Effect of Partially N-acetylate Chitosans to Elicit Resistance Reaction on *Brassica napus* L.

Zhang Xue-kun, Tang Zhang-lin, Chen Li, Guo Yu-hong, Chen Yun-ping, Li Jia-na
(Agronomy Dept, South-West Agricultural University, Chongqing 400716)

Abstract□The effects to elicit resistance reaction on oilseed rape (*Brassica napus* L. cv *Xinongchangjiao*) by four partially N-acetylated chitosan 7B, 8B, 9B and 10B (Degree of acetylation (D.A.) is 30%, 20%, 10%, 0%, respectively) and Glycol chitosan (GC, D.A. is 0%) were tested and compared. Results showed that chitosan were similar to salicylic acid (SA), could induce resistance reaction, but the reaction was influenced by the degree of acetylation of chitosan. Fully deacetylated chitosan, 10B and GC, elicited chitinase activity, but partially acetylated chitosan, 7B, 8B and 9B, inhibited chitinase activity; Phenyalanine ammonia-lyase (PAL) was also elicited, elicitor activity increased with increasing degree of acetylation, 7B induced highest PAL activity among all chitosans; All chitosans induce peroxidase (POD) in similar level. After elicited by glycol chitosan, like SA treatment, the seedlings increased the disease resistance to *Sclerotinia sclerotiorum* significantly.
Keywords: *Brassica napus* L.; Chitosan; Degree of Acetylation; Resistance reaction

Plant respond to various phytopathogenic invading, mechanic wounding and salicylic acid by activating a set of defense responses as hypersensitive response and systematic acquired resistance[1,2]. Some pathogeneses related (PR) protein, such as chitinase, phenylalanine ammonia lyase (PAL) and peroxidase (POD) play a key role in plant defense response[3,4,5,6,].Chitosan can be defined as partially or fully deacetylated chitin containing residues of (1,4)-β-D-Glucosamine. It occurs in the cell wall of many pathogen fungi. Bhaskar et al (1999)[7] and Yu et al(1999)[8] reported chitosan can induce defence reactions in plants and increase the disease resistance against pathogeneses fungi.

It has been shown previously that the structure and degree of acetylation (D. A.) of chitosan can induce different defense response in plants[9,10]. In this report, we studied the resistance reaction elicited by some partially acetylation chitosan on *Brassica napus* L.

1 Materials and methods

1.1 Bioassays for Elicitor Activity.

The cotyledons of 5-d-old oilseed rape (*Brassica napus* L. cv *Xinongchangjiao*) plants grew in automatically regulated growth chambers were sprayed with 0.5% chitosan solutions (PH 6.0) and 5% salicylic acid, 20m mol/L sodium acetate buffer (PH6.0) as control,. After sprayed chitosan solutions, every 12 hours assayed chitinase, PAL and POD activity.

Chitinase extracted with 20 m mol/L sodium phosphate buffer (PH 6.0) at 4 □, and then dialyzed against 20 m mol/L sodium phosphate (PH 6.0, 4□) overnight[11]. Chitosan with a degree of acetylation (D. A. 30%) was used as the substrate in a standard chitinase assay, and the amounts of reducing sugars liberated during the reaction at 37□ for 15 minutes in 20 m mol/L sodium acetate buffer (PH 6.0) were measured as described previously[12], one unit (U) of activity was defined as the amount of enzyme that liberated 1μmol of reducing sugar per minute using GlcN as standard. Peroxidase (POD) extracted with 5% calcium nitrate solution, one unit activity (U) was defined as the amount of enzyme that catalyze 1μmol H_2O_2 per minute[13]. Phenylalanine ammonia lyase (PAL) was determined as described previously by Peter et al[9]., one unit (U) activity was defined as p mol cinnamic acid formed per min per mg protein. Protein content were measured by the method of Brodford[14], using bovine serum album as standard.

1.2 Plant inoculation

Sclerotinia sclerotiorum was isolated from infected rape plants in campus farm by Biotech Center (Southwest Agricultural University). Plant inoculated and disease resistance measured by the method of Hu et al[15]. The fungi was inoculated and cultured on potato dextrose agar (PDA) plates for 4 d at 28□. The 6- to 8- leaf stage rape plants were treated by spraying 0.5% chitosan solutions and 5% salicylic acid on leaves. After sprayed for 48h, the agar disk □Ø□4mm□which contained *Sclerotinia sclerotiorum* mycelium inoculated on the leaves surface, kept in humid growth chambers in 22-25□ for 48h, and measured diameter of disease lesions.

1.3 Chemicals

Chitosan 10B (D. A. 0%), chitosan 9B (D. A. 10%), chitosan 8B (D. A. 20%), and chitosan 7B (D. A. 30%) were purchased from Funakoshi Co. Ltd. (Tokyo, Japan). Glycol chitosan (D.A 0%, polymerization of >400) was purchased from Wako Junyaku Co. Ltd. (Osaka, Japan).

2 Results

2.1 Substrates specificity of rape chitinase

The chitinase cleaved Chitosan 7B with maximum activity, indicated the chitinase specific to chitosan 7B. The chitinase activities declined with decreasing the degree of acetylation of substrates.

2.2 Chitinase activity elicited by partially N-acetylate chitosan

Partiall acetylated chitosan inhibited chitinase activity with increasing degree of acetylation. Fully deaceylated chitosan elicited increased activity. Compared with control, after treated 12 h, chitinase activity declined 42% by chitosan 7B, 25.8% by chitosan 8B and 7.4% by chitosan 9B. Glycol chitosan and chitosan 10B with fully deacetylation elicited increased chitinase activities like salicylic acid. Glycol chitosan treatment chitinase activity occurred peak in 48 h, increased 92.5% than control; chitosan 10B treatment chitinase activity occurred peak in 60 h, increased 80.2% than control.

2.3 Peroxidase activity elicited by partially N-acetylate chitosan

Chitosan could elicited peroxidase activity significantly just like salicylic acid. Compared with control, like salicylic acid, peroxidase activity increased similarly in all treatments from 12h and activity peak occurred in 48 h.

2.4 Phenylalanine ammonia lyase (PAL) activity elicited by partially N-acetylate chitosan

Salicylic acid elicited PAL activity peak in 48 h, and chitosan in 60 h. Chitosan elicited increased PAL activity with increasing degree of acetylation. Chitosan 7B, 8B and 9B increased 333%, 182% and 64.6% than control respectively. Chitosan 10B (D. A., 0%) inhibited PAL activity slightly. But glycol chitosan elicited PAL activity significantly, increased 278% than control, probably the polymerization can affect PAL activity.

2.5 Effects of disease resistance elicited by partially N-acetylate chitosan

Spraying chitosan solution on leaves surface, could elicited diease resistance to Sclerotinia sclerotiorum (Table 1). Glycol chitosan and salicylic acid increased induced resistance 26% and 20.7% than control significantly at 5% level, but other chitosan only increased resistance slightly.

Table 1 Disease resistance of elicited *Xinongchangjiao* by partially N-acetylate chitosan

Treatment	SA	7B	8B	9B	10B	GC	Control
Diameter of disease lesion □cm□	2.12*	2.44	2.55	2.50	2.32	1.98*	2.67
Induced resistance	20.6%	8.6%	4.5%	6.4%	13.1%	25.8%	0%

Notes:* means F value significant at 5% level.

3 Discussion

Schlumbaum et al.□1986a□[3]□Collinge et al. □1993a□[16] reported that plant chitinase can hydrolyze chitin which exist in cell wall of many pathogenic fungi□and lead to inhibit fungi cells growth. The wall structure of *Sclerotinia sclerotiorum* contains about 20% chitin, but no chitosan exist. (Berkey, 1979a)[17]. Our study showed chitinase of rape could hydrolyze high degree of acetalyion chitosan, showed that the chitinase is specific for cell wall components of *sclerotinia sclerotiorum*.

A large amounts of evidence has accumulated suggesting a key role for salicylic acid and chitosan in both systemic acquired resistance signaling and disease resistance. In this paper, the results corroborated some of the earlier findings; chitosan also elicited defense responses as salicylic acid, PAL, POD activity and disease resistance increased. Barber et al□1988a□[10] reported the activity of chitosan was thought to reside in the acetylated regions of the molecule, fully deacetylated chitosan was inactive. Peter et al. [9] suggested that different mechanisms are involved in the elicitation of PAL and POD activities of wheat by partially N-acetaylated chitosan polymers and both enzymes have to be activated for lignin biosynthesis and ensuing necrosis to occur. But Kauss et al. [18]□1989a□reported that elicitors activity should decrease with increasing degree of acetylated of chitosan on *Catharanthus roseus*. It indicate that different plants response to partially acetylated chitosan differently.

In this study, partially acetylated chitosan could inhibit chitinase activities on *Brassia napus* L.. The fully deacetylated (D. A. 0%) chitosan such as glycol chitosan and chitosan 10B elicited increased activities, but partially acetylated chitosan such chitosan 9B, 8B and 7B inhibited chitinase activities. This inhibition phenomenon was reported in chitinase induction on fungi, *Aspergillus sojao* chitinase inhibit by chitosan 7B, 10B and colloid chitin[19], but seldom reported in plants, the mechanism is not clear. We will study this phenomenon further in the future.

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