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ORIGINAL PAPER

Hyaluronic acid of *Streptococcus* sp. as a potent elicitor for induction of systemic resistance against plant diseases

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Abstract The potential of hyaluronic acid (HA) in inducing systemic resistance to cucumber, tomato and pepper was tested *in planta*. In the study, HA was found to be a potent agent for suppressing disease caused by Cucumber Mosaic Virus (CMV) (in pepper), *Pseudomonas syringae* pv. *tomato* (tomato speck disease), *Xanthomonas axonopodis* pv. *vesicatoria* (tomato spot disease), *Pseudomonas syringae* pv. *lachrymans* (cucumber angular leaf spot), and *Colletotrichum orbiculare* (cucumber anthracnose). Disease control was obtained with spraying, injection and drenching of plants with HA. HA did not exhibit direct antimicrobial action against the pathogens tested. Studies carried out in transgenic tobacco indicated that defense genes PR 1a and PDF 1.2 were activated upon treatment with HA, demonstrating salicylic acid (SA) and jasmonic acid (JA) pathways getting activated during defense. Further work is warranted to evaluate the use of HA-mediated disease suppression in crop plants.

Keywords Hyaluronic acid · Elicitor · Induced systemic resistance · Plant disease

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Introduction

Induction of disease resistance following treatment with active microbial and chemical inducers has been reported to provide protection against invasion of pathogens in several plant species (van Loon et al. 1998). A number of chemicals have been demonstrated to induce systemic resistance to plants to combat diseases (Chen et al. 1993; Cohen et al. 1994; Metraux et al. 1991; Davis et al. 1989). The prominent ones are 2,6 dichloro isonicotinic acid (in cucumber) (Staub et al. 1992), benzothiadiazole (Lawton et al. 1996), methyl jasmonate (Epple et al. 1997) and probenazole (Yoshioka et al. 2001). Many of these have been commercialized under different trade names, including BION (BTH by Novartis/Syngnta) (Kunz et al. 1997), Oxycom (Salicylic acid by Redox Chemicals Inc., Burley, ID, USA), and Oryzemate (Probenazole by Meijiseika Co., Tokyo, Japan). The application of these products has been demonstrated to induce expression of defense transcripts in plants (Friedrich et al. 1996; Lawton et al. 1996).

The objective of this study was to determine if bacterially produced hyaluronic acid (HA) could induce systemic disease protection in plants. HA is a naturally occurring biopolymer, which serves important biological functions in bacteria and higher animals including humans. In Gram-positive streptococci it appears as a mucoid capsule surrounding the bacterium. HA is comprised of linear, unbranching, polyanionic disaccharide units consisting of glucuronic acid (GlcUA) an N-acetyl glucosamine (GlcNAc) joined alternately by beta 1–3 and beta 1–4 glycosidic bonds (Fig. 1). Bacterial fermentation methods for large-scale economic production of HA have been well standardized as HA is of used in medical and cosmetic industry extensively (Akasaka et al. 1998; Hasegawa et al. 1999).

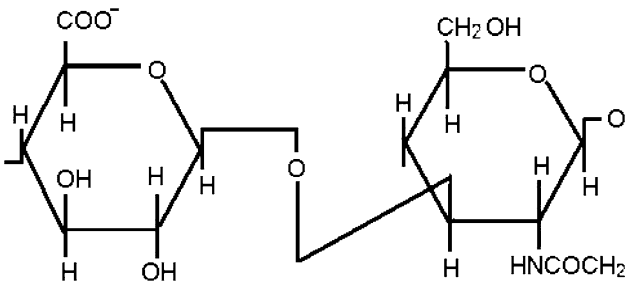

Fig. 1 Hyaluronic acid is comprised of linear, unbranching, polyanionic disaccharide units consisting of GlcUA an GlcNAc joined alternately by beta 1–3 and beta 1–4 glycosidic bonds

In this report, we evaluated the effect of HA on cucumber, tomato, and pepper against one viral, three bacterial, and one fungal pathogen. The pathogens tested were Cucumber Mosaic Virus, *Pseudomonas syringae* pv. *tomato* (which causes tomato speck disease), *Xanthomonas axonopodis* pv. *vesicatoria* (which causes tomato spot disease), *Pseudomonas syringae* pv. *lachrymans* (which causes cucumber angular leaf spot), and *Colletotrichum orbiculare* (which causes cucumber anthracnose).

Materials and methods

The plant materials and the microbes used in the study

Cucumber (*Cucumis sativus*), red pepper (*Capsicum annuum*), and tomato (*Lycopersicum esculentum*) seeds were sown in 10–cm diameter plastic pots filled with a commercial soilless mix (TKS 2, Floragard) containing 10% perlite. The seedlings (one each) were transplanted to fresh cups and watered daily and fertilized weekly with 1% Wuxal Super (12:4:6; Aglukon, Duesseldorf, Germany).

Streptococcus sp. strain, KLO1888 (for production of HA) and the bacterial plant pathogens were maintained in Tryptic Soy Agar (TSA) with 20% glycerol at −80°C. The plant pathogens *viz. P. syringae* pv. *tomato*, *X. axonopodis* pv. *vesicatoria*, and *P. syringae* pv. *lachrymans* were raised in TSA media and cell suspensions were prepared in 10 mM $MgSO_4$. The fungal pathogen, *C. orbiculare* was grown on green bean agar medium for 5 days. The conidial suspension was prepared as 2.5×10^5 conidia per ml. Silwet L-77 (100 µl/l) (Loveland Industries, Greeley, CO) was added to the suspension to enhance the penetration of conidia to the leaf. The CMV suspension was prepared by macerating infected leaves of cucumber in sterile water and the suspension used for challenge inoculation.

Preparation of HA by bacterial fermentation

HA was isolated from culture filtrates of *Streptococcus* sp. Strain, KLO1888 after fermentation in 30 l of media containing 60 g glucose, 5 g yeast extract, 17 g casein, 7 g glutamic acid, 0.7 g magnesium sulfate, 2.5 g calcium phosphate dibasic and 5 g sodium chloride per liter. The media was adjusted to pH 7.0 and sterilized at 121°C for 20 min. Strain, KLO1888 was retrieved from −80°C and the primary inoculum was prepared by streaking onto plates containing TSA, incubating the plates at 28°C for 24 h, and scraping bacterial cells into 0.02 M potassium phosphate buffer, pH 6.8.

The bacteria were grown in the fermentation system for 72 h and the culture filtrate collected were stirred gently after adding 0.02% sodium lauryl sulfate and 0.05 formaldehyde for 3 h in order to separate HA from cell surface. Later, the sample was centrifuged for 20 min at 8000 × g at 4°C. The pH of the supernatant was adjusted to 8.5 by adding 1 N NaOH and stirred with 3% adsorptive resin SP207 for 3 h for elimination of the lipopolysacharides (LPS). NaCl was added to the supernatant to a final concentration of 0.9%. It was mixed with 3% activated charcoal to allow the adsorption of the cell wall enzyme and protein components. The samples were centrifuged for 30 min at 8000 × g at 4°C. To the final supernatant was added acetone for precipitation of pure HA. The samples were stored at 4°C until use in bioassays.

Treatment and challenge inoculation

Various concentrations of HA were sprayed, injected, and drenched onto plants, which were then challenge inoculated with the respective pathogens, 1–2 weeks after treatment with HA. With tobacco, HA was infiltrated in the first leaf. Actigard (from Syngenta) (benzothiadiazole [BTH]) was used as a positive control that induces systemic acquired resistance. The treatment details are given in Table 1.

Bacterial and fungal pathogens were applied as sprays to the entire plant with cell suspensions of bacteria and a spore suspension of *C. orbiculare*. CMV was applied by rubbing onto the leaves, the macerate of an infected cucumber plant. After disease development, the number of lesions was recorded. With CMV, a 0–5 rating was used, where 5 = the most disease, and 0 = no symptoms. The experimental design was a randomized complete block for all tested pathogens. There were six replications for experiments with *P. syringae* pv. *lachrymans* and *C. orbiculare*, 20 replications for experiments with *P. syringae* pv. *tomato*, 10 replications with *X. axonopodis* pv. *vesicatoria*, and seven replications with CMV. Protection against *P. syringae* pv. *lachrymans* was quantified by assessing the number of necrotic lesions per leaf. The severity of anthracnose was determined by estimating the number of anthracnose lesions on each leaf. The number of

Table 1 Treatment details

SI No.	Treatment	Mode of application	Concentration
1	HA	Injection[a]	0.1 ppm
2	HA	Injection	1.0 ppm
3	HA	Injection	10.0 ppm
4	HA	Spray to run off[b]	0.1 ppm
5	HA	Spray to run off	1.0 ppm
6	HA	Spray to run off	10.0 ppm
7	HA	Drench[c]	10.0 ppm
8	*Actigard*	Spray to run off	1.0 mM
9	Untreated control	–	–

[a] 500 µl of HA was infiltrated in the given concentrations to the first leaf using a sterile syringe

[b] The entire foliage was sprayed with the HA solution in the given concentrations

[c] The planting medium in the pots were drenched with 25 ml of a 10 ppm solution of HA

spots (speck lesions) was estimated in the case of bacterial speck of tomato. For tomato spot disease, angular spots on leaves were counted. The study was performed as separate experiments for each pathogen and was repeated twice.

Direct antagonistic property of HA against the pathogens

HA (10 ppm) was tested in vitro against the pathogens, *P. syringae* pv. *tomato*, *X. axonopodis* pv. *vesicatoria*, *P. syringae* pv. *lachrymans*, *E. carotovora* and *C. orbiculare*. The bioassay was carried out by following an agar-disc method in TSA (for bacterial pathogens) and (for *C. orbiculare*). The bacteria were spread plated on to TSA and 10 ppm of HA was pipetted in to agar wells made in the plates with a 3 mm cork borer. Mycelial discs of the fungal pathogen was placed in the center of Potato Dextrose Agar (PDA) and HA was pippeted as above to agar wells made 2 cm away from the disc. Five replicates were maintained for each organism. The plates were incubated at appropriate temperatures and observed for inhibition of growth (growth of colony for bacterial pathogens and mycelial growth for the fungal pathogen) of the organisms.

GUS assay for PR 1a and PDF 1.2 genes

Transgenic tobacco plants expressing either β-glucuronidase (GUS) gene fused to the PR-1a promoter or PDF 1.2 were used for the study. Three-week old seedlings were infiltrated with 0.1, 1.0 and 10.0 ppm of HA. Infiltration of 0.1 mM of Benzothiadiazole (BTH) served as positive control. Another set of plants was sprayed with a solution of HA in the above concentrations. Yet another set of seedlings were germinated in MS medium taken in 24-well cell culture plates and upon two leaved stage of growth, the plants were spotted with HA in the three different concentrations. The plants were sampled 12 h after treatment. GUS activity was measured in leaflets by using a fluorometric assay described by Jefferson (1987) and Park and Kloepper (2000).

All data were analyzed with JMP (a PC-version of SAS). When a significant F test statistic resulted for the experiment, significant treatment differences were determined using Students's T for determining LSD. The significant level tested was 98% ($P = 0.01$). It is most common in publications to use the 95% confidence limit ($P = 0.05$). We chose the 98% limit as a less conservative test to determine potential of the elicitor to induce resistance. All experiments were repeated twice, except for CMV, which was conducted three times.

Results

Effect of HA on ISR in cucumber

Application of HA resulted in significant suppression of cucumber anthracnose, compared to the untreated plants (Fig. 2), and this suppression occurred even at the lowest concentration of HA (0.1 ppm). Among the different modes of application of HA, drenching was found to be more effective than injection and spray. Drenching with 10 ppm of HA brought about 71.79% disease suppression, compared to the control, while *Actigard*, the positive

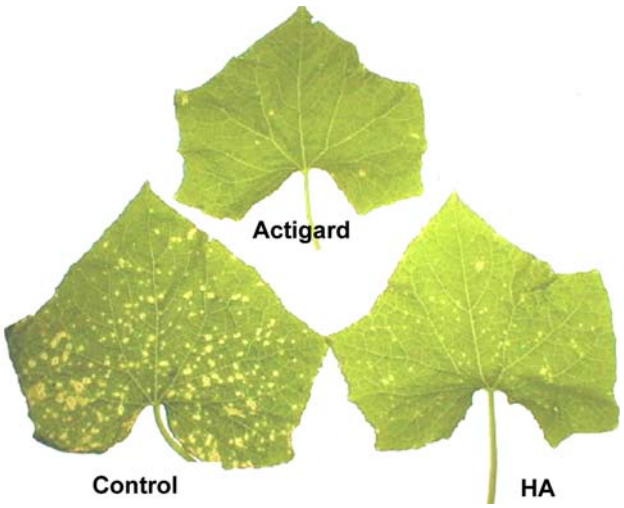

Fig. 2 Application of HA (0.1, 1.0 and 10.0 ppm) (by spray, injection, and soil drench) brought about significant disease (Anthracnose by *C. orbiculare*) suppression in cucumber when compared to the control

control showed 97.14% protection over the control (Table 2).

In the case of cucumber angular leaf spot, drenching with 10 ppm of HA resulted in significant disease suppression. Among the various modes of application of HA, spraying was found to be the least effective followed by injection (Table 2).

Effect of HA on induced systemic resistance (ISR) in tomato

Bacterial speck disease also was suppressed with the application of HA. The positive control, Actigard, induced an 85% disease suppression compared to the control, and HA induced 77% disease suppression. Injection was found to induce more disease resistance than spray or drench (Table 3).

With bacterial spot disease, HA was found to be more effective at inducing resistance than Actigard. Actigard resulted in 43.27% disease suppression, while HA-spray resulted in 45.29% (Table 3).

Effect of HA on ISR against CMV in pepper

HA was found to be effective against CMV in pepper as indicated in Table 4. Up to 35.68% disease suppression was obtained with treatment with HA (Fig. 3).

Table 2 Effect of HA on ISR in cucumber

Treatments	Mean no. of Anthracnose lesions in leaf[a]	Mean no. of angular leaf spots by *P. syringae* in leaf[b]
0.1 ppm HA-spray	187.4[c]	52.8[c]
0.1 ppm HA-injection	235.2[c]	75.7[c]
1 ppm HA-spray	186.3[c]	64.5[c]
1 ppm HA-injection	224.8[c]	85.3
10 ppm HA-spray	205.0[c]	64.7[c]
10 ppm HA-injection	235.8[c]	48.2[c]
10 ppm HA-drench	105.4[c]	33.5[c]
1 mM *Actigard*	10.7[c]	0.2[c]
Control	373.7	97.8
LSD (P = 0.01)	85.6	26.9

[a] Leaves from each plant were severed after symptom development and the lesions were counted using a manual counter

[b] The angular leaf spots were counted using a manual counter

[c] Significant difference compared to the control at $P = 0.01$

Table 3 Effect of HA on ISR in tomato

Treatments	Mean no. of spots (tomato speck disease)[a]	Mean no. of spots (tomato spot disease)[b]
0.1 ppm HA-spray	21.72[c]	11.05[c]
0.1 ppm HA-injection	19.23[c]	10.16[c]
1 ppm HA-spray	21.95[c]	9.30[c]
1 ppm HA-injection	11.43[c]	9.63[c]
10 ppm HA-spray	12.02[c]	8.77[c]
10 ppm HA-injection	20.26[c]	13.95[c]
10 ppm HA-drench	21.84[c]	12.24[c]
1 mM *Actigard*	7.49[c]	11.32[c]
Control	49.97	54.59
LSD (P = 0.01)	10.57	10.07

[a] Upon symptom development, the disease spots (tomato speck caused by *P. syringae*) were counted using a manual counter

[b] Upon symptom development, the disease spots (tomato spot caused by *X. axonopodis*) were counted using a manual counter

[c] Significant difference compared to the control at $P = 0.01$

Direct antagonistic property of HA against the pathogens

There was no antagonistic property observed with HA on the pathogens used in the study (data not given).

GUS assay for gene expression of PR 1a and PDF 1.2

GUS activity in the HA infiltrated plants indicated that the defense genes PR-1a and PDF 1.2 was strongly activated by HA (Table 5). The highest concentration tested, 10 ppm showed maximum GUS activity, irrespective of the mode

Table 4 Effect of HA on ISR against CMV in pepper

Treatments	Disease index (0–5)[a]
0.1 ppm HA-spray	3.32
0.1 ppm HA-injection	3.00
1.0 ppm HA-spray	2.74[b]
1.0 ppm HA-injection	3.89
10.0 ppm HA-spray	2.74[b]
10.0 ppm HA-injection	3.68
10.0 ppm HA-drench	2.68[b]
1.0 mM *Actigard*	1.32[b]
Control	4.26
Mock	0.00
LSD (P = 0.01)	0.98

[a] Disease index was scored from 0 to 5, where 0 = no symptoms and 5 = maximum symptoms.

[b] Significant difference compared to the control at $P = 0.01$

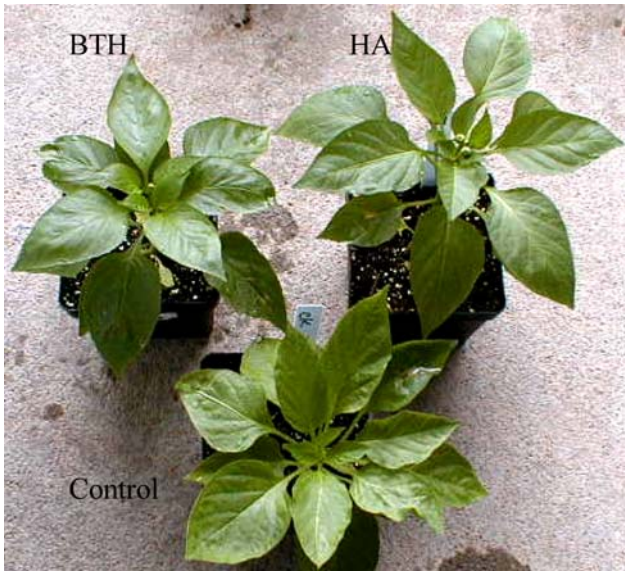

Fig. 3 HA induced significant levels of systemic protection against CMV in bell-pepper when compared to the control. Various concentrations of HA (0.1, 1.0 and 10.0 ppm) were sprayed, injected, and drenched onto plants, which were challenge inoculated after 1–2 weeks with CMV

of application. Low GUS activity occurred with the lowest rate of HA (0.1 ppm), while there was no considerable GUS activity on the control plants. Treatment with BTH induced strong GUS activity at the tested concentration of 0.1 mM in tobacco-PR-1a. The GUS activity was lesser in tobacco-PDF 1.2.

Discussion

Exploitation of a plant's natural defense mechanisms has long been a goal of modern agriculture. Research toward this goal has led to the development of biotic and abiotic agents that can induce systemic resistance in the plant against an invading pathogen. 2,6 dichloro isonicotinic acid, benzothiadiazole, methyl jasmonate, and probenazole are examples for chemical inducers of plant immunity (von Rad et al. 2004). Activation of defense genes in plants has been reported upon induction of the plant with chemical elicitors (Fofana et al. 2002; Yang et al. 2002) proving their molecular mechanisms of action.

The present study demonstrated the potential of another biochemical agent, HA in inducing systemic resistance in cucumber, tomato and pepper. This is the first report demonstrating HA, as an agent for inducing systemic resistance in plants. The experiments conducted revealed that there were significantly higher levels of disease control with HA in all the tested pathosystems irrespective of the mode of application. The fact that drenching of planting medium with HA resulted in significant disease suppression suggests that the mechanism involved is systemic mode and not through direct antagonism. This suggestion is supported by the in vitro test, which showed that HA had no antagonistic effect on any of the tested pathogens.

The mechanisms by which HA elicits systemic protection to the plant are not yet understood fully. But our results demonstrate that the general defense pathways are responsible for the HA-mediated systemic protection. Specifically, HA induced activation of PR-1a and PDF 1.2 genes in tobacco, demonstrating the potential of HA for activating defense responses within the plant. This phenomenon also shows that both SA-mediated and JA-mediated defense mechanisms are activated in the plant with HA. With application of BTH, the expression of PR-1a was more prominent than PDF-1.2, may be that BTH-mediated ISR works mainly through an SA dependant pathway. Similar findings have been reported with application of BTH in tobacco and arabidopsis, where in induced expression of plant defense genes was noted

Table 5 GUS activity in tobacco plants after treatment with HA

Mode of application of HA on tobacco	Concentration of HA (ppm)	GUS activity in tobacco-PR-1a[a]	GUS activity in tobacco-PDF-1.2[a]
Infiltration	0.1	+[b]	+
	1.0	++[c]	++
	10.0	+++[d]	+++
Spray	0.1	+	+
	1.0	++	++
	10.0	+++	+++
Spotting on seedlings in cell culture plates	0.1	+	+
	1.0	++	++
	10.0	+++	+++
Control (sterile water)	–	–	+
BTH (0.1 mM)	–	+++	++

[a] The plants were sampled 12 h after treatment. GUS activity was measured in leaflets by using a fluorometric assay described by Jefferson (1987)

[b] Indicated low GUS activity (below 1,000 nM MU/10 mg fresh weight/h)

[c] Indicated medium GUS activity (below 10,000 nM MU/10 mg fresh weight/h)

[d] Indicated strong GUS activity (below 100,000 nM MU/10 mg fresh weight/h)

(Friedrich et al. 1996; Gorlach et al. 1996). von Rad et al. (2004) have reported the activation of PR-1a and PDF 1.2 upon treatment of Arabidopsis with BION, a commercial formulation of BTH. Systemic increases in activities of β-1,3-glucanase, chitinase, and peroxidase in the leaves and at the infection sites have also been widely reported upon treatment with BTH (Smith et al.1991; van Loon 1997; Ward et al. 1991).

While more work remains to be done to understand how HA activates plant defenses, the broad spectrum of disease protection demonstrated in our study indicates the potential of HA as a chemical inducer of systemic resistance. As emphasized by Kessmann et al. (1994), the use of chemical compounds to initiate the natural defense of plants represents another alternative, potentially promising approach to disease control. As methods have been standardized by many researchers for economic production of HA by bacterial fermentation, viable strategies could be formulated for large-scale production and release.

References

Akasaka H, Seto S, Yanagi M, Fukushima S, Mitsui T (1998) Industrial production of hyaluronic acid by *Streptococcus zooepidemicus*. J Soc Cosmet Chem Jpn 22:35–42

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262:1883–1886

Cohen Y, Niderman T, Mosinger E, Flur R (1994) β-aminobutyric acid induces the accumulation of pathogenesis related proteins in tomato plants and resistance to late blight infection caused by *Phytophthora infestans*. Plant Physiol 104:59–66

Davis KR, Ausubel FM (1989) Characterization of elicitor induced defence responses in suspension-cultured cells of Arabidopsis. Mol Plant Microbe Interact 2:363–368

Epple P, Apel K, Bohlmann H (1997) Over expression of an endogenous thionin enhances resistance of Arabidopsis against *Fusarium oxysporum*. Plant Cell 9:509–520

Fofana B, McNally D, Labbé C, Boulanger R, Benhamou N, Séguin A, Bélanger RR (2002) Milsana-induced resistance in powdery milldew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase. Physiol Mole Plant Pathol 61:121–132

Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Gut Rella M, Meier B, Dincher S, Staub T, Uknes S, Metraux JP, Kessman H. (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J 10(1):61–70

Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8:629–643

Hasegawa S, Nagatsuru M, Shibutani M, Yamamoto S, Hasebe S (1999) Productivity of concentrated hyaluronic acid using a Maxblend® fermentor J Biosci Bioeng 88(1):68–71

Jefferson RA (1987) Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol Biol Rep 5:387–405

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S, Ryals J (1994) Induction of systemic acquired resistance in plants by chemicals. Ann Rev Phytopathol 32: 439–459

Kunz W, Schurter R, Maetzke T (1997) The chemistry of benzothiadiazole plant activators. Pesticide Science 50:275–82

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J (1996) Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. Plant J 10: 71–82

Metraux JP, Ahl GP, Staub T, Speich J, Steinemann A, Ryals J, Ward E (1991) Induced resistance in cucumber in response to 2,6 dichlroisonicotinic acid, pathogens. In: Hennecke H, Verma DPS (eds) Advances in molecular genetics of plant microbe interactions, vol 1. Kluwer academic publishers, Dordrecht pp 432–439

Park KS, Kloepper JW (2000) Activation of PR 1a promoter by rhizobacteria that induce systemic resistance in tobacco against *Pseudomonas syringae* pv. *tabaci*. Biol Control 18:2–9

Smith JA, Hammerschmidt R, Fulbright DW (1991) Rapid induction of systemic resistance in cucumber by *Pseudomonas syringae* pv. syringae. Physiol Mole Plant Pathol 38:223–235

Staub T, Ahl GP, Kessman H (1992) Chemically induced disease resistance in plants. In: Lyr H, Polter C (eds) Proceedings of the 10th international symposium on systemic fungicides and antifungal compounds, pp 239–249

van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol 103:753–65

van Loon LC, Bakker PA, Pieterse CM (1998) Systemic resistance induced by rhizophere bacteria. Annu Rev Phytopathol 36: 453–483

von Rad U, Mueller JM, Durner J (2004) Evaluation of natural and synthetic stimulants of plant immunity by microarray technology. New Phytol 165:191–202

Ward ER, Uknes SJ, Wlilliams SC, Dinche SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux JP, Ryals JA (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3:1085–1094

Yang KY, Blee KA, Zhang S, Anderson AJ (2002) OxycomTM treatment suppresses *Pseudomonas syringiae* infection and activates a mitogen activated protein kinase pathway in tobacco. Physiol Mol Plant Pathol 61:249–256

Yoshioka K, Nakashita H, Klessig DF, Yamaguchi I (2001) Probenazole induces systemic acquired resistance in Arabidopsis with a novel type of action. Plant J 25(2):149–157