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Review

Biostimulant activity of chitosan in horticulture

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ABSTRACT

Chitosan is formed from chitin, a co-polymer of *N*-acetyl-D-glucosamine and D-glucosamine, when over 80% of the acetyl groups of the *N*-acetyl-D-glucosamine residues are removed. Chitosan-based materials exhibit various interesting properties, which make them applicable in many fields, including agriculture, where they are used as biostimulants. Chitosan induces several defensive genes in plants, such as pathogenesis-related genes, like *glucanase* and *chitinase*. It also induces many enzymes in the reactive oxygen species scavenging system, such as superoxide dismutase, catalase and peroxidase. The signal transduction pathway from chitosan that elicits its responses involves hydrogen peroxide and nitric oxide signals, and it may also directly control gene expression by interacting with chromatin. Chitosan has been used both as a biostimulant to stimulate plant growth, and abiotic stress tolerance, and as to induce pathogen resistance; however, these responses are complex and they depend on different chitosan-based structures and concentrations as well as the plant species and developmental stage. This review gathers information on chitosan provided by recent research, especially when it is used as plant biostimulant in horticulture.

Contents

1. Introduction

Chitosan is the deacetylated form of chitin, a biopolymer that occurs naturally as a component of fungal cell walls, insect exoskeletons and crustacean shells. The characterization and application of chitosan have been ongoing for decades, leading to the worldwide use of chitosan in many sectors, including agriculture, industry and medicine.

With respect to agriculture, the application of chitosan has been studied in many crop species, including cereal, ornamental, fruit and medicinal crops. It affects various responses in plants depending on the structure and concentration of the chitosan molecules (Kananont et al., 2010; Limpanavech et al., 2008; Lin et al., 2005), plant species (Ohta et al., 2004) and their developmental stage (Pornpienpakdee et al., 2010). Chitosan has been extensively studied as a means to inhibit microbial growth and decrease microbial

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membrane integrity (Xu et al., 2007a; Palma-Guerrero et al., 2008) reducing disease incidence and severity in many crops (Abd-AllA and Haggag, 2010; Ali et al., 2010, 2012, 2013, 2014, 2015; Bautista-Banõs et al., 2003; Bell et al., 1998; Benhamou and Thériault, 1992; Bhaskara Reddy et al., 1999; Li et al., 2009; Maqbool et al., 2010; Prapagdee et al., 2007). However, this review focuses only on the application of chitosan as a biostimulant. The application of modified chitosan derivatives and the combination of chitosan with other substances has recently been reviewed elsewhere (Bautista-Baños et al., 2013; Das et al., 2015).

This review is structured in three parts. The first part is an overview of the types, properties and production processes of chitosan molecules. The second part covers the biostimulant effects of chitosan on plants and physiological mechanisms, while the last part includes the agronomic responses of horticultural crops.

2. Chitosan structures and physicochemical characteristics

Chitin and chitosan are co-polymers of *N*-acetyl-D-glucosamine and D-glucosamine, where the ratio of each monomer in the polymer chain defines its physical, chemical and biological properties, and whether it is characterized as chitin or chitosan. The *N*-acetyl-D-glucosamine and D-glucosamine residues in chitin and chitosan are linked together via β-1,4-glycosidic linkages similar to cellulose. Although chitin can be found in various sources in nature, it is normally produced from shrimp or crab shells by demineralization and deproteinization (Rinaudo, 2006; Younes and Rinaudo, 2015).

Chitin is distinguished from chitosan by the higher proportion of *N*-acetyl-D-glucosamine over D-glucosamine in the polymer chain, with typically more than 95% *N*-acetyl-D-glucosamine and less than 5% D-glucosamine being found in chitin derived from crab shells, shrimp shells and squid pens (Rinaudo, 2006; Sagheer et al., 2009). Taking into account the arrangement of the chitin polymer in its native form, two major types of chitin can be observed: the α-chitin found in shrimp shells and the β-chitin found in squid pens. These two types of chitin were well characterized (Rinaudo, 2006).

Chitosan is not found abundantly in nature, but is produced from chitin, mostly from crab shells, shrimp shells, squid pens and, in some cases, from filamentous fungi, *via* a heterogeneous deacetylation process (Kumaresapillai et al., 2011; Muñoz et al., 2015; Nwe et al., 2011), where solid chitin is soaked in 40–50% (w/v) NaOH. Chitin is normally deacetylated to remove over 80% of the acetyl groups from the *N*-acetyl-D-glucosamine residues, converting it into D-glucosamine, to yield chitosan. The percentage of the *N*-acetyl-D-glucosamine residues converted to D-glucosamine in chitosan *via* this deacetylation process is normally referred to as the percentage degree of deacetylation (DD) of chitosan, although the percentage degree of acetylation, which is the inverse of DD, is sometimes used. While chitin is insoluble in most solvents, chitosan can be readily solubilized in weak organic acids, such as acetic or lactic acid.

The heterogeneous nature of the production process renders chitosan heterogeneous. The chitosan derived from similar starting materials or preparations can be quite different in terms of the average molecular mass, molecular mass dispersity and DD. The differences in these parameters can greatly affect the physical properties and the biological functions of chitosan, such as the solubility and the ability to stimulate plants. Therefore, these parameters should be well characterized prior to its application. The differences in the starting material can be reduced or eliminated by NaOH pretreatment to modulate DD, and chemical or enzymatic hydrolysis to modulate the size and polydisperisity. Modification methods for these parameters have been well documented elsewhere (Jung and Park, 2014; Kubota et al., 2000; Thadathil and Velappan, 2014).

3. Biostimulant effects on plants

3.1. Physiological, biochemical and growth effects

Chitosan was initially reported as an elicitor of plant responses since it induced phytoalexin (pisatin) production in pea (*Pisum sativum* L.) pods (Walker-Simmons et al., 1983) and induced a proteinase inhibitor in tomato plants (*Solanum lycopersicum* L.) (Walker-Simmons et al., 1983). Since then, the physiological and biochemical responses of chitosan have been investigated and it has been found to act as a stimulator of plant defense responses to both wounding (Doares et al., 1995) and pathogen infections (Bautista-Baños et al., 2003; Bhaskara Reddy et al., 1999; Yu et al., 2012).

Upon wounding of plant tissues, pectic fragments from oligogalacturonides in the cell wall induce the accumulation of reactive oxygen species (ROS) and pathogenesis-related proteins (PRPs) to protect plant tissues against pathogen infection (Ferrari et al., 2013). Chitosan triggers similar responses when applied to plant tissues (Malerba and Cerana, 2015; Mejía-Teniente et al., 2013; Pastor et al., 2013).

An oxidative burst response, with hydrogen peroxide (H_2O_2) production, has been found in many plants treated with chitosan (Lee et al., 1999; Zhao et al., 2007). This led to the induction of plant defense enzymes, including phenylalanine ammonia-lyase (PAL), which is a major enzyme in phenolic compound biosynthesis (Camm and Towers, 1973). The induction of PAL by chitosan correlated well with the accumulation of phenolic compounds after chitosan treatment in many plant species, including papaya (*Carica papaya* L.; Ali et al., 2012), sweet basil (*Ocimum basilicum* L.; Kim et al., 2005), sunflower (*Helianthus annuus* L.; Cho et al., 2008), litchi (*Litchi chinensis* Sonn.; Zhang and Quantick, 1997), grape (*Vitis vinifera* L.; Meng and Tian, 2009), tomato (*Solanum lycopersicum* L.; Badawya and Rabea, 2009; Liu et al., 2007), apricot (*Prunus armeniaca* L.; Ghasemnezhad et al., 2010), loquat (*Eriobotrya japonica* (Thunb.) Lindl.; Ghasemnezhad et al., 2011) and soybean (*Glycine max* (L.) Merr.; Khan et al., 2002).

Moreover, the plant defense system induced by chitosan was triggered *via* the nitric oxide (NO) pathway (Raho et al., 2011; Zhang et al., 2011; Zhao et al., 2007). In tobacco epidermis, oligochitosan could induce both NO and H_2O_2 production (Fu et al., 2011). Chitosan reduced the accumulation of tobacco mosaic virus (TMV) (Pospieszny et al., 1991) and induced TMV-resistance in tobacco (*Nicotiana tabacum* L.; Zhao et al., 2007). When the cells were treated with oligochitosan together with ROS inhibitor diphenyleneiodonium and H_2O_2-degrading enzyme catalase (CAT), the induction of TMV resistance was inhibited. Co-treatment of the NO scavenger, 2,4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CPTIO), with oligochitosan also blocked the induction of TMV resistance (Zhao et al., 2007). These findings suggest that both ROS and NO production lead to defense responses in plants. Zhang et al. (2011) reported that NO was first produced in the chloroplast, then in nucleus and later in the whole cell. Considering that tobacco cells treated with NO synthase and nitrate reductase (NR) inhibitors showed suppressed levels of NO production and several defense-related enzymes, this mechanism is proposed to be regulated by an NO synthase—like enzyme and NR (Zhang et al., 2011).

Although there is a cross-talk between H_2O_2 and NO, which leads to other physiological responses, the interaction between these two molecules remains unclear (Qiao et al., 2014). Additionally, H_2O_2 functions as a signal molecule in both abiotic and biotic stress responses (Choudhury et al., 2013; Frederickson Matika and Loake, 2014; Fürstenberg-Hägg et al., 2013; Qiao et al., 2014). The generation of H_2O_2 in the cell triggers the ROS scavenging system and the expression of other oxidative stress responsive genes