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Review

Oligochitosan: A plant diseases vaccine—A review

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ABSTRACT

Chitosan is one of the most abundant carbohydrate biopolymers in the world. Oligochitosan prepared from chitosan is a potent plant immunity regulator. The efficacy of oligochitosan on plant disease control is presented in this review. This paper summarizes recent progress made on oligochitosan activated plant innate immunity, including: signal perception; signal transduction; oligochitosan response genes and proteins; oligochitosan induced defense-related secondary metabolites accumulation. Based on published papers and our former results, we deduce that the mode of oligochitosan act on plant is similar with general vaccines act on human and animals. So we conclude that oligochitosan is a plant disease vaccine.

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1. Introduction

Chitosan buildup with β -1,4 glucosamines (Fig. 1) is one of the most abundant marine-based biopolymers. Chitosan has been used in agricultural, textile, medicinal and environmental fields due to its unique properties as a polymer and its biodegradable, non-toxic characteristics (Kurita, 2006; Rinaudo, 2006). However, chitosan is insoluble in either organic solvents or water, which greatly limits its application. This problem can be solved by hydrolysis of chi-

tosan to water-soluble oligochitosan perfectly (Kim & Rajapakse, 2005; Yin, Du, & Zhang, 2009). So, substantial efforts have been made worldwide in developing oligochitosan and many impressive accomplishments were achieved from 1980s.

Oligochitosan has shown a wide range of biological applications, including health food, plant growth stimulator, feed additive, antimicrobial agent, etc. In addition, oligochitosan is effective at eliciting plant innate immunity against plant diseases in lots of plants such as tobacco, rapeseed, rice, grapevine, etc. (Agrawal et al., 2002; Cabrera, Messiaen, Cambier, & Van Cutsem, 2006; Chen et al., 2009; Eikemo, Stensvand, & Tronsmo, 2003; Hadwiger & Beckman, 1980; Hadwiger, Ogawa, & Kuyama, 1994; Howe, Lightner, Browse, & Ryan, 1996; Kendra, Christian, & Hadwiger, 1989; Orozco-Cardenas & Ryan, 1999; Rakwal, Tamogami, Agrawal,

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$$H_{2N}$$
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Fig. 1. Chemical structure of chitosan.

& Iwahashi, 2002). Oligochitosan, which is produced in our lab by enzyme hydrolysis and membrane separation coupling technique, is used as bio-pesticide on crop protection in several provinces in China. It has a potent protective effect on several kinds of plants such as crops, fruits, vegetables and trees (Yin, Li, Zhao, Du, & Ma, 2006; Zhao, She, Du, & Liang, 2007). From our former results and other scientific reports, we discover that oligochitosan acts on plants like common vaccines act on human and animals, so we supposed oligochitosan as a plant disease vaccine in this paper. This review aims to present an overview of the efficacy and mechanism of oligochitosan on plant immunity regulation, the latest progress and recent papers will be included.

2. Effect of oligochitosan on plant immunity

Unlike animals, plants are sessile and therefore they have developed sophisticated mechanisms to adapt to various biotic (fungi, bacteria, and insects) and abiotic (wounding, salinity, drought, salt, and cold) stresses. To resist these stresses, plants have evolved the ability to initiate various defense reactions such as hypersensitive responses, production of phytoalexins, and reinforcement of cell walls etc. The above resistant mechanism is named innate immunity (Chisholm, Coaker, Day, & Staskawicz, 2006; Jones & Dangl, 2006; Nurnberger, Brunner, Kemmerling, & Piater, 2004). Plant innate immune system comprises the mechanisms that defend plants from infection by other organisms and abiotic stresses, in a non-specific manner. In this paper, we focus on the effect of oligochitosan on plant immunity. The immunity stimulating activity of oligochitosan has been well documented in many different plant systems. Some important results will be introduced according to plant classification in the following text.

2.1. Food crops

Prof. Hadwiger in Washington State University first reported that oligochitosan can induce plant immunity in soybean plants in 1980 (Hadwiger & Beckman, 1980). When oligochitosan was applied to pea with or prior to *Fusarium solani*, the pea was protected from pathogen infection. Oligochitosan at concentration as low as 0.9 mg/ml and 3 mg/ml elicited phytoalexin induction and inhibited germination of *F. solani*, respectively. This founding leads to an increasing trend on oligochitosan and plant protection research. From then on, much excellent work has been done in this field.

Rice is the most important staple food in the world. Agrawal et al. (2002) reported that oligochitosan has the ability to stimulate defense responses in leaves of rice. They found that reactive oxygen species (ROS) accumulated in rice after treatment with 0.1% oligochitosan. Additionally, the production of phenolic secondary metabolites also was upregulated after oligochitosan treatment. In another study, enhanced resistance against rice blast pathogen *Magnaporthe grisea* was observed in *H7S* rice seedlings treated with oligochitosan. In their experiment, the ability of different concentration of oligochitosan was analyzed; 5 μ g/ml oligochitosan solution showed the best result and the disease control rate was more than 50% (Lin, Hu, Zhang, Rogers, & Cai, 2005).

Wheat is the primary food in cold area, so scientists in cold country such as Russian pay much attention on it. Experiments conducted on wheat indicated that oligochitosan with a molecular weight of 5–10 kDa and 65% degree of acetylation had good efficacy on *Bipolaris sorokiniana* control (Burkhanova, Yarullina, & Maksimov, 2007; Khairullin, Yarullina, Troshina, & Akhmetova, 2001). In our lab, the ability of oligochitosan to promote wheat resistance to pathogenic toxin was validated in greenhouse experiments (Liu, Du, & Bai, 2001).

2.2. Economic crops

Tobacco is an important economic crop and model plant. Many papers reveal that oligochitosan can induce tobacco resistance to tobacco mosaic virus (TMV), tobacco necrosis virus (TNV) and Phytophthora parasitica nicotianae. For example, the effect of different molecular weight and deacetylation degree of oligochitosan for tobacco protection against P. parasitica were studied by Falcon et al. (2008). The results showed that, different kinds of oligochitosan had distinct influence on P. parasitica control: less acetylated oligochitosan were better for inhibition of P. parasitica growth but partially acetylated oligochitosan were more effective to protect tobacco against this pathogen by systemic induction of plant immunity. The effect of oligochitosan on TMV control was studied in our lab from 1990s. Oligochitosan was sprayed on tobacco leaves to inhibit TMV infection and the best disease control effect was observed when TMV inoculation was conducted 24 h after 50 µg/ml oligochitosan application (Fig. 2, Zhao, She, Du, et al., 2007).

Oligochitosan with a molecular weight of 2–6 kDa and 85% degree of deacetylation has the best immunomodulate activity on potato to resist the late blight disease (Ozeretskovskaya, Vasyukova, Panina, & Chalenko, 2006). In another experiment, potato was infected with potato virus X after oligochitosan pretreatment. It was found that, the oligochitosan treatment significantly decreased the number of systemically infected plants compared to control plants and the treated leaves also accumulated less amount of virus than the control leaves (Chirkov et al., 2001).

Rapeseed is widely applied on human food, animal feed, chemical industry and bioenergy. Sclerotinia rot is the most serious disease in rapeseed production. The induced resistance of oligochitosan to Sclerotinia sclerotiorum in rapeseed was investigated in our lab. Whereas oligochitosan did not inhibit radial growth of S. sclerotiorum colonies in vitro, it reduced the size and frequency of rot compared to untreated plants when applied to rapeseed before inoculation with S. sclerotiorum. The best pretreatment time was 72 h before S. sclerotiorum inoculation and the optimum concentration of oligochitosan was 50 µg/ml (Yin, Bai, & Du, 2008). Furthermore, Based on its physical character, oligochitosan could be modulated into steady colloid solution and used as seed coating agent. In a seed coating experiment conducted on rapeseed, oligochitosan did not influence seed sprouting and emerge, but can obviously suppress the occurrence of S. sclerotiorum infection, the control rate was around 40% (Lu, Qian, Peng, & Ma, 2003).

2.3. Vegetable crops

Vasiukova et al. (2001) reported that oligochitosan displayed plant immunomodulate activity by inducing local and systemic resistance of tomato to nematodes and *Phytophthora infestans*. Other studies indicated that oligochitosan had the ability to protect tomato plants against *Colletotrichum* sp. and *Fusarium oxysporum* (Zhang & Chen, 2009). For example, oligochitosan inhibited the radial growth of *Colletotrichum* sp., with marked effect when the concentration exceeds 1.5%. Furthermore, oligochitosan significantly reduced the lesion size of tomato fruits, when tomato was pretreated with 1.0% and 2.5% (w/v) oligochitosan solution 10 days before inoculated with *Colletotrichum* sp. (Munoz, Moret, & Garces, 2009).

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