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Jasmonic acid and salicylic acid induce accumulation of β-1,3-glucanase and thaumatin-like proteins in wheat and enhance resistance against *Stagonospora nodorum*

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Abstract

The effect of application of jasmonic acid (JA) and salicylic acid (SA) on the induction of resistance in wheat to *Stagonospora nodorum* and on the induction of β-1,3-glucanase and thaumatin-like proteins (TLPs) was studied. Western blot analysis revealed that two β-1,3-glucanases with apparent molecular masses of 31 and 33 kDa that cross-reacted with a barley glucanase antiserum were induced in wheat leaves after treatment with JA and SA. When wheat plants were treated with SA and JA, a TLP with an apparent molecular mass of 25 kDa and several other isoforms of TLP were induced. Pre-treatment of wheat plants with SA and JA significantly reduced (up to 56 %) the incidence of leaf blotch disease incited by *S. nodorum* compared with untreated control plants.

Additional key words: leaf blotch disease, pathogenesis-related protein, systemic acquired resistance, *Triticum aestivum*.

Introduction

It is well known that plants can acquire local and systemic resistance to diseases through various biological agents including necrotizing pathogens, non-pathogens and root colonizing rhizobacteria. Induced resistance in plants can be subdivided into two broad categories. The pathogen-induced resistance has been termed systemic acquired resistance (SAR) and the plant growth-promoting rhizobacteria mediated resistance is known as induced systemic resistance (ISR) (Hammerschmidt 1999). SAR is characterized by broad-spectrum disease resistance and is mediated via salicylic acid (Mauch-Mani and Metraux 1998). SAR in plants can also be induced by exogenous application of salicylic acid (SA), acetyl-salicylic acid, polyacrylic acid, methyl salicylate, jasmonic acid and jasmonic methyl ester, benzo-[1,2,3]-thiadiazole-7-carbothioic acid-S-methyl ester (BTH), 2,6-dichloroisonicotinic acid (INA) and DL-β-amino-n-butyric acid (BABA) (Metraux *et al*. 2002).

SA is a natural phenolic compound present in many plants and is an important component in the signal transduction pathway and is involved in local and systemic resistance to pathogens (Delaney *et al*. 1995). In many plants an increase in SA levels precedes the onset of SAR (Malamy *et al*. 1990). Treatment of plants with SA induces disease resistance and expression of genes associated with SAR in plants (Ward *et al*. 1991, Yalpani *et al*. 1991, Malamy and Klessig 1992, Meena *et al*. 2001). It was demonstrated that plants that degrade SA due to the expression of the bacterial gene for salicylate hydroxylase, did not show SAR in response to pathogen infection (Gaffney *et al*. 1993). Methyl jasmonate and jasmonic acids have been found in a large number of plant species (Anderson 1989). They are synthesized in plants from the fatty acid linolenic acid (Anderson 1989). Jasmonate signaling during SAR also has been studied in various plant-pathogen systems (Schweizer *et al*. 1993,

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Abbreviations: ISR - induced systemic resistance; JA - jasmonic acid; PDA - potato dextrose agar; PMSF - phenylmethylsulphonyl fluoride; PVDF - polyvinylene difluoride; SA - salicylic acid; SAR - systemic acquired resistance; TLP - thaumatin-like protein.
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Farmer *et al*. 1998). Pretreatment of crop plants with such chemical "activators" to enhance resistance against a broad range of plant pathogens could be an attractive disease control practice (Lawton *et al*. 1994).

Leaf blotch, caused by *Stagonospora nodorum* is one of the important diseases of wheat worldwide (Shah *et al*. 2000) and none of the commercially available wheat cultivars is immune to this fungus (Nelson and Marshall 1990). Management of this disease by foliar application of fungicides has been successful, but it is not always desirable because of the high cost of application and potential hazards to the environment. To our knowledge there have been no studies demonstrating induction of resistance in wheat plants against *S. nodorum* after application of abiotic inducers. The present study reports the induction of resistance in wheat plants against *Stagonospora nodorum* infection by exogenous application of JA and SA. The effect of JA and SA on accumulation of PR-proteins also was investigated.

Materials and methods

Plants and pathogen: Wheat (*Triticum aestivum* L.) plants (cv. Bob White) were grown in pots (15 cm dia.) in a growth chamber at 12-h photoperiod (irradiance of 600 μmol m^{-2} s^{-1}), day/night temperature of 22/18 °C, and relative humidity of 80 %.

A virulent isolate of *Stagonospora nodorum* (Berk.) E. Castell & Germano, syn. *Septoria nodorum* (Berk.) Berk. & Broome, teleomorph *Phaeosphaeria nodorum* (E. Mull.) Hedjar syn. *Leptosphaeria nodorum* (E. Mull.), was kindly provided by Dr. Willium Bockus, Department of Plant Pathology, Kansas State University, Kansas, USA and maintained on potato dextrose agar (PDA) medium.

Salicylic acid and jasmonic acid treatment: In order to study the efficacy of foliar applications of SA and JA in induction of pathogenesis-related (PR) proteins and in the management of *Stagonospora nodorum* blotch, wheat plants (six weeks after sowing) were sprayed with SA (1 mM) until run-off. Control plants were sprayed with water. JA treatment was undertaken by exposing the plants to 100 μM of JA and the treated plants were then covered with polyethylene bags. Control plants were maintained in an identical condition in the absence of JA. At various times after treatment, leaf samples were collected and analyzed for the induction of β-1,3-glucanase and thaumatin-like proteins.

Pathogen inoculation: The fungus, *Stagnospora nodorum* was cultured on V8 juice agar (200 cm^3 of V8 Juice, 3 g of $CaCO_3$, 15 g of agar and 800 cm^3 of distilled water) at room temperature (26 °C) for 10 - 12 d. The Petri dishes were then flooded with sterile distilled water and the spores were dislodged by gently scraping the surface with a glass rod. The spore concentration was adjusted with distilled water to 10^6 spores per cm^3. *Tween-20* (polyoxyethylene sorbitan monolaurate) was added to the spore suspension at 0.1 cm^3 per 250 cm^3 (Shah *et al*. 2000). One day after JA and SA treatment, wheat plants were challenge inoculated with the spore suspension of *S. nodorum* until run off. The inoculated plants were incubated in a humid chamber for 72 h. Twenty plants were maintained for each treatment. Leaf blotch disease intensities were recorded up to 12 d after inoculation using a 0-9 scale (Saari and Prescott 1975). The experiment was conducted twice under identical conditions.

Western blotting: Protein extracts were prepared by grinding 1 g of plant tissue in liquid nitrogen and homogenizing it in 1 cm^3 of extraction buffer containing 50 mM sodium phosphate (pH 7.0), 10 mM EDTA and 20 mM PMSF. The leaf homogenate was centrifuged at 10 000 *g* for 15 min at 4 °C, and the supernatant was used as enzyme source. Protein content of the crude extracts was determined by the bicinchoninic acid microtitre plate assay kit (*Pierce*, Rockford, IL, USA). Proteins (150 μg) were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in a *Mighty Small II* gel electrophoresis unit (*Hoefer*, San Francisco, CA, USA) with 12 % acrylamide resolving gel and 4 % acrylamide stacking gel according to the method of Laemmli (1970). The gels were electrophoresed for 2 h at a constant current of 20 mA. Proteins were then transferred to a polyvinylene difluoride (PVDF) membrane at 140 mA for 30 min with an electroblotting apparatus (*Bio-Rad* semi-dry transblot apparatus, *Bio-Rad*, Hercules, CA, USA). After transfer, the membrane was blocked with Tris-buffered saline (10 mM Tris-HCl, pH 7.9, 140 mM NaCl) (TBS) containing 0.05 % (v/v) *Tween-20* (TBST) supplemented with 2.5 % (m/v) gelatin for 2 h at room temperature (26 °C). Blots were washed three times for 5 min each in TBST to remove the gelatin and then incubated for overnight in TBST containing the primary antibody at 1:2 000 dilution. Antiserum raised against a barley glucanase (a gift of Dr. M. Ballance, University of Manitoba, Winnipeg, MB) and an antiserum raised against maize zeamatin (a gift of Dr. C.P. Selitrennikoff, University of Colorado Health Sciences Center, Denver, CO, USA) were used as the primary antibodies. Blots were washed three times for 5 min each in TBST and then incubated for 3 h in TBST containing horse radish peroxidase conjugated goat-anti rabbit IgG (*Bio-Rad*) at 1:2 000

dilution. Blots were washed (5 min) thrice with TBST and twice with TBS. Binding of the secondary antibody was detected by reaction of the antibody-HRP-conjugate with a freshly prepared substrate solution consisting of 0.015 cm^3 of 30 % H_2O_2, 5 cm^3 of 0.3 % (m/v) 4-chloro-1-naphthol (*Bio-Rad*) in methanol, and 25 cm^3 of TBS for 3 - 5 min.

Results

Wheat plants were treated with JA and SA and examined for the induction of β-1,3-glucanase and thaumatin-like proteins (TLPs). Western blot analysis of SA and JA treated wheat leaves revealed that the contents of two β-1,3-glucanases with apparent molecular masses of 31 and 33 kDa were markedly elevated in wheat leaves after treatment with SA and JA (Fig. 1). Increase in content of both glucanases was observed as early as 1 h after application of these compounds and remained elevated at 6, 12, 24, and 48 h after treatment. At late times after treatment the thickness of the band increased which suggests a doublet (31 and 33 kDa) contents. Low contents of these glucanases were detected in control wheat plants (Fig. 1). In addition, several minor bands cross-reacting with barley glucanase antiserum were also observed in SA and JA treated plants.

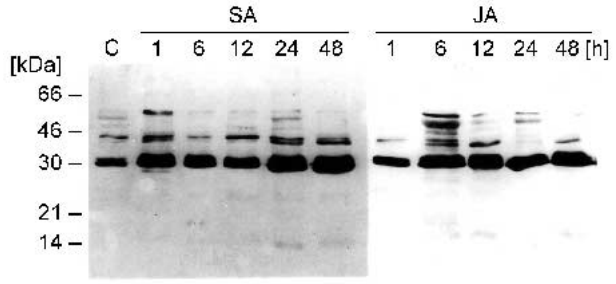

Fig. 1. Western blot showing induction of β-1,3-glucanases in wheat in response to treatment with salicylic acid and jasmonic acid. Protein extracts (150 µg) from SA- and JA-treated or control (C) wheat leaves were separated by 12 % SDS-PAGE and subjected to western blot analysis using barley glucanase antiserum. Numbers indicate hours after treatment. Sizes of marker proteins are indicated on the left.

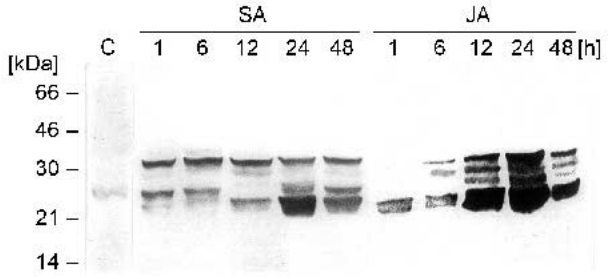

Fig. 2. Immunoblot showing induction of thaumatin-like proteins in wheat in response to treatment with salicylic acid and jasmonic acid. Protein extracts (150 µg) from SA- and JA- treated or control (C) wheat leaves were separated by 12 % SDS-PAGE and subjected to immunoblotting using zeamatin antiserum. Numbers indicate hours after treatment. Sizes of marker proteins are indicated on the left.

Table 1. Effect of application of salicylic acid and jasmonic acid on induction of resistance of wheat to *Stagonospora nodorum*. Plants were treated with 100 µM JA or 1 mM SA for 24 h and then challenge-inoculated with *S. nodorum*. Disease intensity was assessed after 12 d of challenge inoculation with *S. nodorum* using 0 - 9 disease rating scale. Data followed by the same letter in a row do not differ significantly from each other according to Duncan's multiple range test.

Treatment	Disease ratings Experiment I	Experiment II
Control	6.9 a	7.6 a
SA	3.5 b	3.9 b
JA	3.1 b	3.3 b

Upon treatment of wheat leaves with SA and JA, a TLP with an apparent molecular mass of 25 kDa was induced and reached the maximum 24 h after treatment and the intensity of the band decreased thereafter (Fig. 2). However, the content of the 25 kDa TLP was higher in JA-treated plants at all times compared to SA-treated plant, and additional isoforms were observed in JA-treated plants. Healthy wheat leaves had trace amounts of the 25 kDa TLP (Fig. 2).

Pre-treatment of wheat plants with SA and JA significantly reduced infection from a subsequent inoculation with *S. nodorum* (Table 1). Treatment of wheat plants with SA and JA reduced the disease development between 49 and 56 % compared with untreated control plants under greenhouse conditions.

Discussion

The phenomenon of induced resistance in plants against plant pathogens following treatment with abiotic inducers/elicitors is well known (Hammerschmidt 1999). SA and JA have been implicated as inducers of resistance in a variety of pathosystems (Cohen *et al*. 1993, Rakwal *et al*. 1999, Davis *et al*. 2002). In the present study we

have shown that β-1,3-glucanase and TLP are induced in wheat leaves after treatment with SA and JA. Immunoblot analysis of SA- and JA-treated wheat leaves revealed that two β-1,3-glucanases viz., 31 and 33 kDa, were markedly elevated in wheat leaves after treatment with JA and SA. Treatment with SA and JA also induced accumulation of several isoforms of TLPs including a prominant 25 kDa TLP in wheat leaves.

Accumulation of PR-proteins is associated with systemic acquired resistance (SAR) in plants (Ryals *et al*. 1996). Enzymes capable of degrading fungal cell walls directly may represent an important defense mechanism of plants. β-1,3-glucanases (PR-2) catalyze the hydrolysis of β-1,3-glucan, which is a major component of the cell walls of many fungi (Wessels 1993). Velazhahan *et al*. (2003) demonstrated that a β-1,3-glucanase purified from sorghum leaves effectively inhibited growth of *Trichoderma viride in vitro*. Furthermore, β-1,3-glucanases are known to release oligosaccharides from the cell walls of fungi, which in turn, act as signals in the elicitation of host defense responses (Takeuchi *et al*. 1990, Yoshikawa *et al*. 1990, Ham *et al*. 1991). A correlation between constitutive β-1,3-glucanase levels in leaves and resistance to *Alternaria solani* has been established in tomato (Lawrence *et al*. 1996). It has been shown that β-1,3-glucanase is involved in the defense response controlled by the *Lr35* gene in wheat to leaf rust caused by *Puccinia recondita* f. sp. *tritici* (Anguelova *et al*. 1999). The significance of β-1,3-glucanases in plant defense is highlighted by the demonstration that transgenic kiwifruit plants with elevated expression of β-1,3-glucanase have enhanced level of resistance to *Botrytis cinerea* (Nakamura *et al*. 1999). At least four β-1,3-glucanases have been reported in wheat (Muthukrishnan *et al*. 2001). Anguelova *et al*. (1999) reported expression of four isozymes of glucanase with molecular masses of 35, 33, 32 and 31 kDa in wheat leaves infected with *Puccinia recondita* f. sp. *tritici*. In the present study, two β-1,3-glucanases with molecular masses of 31 and 33 kDa were induced in wheat leaves in response to treatment with JA and SA. The accumulation of these β-1,3-glucanases was more rapid in JA-treated plants. These induced glucanases might have involved in the defense of wheat against *S. nodorum*.

Thaumatin-like proteins (TLPs) belong to PR-5 group of PR-proteins that are induced in plants in response to infection by pathogens, osmotic stress, treatment with abscisic acid or ethylene, treatment with *Pseudomonas fluorescens*, application of salicylic acid and wounding (for review, see Velazhahan *et al*. 1999). TLPs show sequence homology to thaumatin, a sweet tasting protein isolated from the fruits of a West African shrub, *Thaumatococcus danielli*. Since purified TLPs from plants exhibit antifungal activity against a variety of fungi (Roberts and Selitrennikoff 1990, Vigers *et al*. 1991, Velazhahan *et al*. 2002), TLPs are postulated to play a role in plant defense. Further, it has been demonstrated that transgenic plants constitutively expressing high levels of PR-5 proteins have enhanced disease resistance (Liu *et al*. 1994, Chen *et al*. 1999, Datta *et al*. 1999, Velazhahan and Muthukrishnan 2003/4). Vigers *et al*. (1991) reported the presence of a 22-kDa TLP (trimatin) in wheat seeds which cross reacted with a maize TLP antiserum. In the present study, it was observed that treatment of wheat leaves with JA and SA induced accumulation of several TLP's including a prominent 25 kDa TLP. These TLPs might have contributed for increased resistance of wheat to *S. nodorum.*

The results of the present study also indicate that pretreatment of wheat leaves with JA and SA significantly reduced subsequent infection by *S. nodorum*. SA and JA treatment reduced the leaf blotch incidence by up to 56 %. The results of this study are in agreement with those of Mills and Wood (1984), Poole and McLeod (1994) and Reglinski *et al*. (1997). Exogenous application of SA induces resistance to *Colletotrichum lagenarium* in cucumber (Mills and Wood 1984). In kiwifruit, pre-harvest application of SA enhanced resistance to wound infection by *Botrytis cinerea* in the immediate post-harvest period (Poole and McLeod 1994). Reglinski *et al*. (1997) demonstrated that pre-treatment of kiwifruit leaves with SA and 4-chlorosalicylic acid caused a reduction in the size of lesions arising from subsequent infection by *Sclerotinia sclerotiorum*. Meena *et al*. (2001) demonstrated that foliar application of SA (1 mM) significantly reduced late leaf spot disease intensity and increased the pod yield in groundnut. Jasmonate is reported to induce resistance to the late blight fungus, *Phytophthora infestans* in tomato and potato plants (Cohen *et al*. 1993).

In summary, the present study demonstrates the induction of β-1,3-glucanases and TLPs in wheat leaves in response to treatment with JA and SA. Pretreatment with SA and JA also induced considerable resistance of wheat to challenge inoculation with *S. nodorum*. Hence, it is possible that increased resistance in wheat to *S. nodorum* after application of SA and JA may be related to the accumulation of PR-proteins.

References

Anderson, J.M.: Membrane-derived fatty acids as precursors to second messengers. - In: Liss, A.R. (ed.): Second Messengers in Plant Growth and Development. Pp. 181-212. Alan R. Liss Inc., New York 1989.

Anguelova, V.S., Van der Westhuizen, A.J., Pretorius, Z.A.: Intercellular proteins and β-1,3-glucanase activity

associated with leaf rust resistance in wheat. - Physiol. Plant. **106**: 393-401, 1999.

Chen, W.P., Chen, P.D., Liu, D.J., Kynast, R., Friebe, B., Velazhahan, R., Muthukrishnan, S., Gill, B.S.: Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. - Theor. appl. Genet. **99**: 755-760, 1999.

Cohen, Y., Gisi, U., Niederman, T.: Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic acid methyl ester. - Phytopathology **83**: 1054-1062, 1993.

Datta, K., Velazhahan, R., Oliva, N., Ona, I., Mew, T., Khush, G.S., Muthukrishnan, S., Datta, S.K.: Overexpression of cloned rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhances environmentally friendly resistance to *Rhizoctonia solani* causing sheath blight disease. - Theor. appl. Genet. **98**: 1138-1145, 1999.

Davis, J.M., Wu, H.G., Cooke, J.E.K., Reed, J.M., Luce, K.S., Michler, C.H.: Pathogen challenge, salicylic acid, and jasmonic acid regulate expression of chitinase gene homologs in pine. - Mol. Plant-Microbe Interact. **15**: 380-387, 2002.

Delaney, T.P., Friedrich, L., Ryals, J.: *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. - Proc. nat. Acad. Sci. USA **92**: 6602-6606, 1995.

Farmer, E.E., Weber, H., Vollenweider, S.: Fatty acid signaling in *Arabidopsis*. - Planta **206**: 167-174, 1998.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., Ryals, J.: Requirement of salicylic acid for the induction of systemic acquired resistance. - Science **261**: 754-756, 1993.

Ham, K.S., Kauffmann, S., Albersheim, P., Darvill, A.G.: Host-pathogen interactions. XXXIX. A soybean pathogenesis-related protein with β-1,3-glucanase activity releases phytoalexin elicitor-active heat-stable fragments from fungal walls. - Mol. Plant-Microbe Interact. **4**: 545-552, 1991.

Hammerschmidt, R.: Induced disease resistance: How do induced plants stop pathogens? - Physiol. mol. Plant Pathol. **55**: 77-84, 1999.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature **277**: 680-684, 1970.

Lawrence, C.B., Joosten, M.H.A.J., Tuzun, S.: Differential induction of pathogenesis-related proteins in tomato by *Alternaria solani* and the association of a basic chitinase isozyme with resistance. - Physiol. mol. Plant Pathol. **48**: 361-377, 1996.

Lawton, K.A., Beck, J., Potter, S., Ward, E., Ryals, J.: Regulation of cucumber class III chitinase gene expression. - Mol. Plant-Microbe Interact. **7**: 48-57, 1994.

Liu, D., Raghothama, K.G., Hasegawa, P.M., Bressan, R.A.: Osmotin overexpression in potato delays development of disease symptoms. - Proc. nat. Acad. Sci. USA **91**: 1888-1892, 1994.

Malamy, J., Carr, J.P., Klessig, D.F., Raskin, I.: Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. - Science **250**: 1002-1004, 1990.

Malamy, J., Klessig, D. F.: Salicylic acid and plant disease resistance. - Plant J. **2**: 643-654, 1992.

Mauch-Mani, B., Metraux, J.P.: Salicylic acid and systemic acquired resistance to pathogen attack. - Ann. Bot. **82**: 535-540, 1998.

Meena, B., Marimuthu, T., Velazhahan, R.: Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum*. - Indian J. Mycol. Plant Pathol. **31**: 139-145, 2001.

Metraux, J.P., Nawrath, C., Genoud, T.: Systemic acquired resistance. - Euphytica **124**: 237-243, 2002.

Mills, P.R., Wood, R.K.S.: The effects of polyacrylic acid, aspirin and salicylic acid on resistance of cucumber to *Colletotrichum lagenarium*. - Phytopathol. Z. **111**: 209-216, 1984.

Muthukrishnan, S., Liang, G.H., Trick, H.N., Gill, B.S.: Pathogenesis-related proteins and their genes in cereals. - Plant Cell Tissue Organ Cult. **64**: 93-114, 2001.

Nakamura, Y., Sawada, H., Kobayashi, S., Nakajima, I., Yoshikawa, M.: Expression of soybean β-1,3-endoglucanase cDNA and effect on disease tolerance in kiwifruit plants. - Plant Cell Rep. **18**: 527-532, 1999.

Nelson, L.R., Marshall, D.: Breeding wheat for resistance to *Septoria nodorum* and *Septoria tritici*. - Adv. Agron. **44**: 257-277, 1990.

Poole, P.R., McLeod, L.C.: Development of post-harvest resistance to picking wound entry *Botrytis cinerea* infection in kiwifruit. - New Zeal. J. Crop hort. Sci. **22**: 387-392, 1994.

Rakwal, R., Agrawal, G.K., Yonekura, M.: Separation of proteins from stressed rice (*Oryza sativa* L.) leaf tissues by two-dimensional polyacrylamide gel electrophoresis: induction of pathogenesis-related and cellular protectant proteins by jasmonic acid, UV irradiation and copper chloride. - Electrophoresis **20**: 3472-3478, 1999.

Reglinski, T., Poole, P.R., Whitaker, G., Hoyte, S.M.: Induced resistance against *Sclerotinia sclerotiorum* in kiwifruit leaves. - Plant Pathol. **46**: 716-721, 1997.

Roberts, W.K., Selitrennikoff, C.P.: Zeamatin, an antifungal protein from maize with membrane permeabilizing activity. - J. gen. Microbiol. **136**: 1771-1778, 1990.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H., Hunt, M.D.: Systemic acquired resistance. - Plant Cell **8**: 1809-1819, 1996.

Saari, E.E., Prescott, J.M.: A scale for appraising the foliar intensity of wheat diseases. - Plant Dis. Rep. **5**: 377-380, 1975.

Schweizer, P., Gees, R. E. M., Mosinger, E.: Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f. sp. *hordei*. - Plant Physiol. **102**: 503-511, 1993.

Shah, D.A., Bergstrom, G.C., Sorrells, M.E.: Differential seed infection of wheat cultivars by *Stagonospora nodorum*. - Plant Dis. **84**: 749-752, 2000.

Takeuchi, Y., Yoshikawa, M., Takeba, G., Tanaka, K., Shibaba, D., Horino, O.: Molecular cloning and ethylene induction of mRNA encoding a phytoalexin elicitor-releasing factor, β-1,3-endoglucanase, in soybean. - Plant Physiol. **93**: 673-682, 1990.

Velazhahan, R., Datta, S. K., Muthukrishnan, S.: The PR-5 family: thaumatin-like proteins. - In: Datta, S.K., Muthukrishnan, S. (ed.): Pathogenesis Related Proteins in Plants. Pp. 107-129. CRC Press, Boca Raton 1999.

Velazhahan, R., Jayaraj, J., Jeoung, J.M., Liang, G.H., Muthukrishnan, S.: Purification and characterization of an antifungal thaumatin-like protein from sorghum (*Sorghum bicolor*) leaves. - J. Plant Dis. Protect. **109**: 452-461, 2002.

Velazhahan, R., Jayaraj, J., Liang, G.H., Muthukrishnan, S.: Partial purification and N-terminal amino acid sequencing of a β-1,3-glucanase from sorghum leaves. - Biol. Plant. **46**: 29-33, 2003.

Velazhahan, R., Muthukrishnan, S.: Transgenic tobacco plants constitutively overexpressing a rice thaumatin-like protein (PR-5) show enhanced resistance to *Alternaria alternata*. - Biol. Plant. **47**: 347-354, 2003/4.

Vigers, A.J., Roberts, W.K., Selitrennikoff, C.P.: A new family of plant antifungal proteins. - Mol. Plant-Microbe Interact. **4**: 315-323, 1991.

Ward, E., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Metraux, J.P., Ryals, J.A.: Coordinate gene activity in response to agents that induce systemic acquired resistance. - Plant Cell **3**: 1085-1094, 1991.

Wessels, J.G.H.: Wall growth, protein extraction and morphogenesis in fungi. - New Phytol. **123**: 397-413, 1993.

Yalpani, N., Silverman, P., Michael, T., Wilson, A., Kleier, D.A., Raskin, I.: Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. - Plant Cell **3**: 809-818, 1991.

Yoshikawa, M., Takeuchi, Y., Horino, O.: A mechanism for ethylene-induced disease resistance in soybean: Enhanced synthesis of an elicitor-releasing factor, β-1,3-endoglucanase. - Physiol. mol. Plant Pathol. **37**: 367-376, 1990.