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CROP PROTECTION

Organic disease control elicitors

INTRODUCTION

It is well known among farmers and crop production specialists that plant diseases significantly reduce crop yield and quality during the seedling phase of plant growth. Often seedlings are vulnerable to diseases that either destroy the plant or cause it to be stunted throughout the growing season. Chemical seed treatments, soil-applied fungicides, and soil fumigants are commonly used to protect crop plants. These represent sources of soil ecosystem/ground water pollution and threats to food safety and public health. There is a well-established need for alternative methods to control crop diseases are environmentally safe.

In January 1994, the authors filed a patent for **Organic Disease Control** (ODC™) (1). The patent identifies any non-damaging stimulus, including concentrations of complex chitin or chitosan, as well as specific chain length oligomers of these polymers, as elicitors of plant disease resistance mechanisms. Our research to be reported elsewhere has shown that activation of genes for two types of pathogenesis resistance (PR) proteins, chitinase and β-1,3-glucanase, occurs when the plant is exposed to chitin and/or chitosan (2). Such elicitors activate natural defense processes that normally occur in response to some soil-borne disease agents.

Specific topics to be discussed in this article include the characterization of oligosaccharide elicitors, plant signal transduction mechanisms, the defense responses raised upon application of the organic elicitors and the advantages of such an approach to disease control over application of pesticides or reliance upon recombinant technologies.

CHARACTERIZATION OF OLIGOSACCHARIDE ELICITORS

Chitin is the primary component of exoskeletons of insects and *Crustacea* and is found in the cell walls of some fungi. Technically, it is called an N-acetylglucan. By treatment of chitin under strong alkaline conditions, the N-acetyl residue may be hydrolyzed to produce chitosan. In actuality, the difference between chitin and chitosan is a continuum of the degree of N-acetylation of the glucosamine residues in the polymer (3). A considerable amount of material in the ODC chitosan-derived elicitor preparation (AgriHouse, Inc., Berthoud, CO, USA) is pentasaccharide, and a lesser amount is tetrasaccharide. The quantity of oligosaccharide with degree of polymerization (DP) 6 is estimated based on standard curve quantitation using high performance liquid chromatographic (HPLC) analysis. The degree of acetylation of the ODC preparation was analyzed by proton NMR spectra. The results indicate that an average of 1 in 5 of the glycosyl residues in the chitosan hydrolysate were acetylated (4).

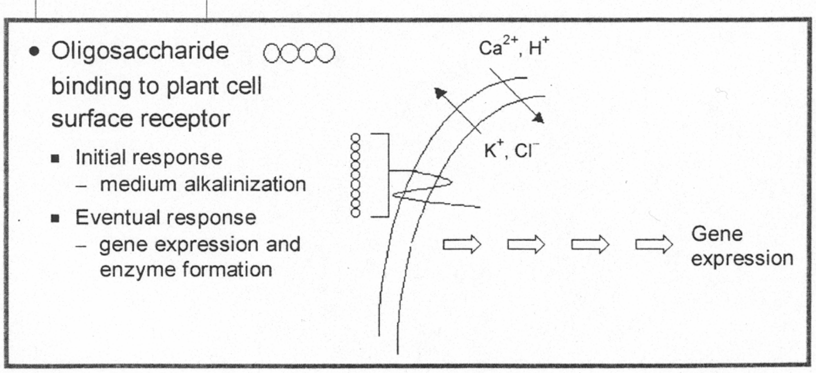
Two types of oligosaccharides, both potentially derived from the chitin cell walls of pathogenic fungi, act as potent elicitors. The first of these, the chitin oligosaccharides are active even at nanomolar (10^{-9} M) ranges (5). Inhibition studies with various other oligosaccharides show specificity of the binding site for oligosaccharides of DP greater than six (6). Chitosan, the deacetylated form of chitin, did not induce phytoalexin formation in rice (5) but is an active elicitor in other plant systems. Chitosan elicitors induce formation of phytoalexins in legumes (soybean, chickpea, bean, alfalfa, pea) and *Solanaceous* sp. (potato, sweet pepper) (7).

SIGNAL TRANSDUCTION MECHANISMS

The Agrihouse patents covers any non-damaging stimulus, which when placed in the vicinity of a seed or emerging seedling, causes naturally defensive substances to be produced. When chitin and/or chitosan are placed near the seed or young plant, some of the polymer or oligomers are sloughed from the polymer due to physical disruption or enzymatic hydrolysis by the constitutive seedling chitinases or chitinases from soil-borne microorganisms. These oligomers may diffuse to the emerging seedling and induce PR responses in the epicotyl and hypocotyl. The chitin or chitosan oligomers impinge upon receptors in the plasmalemma (membrane that surrounds the plant cell) that initiate reactions in the signal transduction system. Binding sites for N-acetylchitooligosaccharide elicitors have been characterized in plasma membranes of suspension-cultured rice cells, for which specific PR responses are induced (8-10). This same principle of disease resistance exists in other major crop plant species such as wheat, barley, soybeans, tomato and potato (11,12).

Upon elicitor binding, a sequence of reactions in the plant signal transduction pathway involves activation of phosphoinositide hydrolysis and stimulation of calcium fluxes in the cell. Release of calcium from the endoplasmic reticulum and/or extracellular environment into the cytosol can stimulate kinase reactions, which phosphorylate proteins that may directly or indirectly interact with DNA in the cell nucleus to induce formation of specific types of mRNA (13). The mRNA may code for chitinase, β-1,3-glucanase, and protease inhibitors, which individually or in combination with other substances or enzymes are released through the plasmalemma and cell wall (14). Through this process, the elicitor acts to induce the release of plant-derived chitinases and β-1,3-glucanases.

Signal transduction



DEFENSE RESPONSES

Protection from fungal pathogens occurs in at least two ways. Ryan (15) proposed that the action of these enzymes on fungal cell walls (which contain chitin) releases oligosaccharide signal molecules that can activate a variety of plant defenses, such as hydrogen peroxide production and cell wall lignification at the site of elicitation. Systemic mechanisms of pathogenesis defense response are also mounted to protect other parts of the plant. Secondly, chitinases and β-1,3-glucanases can also directly lyse the cells of hyphal tips (16). Chitosan stimulated the activities of both β-1,3-glucanase and chitinase, but did not affect chitin deacetylase activities (17).

AgriHouse, Inc. sponsored soil pathogen research in 1995 at two independent locations using Russet Burbank minitubers. Certified greenhouse grown minitubers were exposed to 960 milligrams of chitin per minituber and compared against untreated minitubers. A study on seed piece decay from soil pathogens was conducted by Dr. Dave Douches, Michigan State University, which resulted in a statistical reduction of *Fusarium/Erwinia*. In the same year Dr. Gary Beaver, Southern Idaho Agricultural Researchers & Consultants, found a statistically significant reduction of *Rhizoctonia* Stem Cankering. Both results are shown in Table I.

Another study using Russet Ranger seed potatoes showed resistance to soil-borne pathogens resulting from both chitin and chitosan application to cut seed pieces. The treated and control seed pieces were planted in a random block test at the Colorado State University Horticultural Farm. After emergence the potato plants were inspected for *Rhizoctonia* Stem Cankering. The data showed a statistically significant reduction on *Rhizoctonia* Stem Cankering resulting from chitin and chitosan encapsulation treatments on cut seed potatoes (Table II).

Chitinases from plants are found in many forms: there are acidic and basic chitinases, depending on their pH optima, and there are five classes of chitinases, I-V, which are dependent on whether the enzyme acts as an endoglucanase or an exoglucanase and whether the structure of the protein is consistent with other chitinases that have been characterized from other plant sources. For example, the best characterized group are basic Class I endochitinases. Genes for many of these have been isolated from potato (18), pea (19), loblolly pine (20), *Brassica* (21), tomato (22), carrot (23), tobacco (24), *Arabidopsis* (25), and rice (26).

In addition to this diversity, a particular basic Class I endochitinase (e.g., from potato) may be represented by four different isoforms derived from four distinct genes. This group includes a family of acidic and basic chitinases produced in varying amounts, at different stages of development, in local and remote tissues,

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Table I - 1995 field studies of chitin application to minitubers as method to control of soil pathogens by Michigan State University and Southern Idaho Agricultural Researchers & Consultants

Treatments	Pathogen: Fusarium/Erwinia % seed piece decay * Mean	Rhizoctonia % of Stem Cankering ** Mean
Control Russet Burbank Minitubers	100 a	3.07 a
0.96 grams chitin per Russet Burbank Minituber	40 b	0.39 b

Values followed by different letters are statistically different at P=0.01; * MSU; ** SIARCO)

and in response to different fungal pathogens. Pathogen challenge induces the upregulation of class I basic, vacuolar chitinases as well as class II acidic, extracellular chitinases. This Local Acquired Resistance (LAR) provides the plant with specific and non-specific protection from the spread of infection. After initial infection, the coordinate induction of nine classes of Systemic Acquired Resistance (SAR) genes, including class II chitinases, in remote uninfected tissues provides a non-specific immunity to subsequent infection (27).

ADVANTAGES

Experiments in many laboratories around the world are being performed with molecular biological approaches for control of fungal diseases by creating transgenic plants. Chitinase genes are introduced under the control of constitutive or inducible promoters in transgenic potato plants (14,28,29). However, the synergistic action of *many* chitinase isoforms and β-1,3-glucanases appears to be important in enhancing resistance to fungal pathogens, without which there is limited usefulness of creating transgenic plants (10).

Without the introduction of transgenic plants, environmentally-safe elicitors can be used to significantly replace many of the fungicides currently applied to protect crop yields. The potential benefits of using natural, non-damaging stimulus approaches to crop production and plant propagation are enormous. Reduction of pesticide use is consistent with current public concern about the toxic effects of pesticides on humans and the ecosystem in general. In addition, the practical and relatively inexpensive technology outlined in this paper can have broad applications and economic benefits to many sectors of commercial agriculture and home gardeners.

Table II - 1995 field study of chitin and chitosan application to cut seed potatoes to control *Rhizoctonia* stem cankering at the Colorado State University Horticultural Farm

Treatments	Mean
Control - cut seed piece alone	5.51 a
0.60 grams chitin per cut seed piece	3.21 b
0.60 grams chitosan per cut seed piece	3.30 b

Values followed by different letters are statistically different at P=0.05

The principles, which have been described for molecular effects of ODC elicitors to potatoes, have also been studied and confirmed using aduzi beans in the laboratory (30) and by NASA on the Mir space station (31). AgriHouse offers ODC natural elicitors in the form of liquid concentrates, which when diluted, can be applied directly to seeds and plants.

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