety of plant systems as cell suspensions (Lin et al., 2005; Cabrera et al., 2006; Zhao et al., 2007), plant organs elicited (Vander et al., 1998; Trotel-Aziz et al., 2006) and roots and leaves of whole plants (Mandal & Mitra, 2007; Falcón et al., 2009; 2011). In several reports the role of PAL induced by chitosaccharides as cardinal protecting factor against pathogens has been stated in different plant-pathogen systems (Chen et al., 2005; Yafei et al., 2009; Falcón et al., 2011).

Concerning chitosan dose responses of PAL enzymatic activity, it depends on plant sample system treated; working with cell suspensions the highest PAL activity required about 0.1 $mg.mL^{-1}$ of high oligochitosan molecular weight mixture in *Arabidopsis thaliana* cells (Cabrera et al., 2006), while directly treating plant organs as grapevine leaves and tobacco roots the dose requirements were between 75-250 $mg.L^{-1}$ (depending on time course) for PAL activity in leaves treated with low molecular weight chitosan (Trotel-Aziz et al., 2006) and 100 $mg.L^{-1}$ to achieve the highest activity in roots directly treated with a DP 5-9 of oligochitosan mixture (Falcón et al., 2009). Moreover, when using a foliar spray of whole plants the best results detected in rice leaves for this enzyme activity required 500 $mg.L^{-1}$ of chitosan hydrolysate elicitor (Rodríguez et al., 2007) and 1000 $mg.L^{-1}$ of the DP 5-9 oligochitosan mixture in tobacco leaves (Falcón et al., 2011).

β 1-3 glucanase (EC 3.2.1.39) and chitinase (EC 3.2.1.14) catalyze the degradation of β 1-3 glucan and chitin polymers, majors cell wall components of many pathogens (Arlorio et al., 1992). Chitosaccharides also induce these PR proteins in inoculated and non inoculated monocots and dicots plants, and in different plant biological systems (Table 2). Rodríguez and coworkers evaluated in separate trials the induction PAL, β 1-3 glucanase, chitinase and chitosanase in leaves of rice plants previously treated, by seed immersion before planting, with a chitosan polymer and with a hydrolysate obtained from the same chitosan polymer in non inoculated plants (Rodríguez et al., 2004; 2006) and also in inoculated (*Pyricularia grisea*) plants (Rodríguez et al., 2007). It was observed significant increments above the control in all enzymes tested in leaves of non inoculated plants at 18, 25, 32 and 39 days after seed germination (Rodríguez et al., 2004; 2006). The highest increments in the four enzymatic activities required 1000 $mg.L^{-1}$ of the chitosan polymer (Rodríguez et al., 2004), while the requirements for maximal activities in plants treated with the hydrolysate were lower (Rodríguez et al., 2006). For both elicitors and in all enzymes tested, activities were increased 2-3 times above control depending on each enzyme and the evaluated moment. It means that chitosan seeds immersion can cause high increments of defense responses in non inoculated plants several weeks after treatment. In a different trial, the same authors comparatively evaluated the eliciting of the above studied enzymes in *P. grisea* inoculated rice plants and monitoring enzymatic activities at 1, 3, 5 and 7 days after inoculation (Rodríguez et al., 2007). As in the former reports, the chitosan polymer caused 2-3 times increments above control activity in the four enzymes tested and similar results were found with the hydrolysate in all the enzymes, except for PAL activity that increased 3-4 times above the level of control at 72 hours with all three concentrations tested. This result was consistent with the infection degree found at seven days in rice plants, where all concentration of the hydrolysate clearly protected the plants against the infection, while only the two highest concentration of the polymer significantly reduced plant infection (Rodríguez et al., 2007).

Activation by chitosaccharides of defensive enzymes has also been reported in dicots (Trotel-Aziz et al., 2006; Falcón et al., 2009). By applying different oligochitosan concentrations (75-300 mg.L^{-1}) through the petiole of grapevine leaves it was shown high increments of LOX, PAL and chitinase activities in the leaves directly applied (Trotel-Aziz et al., 2006). The best induced protection against *Botrytis cinerea* occurred with lowest chitosan concentration evaluated (Trotel-Aziz et al., 2006). Working with whole tobacco plants Falcón and coworkers (2011) also demonstrated defensive enzymes activation in non inoculated plantlets. They showed that foliar spray of chitosan polymers and oligochitosans caused activation of β 1-3 glucanase, PAL and peroxidase activities in tobacco leaves. Depending on dose and type of chitosan tested, it was observed increments of 2-3 times above the control for β 1-3 glucanase activity, while for PAL ranged between 2 and 10 times and for peroxidase ranged between 4 and 10 times. Ulterior infection assays performed against *Phytophthora nicotianae* showed a significant relation between the plant protections achieved and the PAL and β 1-3 glucanase activities detected (Falcón et al., 2011).

3.4 Influence of chitosaccharides physico-chemical properties on their bioactivity

Since the biological activities of chitosaccharides have often been determined using heterogeneous and/or uncharacterised oligosaccharide or polymer mixtures, the size and structure requirements for oligochitins and chitosan oligomers to have a biological activity are difficult to ascertain. Additionally, structure-bioactivity relationships depend on the experimental systems (Shibuya and Minami 2001). The oligosaccharides generally must have a DP>4 to induce a biological response, but beyond that requirement, it is not possible to generalise about structural features essential for their biological activity (Côté and Hahn 1994). The concentrations of oligosaccharides that are effective in plant bioassays seem also to be different for both elicitors and dependent on the plant model used. The concentrations of chitosan derived oligosaccharides required to trigger defence responses are usually much higher than those necessary for chitin oligosaccharides to elicit similar defence responses (Yamaguchi et al. 2000).

Different chitosan MW caused a differential activation of H_2O_2 in plant cell. Lin and co-workers (2005) demonstrated that reduction of polymeric chitosan MW (50 kDa) to oligomeric structures (1.3 and 2.7 kDa) benefited the production of H_2O_2 in the cell. However, in arabidopsis cells, chitosan oligomers with higher MW caused the maximum increment of H_2O_2 production (Cabrera et al., 2006). In addition, the DA of chitosan oligomers also affected H_2O_2 accumulation in the same biological system. As lower was de N-acetylation, as higher was the H_2O_2 production, being the oligochitosans with 0% acetylation the best elicitors for H_2O_2 induction (Cabrera et al., 2006).

High molecular weight chitosan induces more callose formation in cell suspensions and protoplast of *Catharanthus roseus* than chitosan oligomers (Kauss et al., 1989). When analysing the effect of the N-acetylation in comparables molecular weight (MW) chitosans, it was found that a partially N-acetylated chitosan was less effective to elicit callose synthesis than a 0% acetylated chitosan; so, it means that in this biological system callose formation is favoured with high MW and non acetylated chitosans (Kauss et al., 1989). These results were also corroborated by Faoro & Iriti (2007) in leaves fragment of *Phaseolus vulgaris* treated by floating in solutions chitosan. They observed that chitosan of medium MW (76, 120 and 139 kDa) caused higher callose deposition than those of low MW (6 and 22 kDa). In

addition, they found that the highest MW (322 and 753 kDa) chitosan tested did not caused callose deposition, probably because of these polymers scarcely penetrated into the leaf and as a consequence there was not direct interaction between polymers and leaf cells membranes (Faoro & Iriti, 2007).

Oligochitosans were better elicitors of pisatin phytoalexin in pea pods than chitosan polymer (Kendra and Hadwiger, 1984; Hadwiger et al., 1994). This behaviour was corroborate in grapevine leaves where it was showed that the oligochitosans of lowest MW (1.5 kDa) induced higher levels of phytoalexins than higher MW (3 and 10 kDa) oligochitosan. In this work, the influence of degree of acetylation (DA) was also evaluated. Intermediate DA tested (20%) caused higher increments of the three phytoalexins evaluated than other ones of lower (2 and 10%) and higher (30%) DA (Aziz et al., 2006).

The chitosan MW and DA, also affect the activation of enzymes and defence proteins. Rodríguez and co-workers (2007) demonstrated that lower MW caused higher increments of defence enzymes activities in leaves of rice plants previously treated by seed immersion with chitosan polymer and its hydrolysate. The polymer induced 2 times above controls PAL, chitinase and chitosanase enzymatic activity, while the hydrolysate did it 7 times for PAL and 3 times for chitinase and chitosanase. These differences observed in enzymatic activity, also provoked, a better plant protection of rice plants against *Pyricularia grisea* (Rodríguez et al., 2007). It demonstrated the chitosan MW influence in a long-lasting induced resistance. The best efficiency with lower MW chitosans on the activation of induced resistance in whole plants was corroborated in tobacco treated by foliar spray. β-1,3-glucanase activity in tobacco leaves required 10 times higher polymer concentration than oligochitosan mixture to induce the highest activity detected above control and this enzyme was significant related to the plant protection found against the infection with *Phytophthora nicotianae* (Falcón et al., 2011).

Different DA also caused differences in enzyme activation. Vander and co-workers (1998), working with directly treated wheat leaves, demonstrated that as increasing the DA until intermediate values it was increased the PAL and peroxidase activity in this organ. Similar behaviour for PAL activity was found in roots and leaves of tobacco plants directly applied by root immersion and foliar spray, respectively. Conversely, POD activity was benefited by the less acetylated polymer for both organs and application forms (Falcón et al., 2009 & 2011). In addition, the influence of the DA in plants applied by root immersion was detected in both roots and leaves for the peroxidase enzymatic activity, indicating that the effect of the DA was systemically transmitted to the leaves (Falcón et al., 2009). From all before described it is clear that MW and DA are important structural parameters that affect biological responses of plant resistance against pathogens, as a consequence, they must be taken into account for a practical approach to develop chitosaccharides as natural pesticides.

4. Antimicrobial activity of chitosaccharides

This section is going to focus on antimicrobial properties of chitosaccharides, how this molecule can affect the microbial cells, the relation of the macromolecule structure and the antimicrobial activity and its action mode; in a few words, how it works.

Chitosan exhibits high antimicrobial activity against a wide variety of microorganisms. An antimicrobial is defined as a substance that kills or inhibits the growth of microorganisms

such as bacteria, fungi, or protozoan (Andrews 2001). The most widely known type of antimicrobial are antibiotics but there is currently growing concern about them because bacteria are becoming resistant. This leads to a demand for effective antimicrobial agents that are less prone to stimulate the development of resistance such as chitosan. Chitosan has been proven able to control different plant pathogenic microorganisms (pre and post harvest disease) on different cultures (Bautista-Banos, et al. 2006).

Although the exact mechanism of the antimicrobial effect is still unknown, several hypothesis have been formulated and chitosan's action is believed to act at different levels depending on circumstances. The presence of a polycationic structure is the main reason of the antibacterial effect of chitosan below pH 6. Below its pKa (6,3-6,5), the amino group (C2 of chitosan glucosamine) is positively charged. This charge is capable to interact with the negatively charged components at the surface of the bacterial cell walls. This binding or interaction leads to a rupture or leakage of proteins and intracellular constituents of the microorganism in the medium (Shahidi, et al. 1999). In a way the more the positive charge density is important along the polymeric chain, the more the antimicrobial properties of chitosan will be important. The charge density is also associated with the DA of the molecule (explained above), as the number of amino groups linked on the chitosan structure impacts the electrostatic interactions. A high amount of amino groups therefore enhance the antimicrobial activity.

When the pH is above the pKa, there is still an antimicrobial effect but the later can no more be explained by electrostatic effect. In those conditions the antimicrobial effect of chitosan only relies on its chelating and hydrophobic capacities that work beyond any pH limit. When using native chitosan, lacking hydrophobic capacities, its antimicrobial effect above pH 6 is therefore principally due to its chelating capacity. Chitosan also has high chelating potential. It can bond to a lot of metal ions (as Ni2+, Zn2+, Co2+, Fe2+, Mg2+ and Cu2+), reason of its use in industry in the recovery of several metal ions (Kurita 1998). Those metallic ions are vital for the stability of the microbial cell wall, the chelation of those ions in acidic but also, in neutral conditions account for a part of the antimicrobial potential of chitosan. This action as a chelating agent that selectively binds trace metals is the reason of the inhibition of the production of certain toxin and microbial growth (Cuero, et al. 1991).

The physical state of chitosan and its Mw are also of great importance in its action mode. This is mainly due to the poor solubility of chitosan. For instance it has been reported that the use of a low molecular weight (LMw) water soluble chitosan or nanoparticles that can penetrate the microbial cell wall, exhibit another form of antimicrobial activity, as they combine to DNA and inhibit mRNA synthesis and DNA transcription (Hadwiger, et al. 1986; Ignatova, et al. 2006; Qi, et al. 2004).

4.1 Factors influencing antimicrobial efficiency

Despite inherent properties of chitosaccharides molecules, the antimicrobial activity is also depending on others factors such as type of microorganisms, environmental conditions and physical states of chitosaccharides.

Microorganisms: Although an inhibitory effect of chitosan has been reported on viruses and viroids (Pospieszny 1997), the majority of the literature is focused on fungi and bacteria.

Bacteria are not the biggest pathogens of plants but really are the most studied type of microorganisms. In fact, most bacteria associated with plants are generally saprotrophic, and do no harm the plant itself. Only a small number of bacteria such as *Agrobacterium tumefaciens, Pantoea stewartii, Erwinia carotovora, Ralstonia solanacearum, Pseudomonas syringae, Pseudomonas aeruginosa, or Xanthomonas campestris* are able to cause plant diseases. In order to be able to colonize the plant, bacteria have specific pathogenicity factors, which include the production of toxins, extracellular polysaccharides, degradative enzymes, effector protein or the secretion of phytohormones. The production of those pathogenicity factors are controlled within the bacterial population via quorum sensing (Von Bodman, et al. 2003).

Although chitosan is a wide spectrum antimicrobial, it exhibits different efficiencies against different types of microorganisms. In this field, contradictory results have been reported. For instance, (Chung, et al. 2004; No, et al. 2002) have reported more bactericidal effects against Gram-positive than on Gram-negative bacteria (in the presence of 0.1% chitosan), while (Zhong, et al. 2008) have reported that Gram-negative bacteria where more sensitive to chitosan than Gram-positive bacteria. Other authors have reported that there were no obvious differences observed between gram positive and gram negative bacteria (Wang, et al. 2004). Two points on which authors appear to all be in agreement is that fungi are more sensitive to chitosan's action than bacteria. Secondly, that the theoretical mode of action seems indeed to be different between Gram-positive and Gram-negative bacteria.

One other point influencing the antimicrobial activity is the age of the cells. Tsai and Su (1999) suggested that the differences of electronic negativity of cell surface vary with the phase of growth leadings to differences in sensitivity towards chitosan. Surface of microorganisms varying from species to species, this explains differences in results, for example, S. aureus CCRC 12657 was found to be more susceptible to chitosan in late exponential phase (Chen & Chou 2005); but E. coli O157:H7, on the contrary, was found to be the most sensitive to chitosan action in its mid-exponential phase (Yang, et al. 2007).

Fungi or molds are parasite on all types of eukaryotic organisms and plants are no exception. Using fungicides can help to control a lot of fungal diseases, but strains often evolve and resistance appears making the use of fungicides inappropriate (reason why the use of chistosan can be a new sustainable solution). The fungicidal activity of chitosan has also been documented. The mecanism of chitosan's action on fungi is believed to be similar to the action on bacteria: amino groups interact with macromolecule's negative charges at the surface of the fungal cell wall (Leuba & Stossel 1986). Other mechanisms of lower importance similar to those presented for the bacteria are also discussed in the literature (interaction with microbial DMN and chelation of metals). In addition, it has been shown that chitosan also has an inhibition effect on several fungal enzymes which slows their growth (El Ghaouth, et al. 1992).

Like for bacteria, the inhibition is dependant on the strain of fungi, the type and the concentration of chitosan used (Benhamou 1992). On the other hand, some fungi such as *Rhizopus nigricans* (bread mold), have been reported to be unaffected by chitosan (El Ghaouth, et al. 1992). Chitosan is able to alter the fungal cells. Fungal morphological changes (cells disorganisation, thinner hyphae (Benhamou 1996; Arlorio, et al. 1992), excessive mycelial branching, hyphal swelling or abnormal shapes (Benhamou 1992; Cheah, et al. 1997) have been observed. Cells exposed to chitosan look like cells displaying signs of nutriment depravation (Barka, et al. 2004).

Fungal sporulation is lower or completely inhibited when fungi are in contact with chitosan. In some cases however, the opposite effect have been reported as well. For instance chitosan is reported to stimulate sporulation on *Penicillium digitatum* (postharvest fungal disease of citrus, green mold) (Bautista-Banos et Hernandez-Lopez 2004) but this might only be a stress response due to the presence of the antimicrobial. The long term effect of chitosan on spore viability has also been proved on *Puccinia arachidis* (peanut rust) (Sathiyabama et Balasubramanian 1998).

Physical state: The antimicrobial reaction, as explained, takes place between the cell wall of the microorganism and the chitosan molecule. The physical state of the later is then highly relevant for the efficacy of the microbial effect.

Chitosan in solution is more effective in inhibiting bacterial growth. This is explained by the fact that in this dissociated form, enables a reaction with the counter-parts to a sufficient degree, enabling the full potential of the molecule (Phaechamud 2008). The Mw and DA of chitosan play an important role as improving solubility can be done by reducing Mw or controlling deacetylation. Last but not least, pH is another important parameter. Firstly pH acts directly its solubility. Secondly, the antimicrobial activity of chitosan is only exhibited when pH is below its pKa (protonation) when the molecule is dissociated as ion (as explained above). Totally dry samples are incapable of inhibiting the growth of microorganisms because they cannot release their energy stored in chemical bond to initiate interaction. In solid state, chitosan can then only react when in contact with solution, therefore at the surface of the material.

Environmental conditions: (Lim & Hudson 2004) rightly stipulated that the antimicrobial activity of chitosan is dependent of the environmental pH. Chitosan has its microbial inhibition activity reducing as pH increases. This is due to two factors, the presence of a majority of uncharged amino groups from pH 7 and onwards and its poor solubility in non-acidic environment (Aiedeh & Taha 2001; Papineau, et al. 1991; Sudarshan, et al. 1992).

The modification of the ionic strength of the medium can impact on the antimicrobial activity in two ways. By increasing the presence of divalent metallic cations, the chelatant power of chitosan is reduced, this leading to a reduced antimicrobial activity (Kong, et al. 2008).Thus, the cations in the medium interact competitively with the negative components of the microbial membrane therefore also reducing the antimicrobial activity of chitosan. (Xing, et al. 2009) also demonstrated that the addition of anions also, in their experiment phospate groups, decreased the antimicrobial activity.

The temperature also plays an important role in the antimicrobial effect on *E. coli*. (Tsai & Su, 1999). At low temperature, such as 4°C, the cell wall structures of the microorganisms are impacted in a way that the number of potential binding sites for chitosan is decreased consequently lowering the antimicrobial effect of chitosan.

5. Chitosaccharides in crop protection

On spite of chitosaccharides research studies, as inhibitor of microbial development and plant defence inducer, have been broadly performed during the last 3 decades, most of them have investigated the basic insights concerning the effects and action mode of these compounds on plants and microorganisms (Vander et al., 1998; Xu et al., 2007) while

practical approaches have been much less reported. This could be related with the fact that trials to evaluated protective efficacy of chitosaccharides against pathogenic diseases are difficult to do. In addition, as several chitosaccharides have been reported as growth and yield enhancers (Boonlertnirun et al., 2008; Abdel-Mawgoud et al., 2010), most of studies out of labs and controlled conditions evaluated their capabilities to improve crops while monitoring the control of natural pathogen incidence. In this sense, the influence of chitosaccharides used to control plant pathogens, being in control or uncontrolled trials, depends on the pathosystem, the type of used derivative, concentration and formulation.

Crop protection by applying chitosaccharides have been extensively reported worldwide against diverse pathogens including virus, fungi, oomycetes and bacteria (Rodríguez et al., 2007; El Haldrami et al., 2010; Falcón et al., 2011). Viral infections cannot be controlled by chemicals; however, several reports demonstrated reduction of virus in inoculated plants previously treated with chitosaccharides. Chitosan inhibited potato spindle tuber viroid infection when added to the inoculum and when sprayed into the leaves of tomato plants prior to viroid inoculation. Chitosan was also effective when sprayed into viroid inoculated leaves not later than 1-3 h after inoculation (Popieskny, 1997). In addition, the degradation of chitosan polymer affect the antiviral activity depending on the pathosystem tested. Using doses between 0.01 and 0.25%, a partially degraded chitosan highly reduced the % of TMV infection in tobacco leaves respect to the original polymer; while conversely, polymer was more effective than its partially degraded derivative to reduce the % of infection in bean by alfalfa mosaic virus (Struszczyk et al., 1999).

Correlation between defenses responses and antiviral activity has been detected. In bean plants inoculated with Tobacco necrosis virus (TNV), the efficacy of the antiviroid activity of chitosans positively correlated with their ability in inducing callose apposition (Faoro & Iriti, 2007). The same authors working with a tobacco-TNV pathosystem demonstrated antiviral resistance in plants previously treated with 0.1% chitosan. The resistance induced was associated with callose deposition, micro-oxidative burst and micro-hypersensitive response (Iriti et al., 2006). Moreover, an oligochitosan mixture sprayed in tobacco plants caused antiviral activity against tobacco mosaic virus and this resistance was related to nitric oxide production and increments in PAL enzymatic activity (Zhao et al., 2007).

Among pathogens, fungi cause the most destructive diseases and the highest losses in agriculture. Most plant protection by using chitosaccharides have been reported against this group of pathogens. In monocots, several results showed chitosan potentialities to protect against fungal diseases; Lin and coworkers (2005) observed a differentiate protective behavior when rice seedlings, inoculated with *Pyricularia grisea*, were previously treated by chitosans of different MW and favoring the lowest MW chitosan tested. In the same pathosystem, similar results were obtained by using polymeric and oligomeric chitosans applied by seed immersion before planting and testing plant infection in 25 and 32 days old plant seedlings. It demonstrated rice plant protection by lasting induced resistance (Rodríguez et al., 2007). Comparison of the effect of different mode of chitosan applications in plant protection were studied using a commercial chitosan product (Elexa) in another pathosystem with monocot specie. Seed treatment, foliar spray and the combination of both were tested for control of downy mildew caused by *Sclerospora graminicola* in Pearl millet and at greenhouse and field conditions (Sharathchandra et al., 2004). Under greenhouse conditions seed treatment offered 48% protection while maximum protection of 67% was

recorded with foliar spray to 2-day-old seedlings. However, the combination of both methods allowed achieving 71% protection. At field conditions foliar spray and the combination of both methods showed the best results to reduce disease incidence and severity. An additional benefit of increase in plant height and yield was obtained at greenhouses experiments (Sharathchandra et al., 2004).

An important number of reports showed chitosaccharides protection of dicot crops against fungal pathogens (for reviewing see Bautista-Baños et al., 2006; El Haldrami et al., 2010). Many studies have been developed under controlled bioassays. In this sense, Trotel-Aziz and coworkers (2006) demonstrated protection on grapevine against *Botrystis cinerea* working with detached leaves incubated with chitosan oligomers and subsequently inoculated with the pathogen. Similar method of floating leaves in chitosan solutions or leaves spraying before inoculation were performed to demonstrated chitosan protection against *B. cinerea* and *Plasmopara viticola* in grapevine and synergistic activity with copper sulfate (Aziz et al., 2006). Dose responses in chitosan plant protection depend on biological target applied. While plant organs require lower doses than whole plants, in general, doses are between hundreds and thousands of mg per liter (Aziz et al., 2006; Falcón et al., 2011).

As before explained, the action mode of chitosaccharides is through direct inhibition of pathogen development or by activation of plant induced resistance. Depending on the mode of chitosan application and the type of pathogen (being aerial or soilborne pathogen) one or both action way takes place in plant protection. For instance, mostly against aerial pathogens, those penetrate plants through the leaves; both mechanisms take place when chitosaccharides are applied by spraying. There are several reports for this action way (Sathiyabama & Balasubramanian, 1998; Ben-Shalom et al., 2003). Conversely, when chitosan derivatives have been applied by foliar spray against a soilborne pathogen or as seed immersion before planting the protective action manifested is the activation of induced resistance. Several reports for this action way have informed protection against fungal and oomycetes pathogens (Sharathchandra et al., 2004; Rodríguez et al., 2007; Falcón et al., 2011).

The preventive character of chitosaccharide applications have been clearly demonstrated in several reports. In the pathosystem cucumber-*Botrytis cinerea* it was shown that spraying chitosan one hour before inoculation decreased gray mold incidence by 65% while spraying 4 or 24 hours before inoculation caused a reduction of disease incidence of 82 and 87%, respectively (Ben-Shalom et al., 2003). In this example it must take into account that part of protective action achieved is the result of direct inhibition of the pathogen when inoculated in the leaves previously treated with chitosan, as a consequence, part of it remains on leaves and performed an antifungal activity against the pathogen (Ben-Shalom et al., 2003). Chitosan preventive action was also demonstrated in the carrot-*Sclerotinia sclerotiorum* interaction, where disease incidence and rot size on carrots decreased as time of inoculation increased (Molloy et al., 2004).

Considerable postharvest losses of fruit and vegetables are brought about by decay caused by fungal plant pathogens. Fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which cause rots and also produce mycotoxins (Moss, 2002). An additional positive effect of chitosan occurs in postharvest protection by its ability to extend the storage life of fruits and vegetables when it is applied as coatings of agricultural commodities. Chitosan can forms

a semipermeable film that, depending on molecular weight and viscosity of solution, regulates the gas exchange, reduces transpiration rate, ethylene production and water loss and prevents pathogens entry, as a consequence, fruit ripening and ulterior degradation is slowed down causing a benefic extension of commodities shelf life. These effects and their antimicrobial benefits has been reported for several authors in postharvest of numerous crops such as cucumber, bell pepper, tomatoes, strawberries, papaya, apples, grapevine, among others (El Ghaouth et al., 1991; 1992a; 1992b; Du et al., 1998; Romanazzi et al., 2002; Bautista-Baños et al., 2003; Bautista-Baños & Bravo-Luna, 2004; Liu et al., 2007; González-Aguilar et al., 2009).

Chitosaccharides applications are not in contradiction with the use of biological controls to protect crops. In a postharvest study, El Ghaouth and coworkers (2000) demonstrated that the combination of glycolchitosan (0.2%) with the antagonist *Candida saitoana* was more effective in controlling gray and blue mold of apple caused by *Botrytis cinerea* and *Penicillium expansum*, respectively, and green mold of orange and lemons caused by *Penicillium digitatum* than both components of the combination when tested each one alone. In addition, it was observe, that pretreatment of fruits with sodium carbonate followed by the combination of *C. saitoana* with 0.2% glycolchitosan was the most effective treatment in controlling green mold of both light green and yellow lemons (El Ghaouth et al., 2000).

All examples afore mentioned demonstrated the efficacy of chitosaccharides as preventive agent to protect crops against pathogenic diseases with the additional benefits of growth and yield enhancing. Perspective work must evaluate the influence of concentration and physicochemical properties of chitosan employed in greenhouse and field experiments on plant induced resistance, in order to determine the activation of priming (capacity for inducing augmented defense expression and resistance in plant after pathogen challenge) or the activation of plant direct defenses, although, the latter could be more costly in term of plant fitness.

6. Conclusion

Chitosaccharides has profitable advantages as plant resistance inducers: Chitosaccharides can protect a broad range of plants either as activator of plant innate immunity or by the inhibiting effect of its antimicrobial activity on a wide array of plant pathogens. These bioactives also stimulate plant growth and improve crop yield and quality in many species. The 90% of chitin and chitosan commercialized is obtained from polluting byproducts from fishing industry. Preparation methodologies have no o very low polluting impact. Additionally, chitosaccharides not disrupt beneficial predators and parasites. Thus, chitosan applications are compatible with the simultaneously use of biofertilizers and biological agents for diseases control.

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8. References

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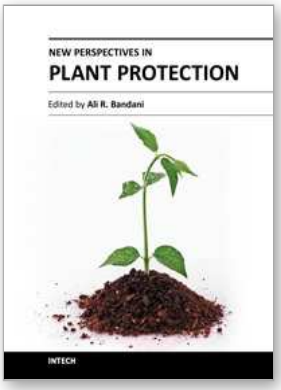

New Perspectives in Plant Protection

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Crop losses by pests (insects, diseases and weeds) are as old as plant themselves but as agriculture are intensified and cropping patterns including the cultivation of high yielding varieties and hybrids are changing over time the impact of the pests becoming increasingly important. Approximately less than 1000 insect species (roughly 600-800 species), 1500 -2000 plant species, numerous fungal, bacterial and nematode species as well as viruses are considered serious pests in agriculture. If these pests were not properly controlled, crop yields and their quality would drop, considerably. In addition production costs as well as food and fiber prices are increased. The current book is going to put Plant Protection approaches in perspective.

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