BIOLOGIA PLANTARUM 35 (1): 95-101, 1993

Glucanase, glucan synthase and chitinase activity in barley genotypes susceptible or resistant to *Erysiphe graminis* f.sp. *hordei*

F. FRIČ and J. HUTTOVÁ

Botanical Institute, Slovak Academy of Sciences, Dubravská 14, 842 23 Bratislava, Slovak Republic

Abstract

In barley genotype susceptible to *Erysiphe graminis* f. sp. *hordei* 1,3-β-glucan synthase activity in whole leaf extracts was higher in comparision with healthy plants. A positive correlation was found between the activity of 1,3-β-glucan synthase and the degree of barley resistance. On the contrary, the 1,3-β-D-glucanase activity in whole leaves was negatively correlated to host plant resistance. This phenomen is evident only in the early phase of plant pathogen interaction. However, in epidermal cells the 1,3-glucanase activity was not significantly changed after attack and the 1,3-glucan synthase activity was practically zero. Chitinase activity in inoculated leaves and epidermis was higher than in healthy ones, but no unambigous correlation was found between the enzyme activity and host resistance.

Introduction

Plant 1,3-β-glucanase and chitinase are thought to play an important role in plant resistance against phytopathogenic microorganisms (Boller 1987, Mauch *et al.* 1988). Plant 1,3-β-glucanase have been shown to attack cell walls of pathogenic fungi. The degradation of fungal cell walls by 1,3-glucanases is generally synergistically stimulated by chitinase (Young and Pegg 1982). Plant chitinase is reported to be a potential ihibitor of fungal growth (Schlumbaum *et al.* 1986, Broekaert *et al.* 1988, Toyoda *et al.* 1991).

Plant 1,3-β-glucan synthase is responsible for 1,3-β-glucan (callose) deposition in periplasmatic spaces of plant cells after wounding or stress and therefore could be also of interest from the point of view of plant resistance. Chitinase and 1,3-β-glucanase are localized not only in vacuole (Boller and Vögeli 1984), but also in apoplastic compartment (Fink *et al.* 1988, 1990).

Received 24 November 1991, *accepted* 17 March 1992.

They could so interact with the surface of the differentiating fungal infection hypha, containing chitin and 1,3-glucan (Bartnicki-Garcia 1968).

In this work we studied the activities of the mentioned enzymes in inoculated barley leaves after fungal attack at host-pathogen compatibility and incompatibility to elucidate their role in preventing fungal growth.

Material and methods

Two susceptible barley cultivars (*Hordeum vulgare* L. cvs. Slovenský dunajský trh and Dvoran, infection type 3-4), and two resistant ones (cvs. Koral and Rupee, infection type 0-2) to *Eryshipe graminis* f. sp. *hordei* Marchal race C_6 were used for investigations. The infection types were judged according to Nover (1972). Seedlings of barley plants were grown in phytotron under controlled conditions as reported previously (Frič 1993).

Leaves of 7 d old seedlings were inoculated with freshly harvested conidia of *E. graminis* f. sp. *hordei*. The inoculated primary leaves were taken for analysis in appropriate time intervals.

Proteins: Barley leaves or abaxial epidermal stripes, were homogenised in 0.05M Tris-HCl buffer (pH 7.2). The homogenate was centrifugated at 105 000 *g* for 20 min and the supernatant was used for analyses. Total protein content in the extracts was determined according to the method of Bradford (1976).

Chitinase (EC 3.2.1.14) and 1,3-β-D-glucanase (EC 3.2.1.39): The activity of enzymes was determined by means of chromogenic substrates CM-Pachyman-RBB, (1,3-β-glucan), CM-chitin-RBV (polyacetylglucosamine 1,4-glucan) (for detail see Wirth and Wolf 1990, Frič and Wolf 1993). Glucanase activity was also determined in acetone dried leaf or epidermis powder according to Jones (1971). As substrate, laminarin (β-1,3-glucan) at pH 5,0 was used, and the hydrolytic product (glucose) was determined by the methods of Somogyi and Nelson (Somogyi 1952). An "*Easy Reader*" (*STL-Laborinstruments Austria*) with a *96-well EIA-plate* (*Costar USA*) of 200 µl capacity was used for all photometric measurements. Heat denaturated samples were used as blanks.

Preparation of acetone dried powder: 1 g of freshly cut leaves or of epidermal cells was ground in 20 ml of cold (20 °C) acetone, in chilled mortar and pestle, with acid-washed sand. The homogenate was filtetred through glass sinter *No.2* under vacuum. The insoluble material was repeatedly handled two or three times in the same way, until the chlorophylls were completely extracted. The acetone dried powder was dried on open air 30 min and stored at -20 °C for several weeks.

Glucan synthase (EC 2.4.1.34): The 1,3-β-D-glucan synthase activity was determined radiometrically in isolated plasma membrane fragments using UDP-glucose (U-^{14}C) as a substrate according to the method described by Morrow and Lucas (1986).

Radioactive samples were measured in a liquid scintillation counter (*LKB-Wallac, 1217 Rack beta*).

The figures in this paper represent one of three completely independent experiments for each host-pathogen combination, which all showed the same or similar trend in enzyme activity changes in the studied rank of pathogenesis. Changes in enzyme activities are expressed as the percentage of values determined for uninoculated (healthy) plants.

Results

1,3.-β-glucan synthase: In inoculated barley leaves of incompatible host-pathogen genotype combinations the plasma membrane bound 1,3-β-glucan synthase activity was significantly increased in contrast to compatible ones.

In compatible host-pathogen genotype combinations no significant activity changes were determined in the early phase of pathogenesis (up to 48 h). Later in some cases the leaf 1,3-glucan synthase activity was even significantly lower in compatible host-pathogen interactions than in healthy-control plants (Fig. 1).

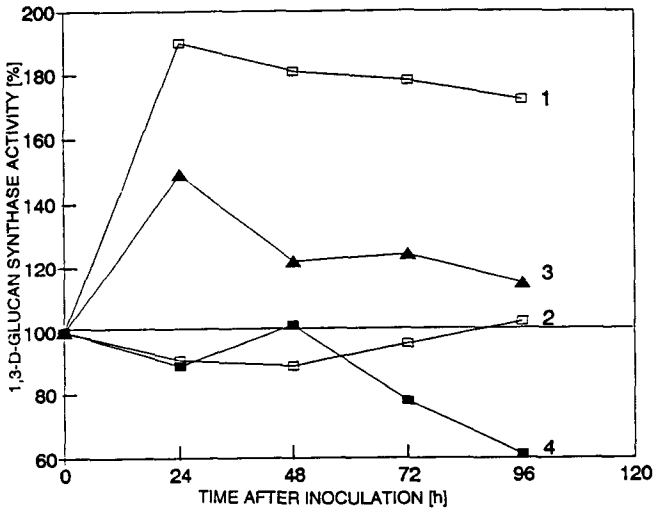

Fig. 1. 1,3-D-glucan synthase activity in powdery mildewed (race C_6) barley leaves (in % of that in inoculated - healthy plants). *1*-cv. Rupee-race C_6 (incompatibility), 2-cv. Slovensky dunajsky trh - race C_6 (compatibility), *3*-cv. Koral-race C_6 (incompatibility), *4*-cv. Dvoran-race C_6 (compatibility).

In epidermal cell extracts, with the method used we were unable to detect unambiguously this enzyme, probably because of its trace amount in extracts.

1.3-β-glucanase: 1,3-β-glucanase in leaves at barley-powdery mildew compatibility is strongly activated only in the very early phase of pathogenesis. Later in the morbide

phase the activity is continually descreasing. At the begining of the fungal sporulation the 1,3-glucanase activity is even lower than that in the healthy leaves. Incompatible host-pathogen combinations show an oposite trend (Fig. 2). The results indicate that the activation of leaf 1,3-β-glucanase activity is different at host-pathogen compatibility and at incompatibility.

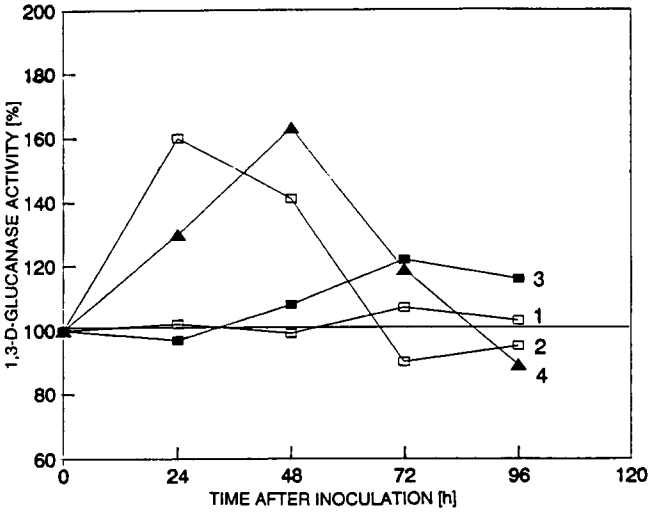

Fig. 2. 1,3-glucanase activity in powdery mildewed (race C_6) barley leaves. Legend as in Fig. 1.

In epidermal cells this phenomenon could not be noticed. With regard to control plants, no significant changes of 1,3-glucanase activity could be determined in epidermal cells after fungal attack neither at host-parasite compatibility nor at incompatibility (data not shown).

Chitinase: Chitinase activity can be easily detected in barley leaves and epidermal cell extracts. Considerable differences in enzyme activity were found in different barley cultivars. Leaf extracts of powdery mildewed leaves exibit an increased chitinase activity which was not caused by fungal chitinase contamination. Chitinase activity in inoculated barley leaves is higher than in healthy ones, regardless to plant compatibility or incompability to the given race of pathogen. No correlation was therefore found between leaf chitinase activation and host plant resistance.

In powdery mildewed epidermal cells at host-pathogen compatibility as well as at incompatibility no significant increase of chitinase activity was determined (data not shown).

Discussion

Plants respond to pathogen attack by an array of constitutive and inducible biochemical reactions, which to the end contribute to a determinate degree of plant

resistance. Activation of plant hydrolases especially of chitinase, 1,3-β-glucanase after pathogen attack has proposed to play an important role in plant resistance (Boller 1987, Fink *et al.* 1988). The activity of 1,3-β-glucan which is involved in callose (1,3-β-glucane) deposition in plants is therefore also of interest. In some callose was observed around penetrating pegs and is suggested as a probable constituent of papillae (Stanbridge *et al.* 1971, Bassi *et al.* 1984). It is postulated that papillae in invated host cells may act as a mechanical barrier to penetration of the infection peg (Stolzenberg *et al.* 1984, Aist and Israel 1986). Callose can be rapidly deposited in papilla and in periplasmatic places after pathogen attack. Principally the rate of callose synthesis and deposition in papillae may play an important role in papillae maturation. Callose deposition in periplasmatic spaces may influence *i.e.* lower the symplastic transport of assimilates. Callose synthesis can be therefore regarded as a defence reaction of the host cels. According to this working hypothesis, in incompatible host-pathogen genotype combinations an increased activity of 1,3-β-glucan synthase is to be expected. Increased activity of the mentioned enzyme in whole leaves at incompability in contrast to compatibility indicate that the enzyme may be involved in plant defence reactions. In epidermal cell extracts the used analytical method failed and so we were not able to confirm the results obtained by whole leaf extracts.

Callose is hydrolyzed by 1,3-β-glucanase (Wong and Maclachlan 1980) and hydrolyzing callose round plasmodesmata and sieve plate, may be involved in the regulation of the symplastic transport (Eschrich 1975, Fincher and Stone 1981) and may so influence the accumulation of various substances especially in mesophyll cells. This process is important for the nutrition of the obligate parasite.

Theoretically the enzyme could slow down the intensity of callose deposition in papillae, this assumption, howewer, is rather speculative. According to working hypothesis 1,3-glucanase in the infected barley leaves at host-pathogen compatibility should be more activated than at incompability. This assumption was confirmed, but only in whole leaf extracts. In epidermis the mentioned correlation was not so unambiguous.

1,3-glucanase is abundant not only in higher plants but also in fungi. Plant 1,3-glucanases can attact and partially digest fungal cell walls too (Young and Pegg 1982). According to low and nonsignificant glucanase activity changes in epidermal cells, in seems not probable that the enzyme may cause a deep degradation of the fungal cell wal, leading to growth inhibition. This process, however, beside beeing "harmless" might trigger metabolic responses (in incompatible cultivars) by the degradation products of 1,3-glucans leading to a district grade of resistance. Similar views sere suggested by other authors too (Mazau *et al.* 1967).

Chitinase activity is present in plant cells and in extracellular spaces. it is stated in many reports that the enzyme is strongly induced in respons to pathogens (Boller 1987, 1988, Metraux *et al.* 1988). This ascertainment, the fact that no chitinase substrate(s) has been found in plants, and the notion that chitinases exhibit antifungal properties (Shlumbaum *et al.* 1986, Broekaert *et al.* 1988) led to an assumption that plant chitinases are directed against the pathogens. We found no unambiguous correlation between barley chitinase activity in powdery mildew infected leaves,

epidermal cells and the host resistance. Chitinase while present in extracellular spaces of barley leaves may come into contact with powdery mildew cell chitin. At present we have no evidence that barley chitinase hydrolysing hyphal wall could inhibit fungal growth. There is also a possibility that plant chitinase could release elicitors (degradation products of fungal chitin) which can trigger defence reactions. Owing to the fact that chitinase is present and is very active in cell walls of many fungi (Boller 1987, Roberts and Selitrannikoff 1988, Frič and Wolf 1993) the above mentioned functions of plant chitinases are doubtful and highly speculative.

References

Aist, J.R., Israel, H.W.: Elemental composition of barley coleoptile papillae in relation to their ability to prevent penetration by *Erysiphe graminis*. - Physiol. mol. Plant Pathol. **29**: 67-78, 1986.

Bartnicki-Garcia, S.: Cell wall chemistry, morphogenesis, and taxonomy of fungi. - Annu. Rev. Microbiol. **22**: 87-108, 1968.

Bassi M., Conti, G.G., Maffi, D., Bocci, A.M.: Preliminary observations on *Sphaerotheca pannosa* development in a susceptible and resistant rose cultivar. - Rev. Pathol. veg. **20**: 134-137, 1984.

Boller, T.: Extracellular localization of chitinase in cucumber. - Physiol. mol. Plant Pathol. **33**: 11-16, 1988.

Boller, T.: Hydrolytic enzymes in plant disease. - In: Kosuge, T., Nester, E.W. (ed): Plant-Microbe Intertactions, Molecular and Genetic Perspectives. Vol. II. Pp. 385-413. Macmillan, New York 1987.

Boller, T., Vögeli, U.: Vacuolar localization of ethylene-induced chitinase in bean leaves. - Plant Physiol. **74**: 442-444, 1984.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Broekaert, W.F., Parijs, J.V., Allen, K.A., Peumans, W.J.: Comparation of some molecular, enzymatic and antifungal properties of chitinase from thorn-apple, tobacco and wheat. - Physiol. mol. Plant. Pathol. **33**: 319-331, 1988.

Eschrich, W.: Sealing systems in phloem. In: Zimmermann, M.H., Milburn, J.A. (ed.): Transport in Plants. Encyclopedia of Plant Physiology, New Series Vol. I. Pp. 39-56. Springer-Verlag, Berlin - Heidelberg - New York 1975.

Fincher, G.B., Stone, B.A.: Metabolism of noncellulosic polysaccharides. - In: Tanner, W., Loewus, F.A. (ed.): Plant Carbohydrates II. Encyclopedia of Plant Physiology, New Series Vol. 13 B. Pp. 68-132. Springer-Verlag, Berlin, Heidelberg - New York 1981.

Fink, W., Liefland, M., Mendgen, K.: Chitinases and B 1,3-glucanases in the apoplastic compartment of oat leaves (*Avena sativa*). - Plant Physiol. **88**: 270-275, 1988.

Fink, W., Liefland, M., Mendgen, K.: Comparation of various stress responses in oat in compatible and nonhost resistant interactions with rust fungi. - Physiol. mol. Plant Pathol. **37**: 309-321, 1990.

Frič, F.: Biochemical changes in barley plants in the preparasitic stage of powdery mildew-barley interaction. - Acta phytopathol. Acad. Sci. hung. **19**: 183-191, 1984.

Frič, F.: Lipoxigenase and superoxidismutase activity in powdery mildewed barley leaves. - Acta phytopathol. entomol. hung. 1993 (in press).

Frič, F., Wolf, G.: Protein changes in the intercellular spaces of barley leaves in the early stage of mildew pathogenesis. - Proc. of the European ESNA Soc. of Nuclear Methods in Agriculture. Working Group 2. Pp. 47-57. Beograd 1979.

Frič, F., Wolf, G.: Some hydrolytic enzymes of ungerminated and germinated conidia of *Erysiphe graminis, hordei* Marchal. - J. Phytopathol. 1992 (in press).

Jones, R.L.: Gibberelic acid-enhanced release of beta 1,3-glucanase from barley aleurone cells. - Plant Physiol. **47**: 412-416, 1971.

Mazau, D., Rumeau, D., Esquerre-Tugaye, M.T.: Molecular approaches to understanding cell surface interactions between plants and fungal pathogens. - Plant Biochem. **25**: 337-343, 1987.

Metraux, J.P., Streit, L., Staub, T.: A pathogenesis-related protein in cucumber is a chitinase. - Physiol. mol. Plant Pathol. **33**: 1-9, 1988.

Morrow, D.L., Lucas, W.J.: (1,3)beta D-glucan synthase from sugar beet I. Isolation and solubilisation. - Plant Physiol. **81**: 171-176, 1986.

Nover, J.: Untersuchungen mit einer für den Rezistenzträger "Lyallpur 3645" virulenten Rasse von *Erysiphe graminis* DC f. sp. *hordei* Marchal. - Arch. Pflanzenschutz **8**: 436-445, 1972.

Oku, H., Ouchi, S., Shiraishi, T., Baba, T., Miyagawa, H.: Phytoalexin production in barley powdery mildew as affected by thermal and biological predispositions. - Proc. Jap. Acad. **51**: 198-201, 1975.

Roberts, W.K., Selitrennikoff, C.P.: Plant and bacterial chitinases differ in antifungal activity. - J. gen. Microbiol. **134**: 169-176, 1988.

Schlumbaum, A., Mauch, F., Vögeli, U., Boller, T.: Plant chitinases are potent inhibitors of fungal growth. - Nature **324**: 365-367, 1986.

Somogyi, M.: Notes on sugar determination. - J. biol. Chem. **1295**: 19-23, 1952.

Stanbridge, B., Gay, J.L., Wood, R.K.S.: Gross and fine structural changes in *Erysiphe graminis* and barley before and during infection. - In: Preece, T.E., Dickinson, C.H. (ed): Ecology of Leaf Surface Microorganisms. Pp. 367-379. Academic Press, London - New York 1971.

Stolzenburg, M.C., Aist, J.R., Israel, H.W.: The role of papillae in resistance to powdery mildew conditioned by the *ml-o* gene in barley. I. Correlative evidence. - Physiol. Plant Pathol. **25**: 337-346, 1984.

Toyoda, H., Matsuda, Y., Yamaga, T., Ikeda, S., Morita, M., Tamai, T., Ouchi, S.: Suppression of the powdery mildew pathogen by chitinase microinjected into barley coleoptile epidermal cells. - Plant Cell Rep. **10**: 217-220, 1991.

Wirth, S.J., Wolf, G.: Dye - labelled substrates for the assay and detection of chitinase and lysozyme activity. - J. microbiol. Methods **12**: 197-205, 1990.

Wong, Y.S., Maclachlan, G.A.: 1,3-beta-D-glucanases from *Pisum sativum* seedlings III. Development and distribution of endogenous substrates. - Plant Physiol. **65**: 222-228, 1980.

Young, D.H., Pegg, G.F.: The action of tomato and *Verticillium albo-atrum* glucosidases on the hyphal wall of *V. albo-atrum*. - Physiol. Plant Pathol. **21**: 411-423, 1982.