

Chitinase isoenzymes in near-isogenic wheat lines challenged with Russian wheat aphid, exogenous ethylene, and mechanical wounding

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Abstract. Russian wheat aphid (RWA) infestation, ethylene exposure, and mechanical wounding resulted in differential expression of chitinase (EC 3.2.1.14) isoenzymes in near-isogenic wheat lines susceptible and resistant to the RWA. Isoelectric focusing (IEF) and non-denaturing IEF gels revealed the existence of multiple isoenzymes of chitinase with a wide range of isoelectric points, ranging from 9.7 (basic) to 3.4 (acidic). Seven bands were observed in the resistant and susceptible untreated plants (pI values of approximately 9.5, 9.2, 8.8, 8.5, 8.4, 7.8 and 5.0) on IEF gels. RWA infestation resulted in the expression of an additional band (approximately pI 5.5) whereas ethylene exposure resulted in the induction of two additional isoenzymes (approximately pI 4.1 and 6.8). Mechanical wounding also induced the isoenzyme with pI of 4.1. Isoelectrical focusing (IEF) in liquid medium confirmed the multiple isoenzymes obtained with IEF gels. Five different peaks with chitinase activity were detected in control plants (pI values of 9.2, 7.8, 5.0, 4.1, and 3.6) after IEF. RWA infestation, ethylene and mechanical wounding caused the induction of additional isoenzyme peaks. Differential expression of chitinolytic activity was also observed between the near-isogenic wheat lines.

Keywords: Chitinase expression; Ethylene; Hydrolase; Isoelectric focusing; Isoenzymes; Mechanical wounding; Russian wheat aphid infestation.

Abbreviations: GlcNAc, β -1,4-linked 2-acetamino-2-deoxy-D-glucose; IEF, isoelectric focusing; IWF, intercellular washing fluid; NAD MDH, Malate dehydrogenase (NAD: EC 1.1.1.37); PR, pathogenesis related; RWA, Russian wheat aphid.

Introduction

Chitinase [poly{1,4-(N-acetyl)- β -D-glucosaminide}] glycanohydrolase], (EC 3.2.1.14) catalyses the hydrolysis of chitin, a polymer of unbranched chains of β -1,4-linked 2-acetamido-2-deoxy-D-glucose (GlcNAc; N-acetylglucosamine) (Jeuniaux, 1966; Mauch and Staehelin, 1989). This enzymatic degradation of chitin to produce GlcNAc is performed by a chitinolytic system, which has been found in micro-organisms, plants, and animals (Flach et al., 1992). The chitinolytic enzymes are traditionally divided into two main classes: (1) endochitinases and (2) N-acetyl glucosaminidases (sometimes termed chitinase, EC 3.2.1.30). The existence of a third class of enzyme, exochitinase, has been suggested (Robbins et al., 1988). Endochitinases randomly hydrolyse GlcNAc polymers, eventually giving diacetylchitobiose as

the major product. N-acetyl glucosaminidases preferentially act on a dimer (Gooday, 1990). The exochitinase also catalyses progressive release of diacetylchitobiose units from the non-reducing ends of chitin chains.

The physiological role of chitinases in the general metabolism of plant cells has not been documented. Indeed, a general role is difficult to envisage, because its substrate, chitin, does not occur in higher plants. It has been postulated that plants produce chitinase in order to protect themselves from chitin-containing parasites (Abeles et al., 1970; Bell, 1981; Boller, 1985) but, until recently, direct evidence supporting this hypothesis was lacking (Boller, 1985). However, in 1988, Roberts and Selitrennikoff reported that an endochitinase purified from barley, was capable of inhibiting the growth of *Trichoderma reesei*, *Alternaria alternata*, and *Neurospora crassa*. In addition, Mauch et al. (1988) reported that, in combination, chitinase and β -1,3-glucanase act synergistically to inhibit fungal growth. These results, therefore, support the hypothesis that the in vivo role of these pathogenesis-related proteins is to protect the host from invasion by fungal pathogens and that, as such, they are an integral component of a general disease resistance mechanism.

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Numerous studies have compared rates of chitinase induction and final chitinase concentrations in tissues that are resistant or susceptible to a pathogen; however, the results of these studies are not clear. In some plant species, resistant tissues accumulated chitinases more rapidly and, in some instances, to higher final concentrations than in susceptible tissues (Bernasconi et al., 1987; Daugrois et al., 1990; Hedrick et al., 1988; Irving and Kuc, 1990; Joosten and De Wit, 1989; Vad et al., 1991; Wyatt et al., 1991). In many of these cases, the resistant response was initially a hypersensitive reaction, with very rapid localised cell death (Bol et al., 1990; Hedrick et al., 1988; Schröder et al., 1992; Vogeli-Lange et al., 1988). Broglie et al. (1991) observed that transgenic plants, with elevated levels of chitinase expression, were more resistant to fungal pathogens. In other plant species, however, there was no difference between chitinase accumulation in susceptible and resistant tissues, or else, paradoxically, the susceptible tissues accumulated higher levels of the enzyme (Bama and Balasubramanian, 1991; Kragh et al., 1990; Vogelsang and Barz, 1993). Mauch et al. (1984) found that there were no significant differences in the induction of chitinase between compatible and incompatible interactions. Thus, the enzyme does not seem to be directly involved in determining disease specificity in the pea-*Fusarium* interactions. This, however, does not exclude the possibility that it is important for resistance against the incompatible fungus. Resistance of pea tissue to *F. solani* f. sp. *phaseoli* appears to be associated with the synthesis of about 20 major pea proteins (Pegg, 1976). If the enhanced synthesis of these "resistance-response proteins" is blocked, or altered by inhibitors of protein synthesis, the tissue becomes susceptible to infestation. Indications are that chitinase may also be involved in the defence of wheat against Russian wheat aphid (RWA), as substantial levels of chitinase protein were present in resistant wheat (TugelaDN) after RWA infestation, in comparison to the chitinase protein levels present in the susceptible near-isogenic Tugela cultivar (Botha et al., 1995; Van der Westhuizen and Pretorius, 1996).

There is little information about the isoforms of chitinase, and how expression of these isoforms may be enhanced by abiotic factors, treatment with elicitors, pathogens or insect infestation. Multiple isoforms (up to 13) of chitinase have been described in germinating seeds of cucumber (Majeau et al., 1990). In tobacco, seven chitinase isoforms were constitutively expressed, and a new isoform was induced by fungal infection (Pan et al., 1992). Four chitinases were induced in peanut by treatment with elicitors, yeast extract, and UV irradiation (Herget et al., 1990).

The aim of this study was to investigate correlations between insect infestation and the resistance response to the Russian wheat aphid (RWA), which may be useful to identify markers for RWA resistance. In order to address this, near isogenic wheat lines, resistant (TugelaDN) and susceptible (Tugela) to the RWA were exposed to RWA infestation. We also aimed to obtain more information on

the effect of infestation by insects on PR-protein expression, more specifically chitinase isoform expression, as these may serve as potential resistance markers for wheat breeders. The observed changes in chitinase isoforms after RWA infestation were also compared with ethylene treated plants and mechanically wounded plants. The RWA inserts a stylet into the plant cell wall during the feeding process (Westphal et al., 1989), and the stylet appears to secrete a sheath (containing lipoproteins and glucans, both known elicitors). We also aimed to investigate whether the observed responses were due to an elicitor secreted by the RWA, or due to mechanical wounding during phloem probing and the associated wound ethylene production.

Materials and Methods

Plant Material

Wheat plants (*Triticum aestivum* L.) resistant (cv. PI137739/5 TugelaDN selection 22 [Du Toit, 1989]) and susceptible (cv. Tugela) to the Russian wheat aphid, *Diuraphis noxia*, were supplied by the Small Grain Institute, Bethlehem, South Africa. Resistant TugelaDN (SA1684/*4Tugela) was bred by crossing Tugela and the RWA resistant SA1684 (*Dn1*), thereafter the resulting population was backcrossed with Tugela, to give near-isogenic lines (Du Toit, 1989). The plants were cultivated under greenhouse conditions as described by Du Toit (1988). Ten days after planting, the plants were incubated in tightly closed glass chambers. The containers were connected to an air pump which circulated the air (flow rate: 478 mL min⁻¹) through the chamber. The plants were infested as described by Tolmay (1995).

Chitin (poly-N-acetylglucosamine), chitosan, glycol chitosan, N-acetyl-D-glucosamine, fluorescent brightner 28, ampholines (pH 3.5–10), and cytohelicase were purchased from Sigma. Ethylene gas was obtained from Fedgas, South Africa (99.7% purity). All other chemicals were of analytical grade.

Ethylene Treatment and Measurement

Ten day-old plants were incubated in glass chambers, as described above, except that the circulated air contained 15 nL ethylene L⁻¹. Control plants were incubated in an identical chamber in the absence of ethylene. The maximal induction of endo- and exochitinase activity was observed after addition of an exogenous supply of 15 nL ethylene L⁻¹ (results not shown). Higher concentrations of exogenously applied ethylene suppressed chitinase activity. Therefore, all further treatments were conducted with 15 nL ethylene L⁻¹. Ethylene was measured according the method of Sanders et al. (1989).

Mechanical Wounding

The induction of chitinase after mechanical wounding was analysed after the leaves of the plants were punctured with a device containing 20 needles. The needles were

0.5 mm in diameter and 2 mm in length, and were spaced in two rows, 4 mm apart. The total length of the apparatus was 40 mm.

Extraction of IWF

Infiltration of leaves and extraction of intercellular washing fluid (IWF) was carried out according to Rohringer et al. (1983). Leaves were cut into 80 mm long pieces and the cut ends were washed extensively in water to remove all the intracellular contamination due to mechanical wounding. The leaf pieces (1 g fresh wt) were then vacuum-infiltrated for 4 min with 80 mL of a 50 mM Tris buffer (pH 7.8) containing 0.5 mM PMSF and 5 mM 2-mercaptoethanol. To obtain the IWF, leaves were centrifuged at 500 g in a Beckman JA 20 centrifuge with their tips pointing downwards. The IWF was collected and frozen at -20°C.

For multiple infiltration, the leaves were infiltrated and centrifuged up to 4 times. The degree of purity of the IWF was quantified by using the marker enzyme NAD Malate dehydrogenase (EC 1.1.1.37; NAD MDH). The activity was determined according to the method of Rohringer et al. (1983). The NAD MDH activity was less than 2% (results not shown), indicating little symplastic contamination in the IWF.

Chitinase Assay

For the assay of endochitinase activity, 0.5 mL of the reaction mixture contained: enzyme, 1 mg colloidal chitin, 0.6 mM sodium azide, and 28 mM sodium acetate buffer (pH 6.5). This was incubated at 37°C for 1 h. The reaction was stopped by centrifugation (1,000 g for 2 min); 0.3 mL of the supernatant was incubated at 37°C with 0.02 mL 3% (m/v) desalted snail gut enzyme (Cytohellicase) and 0.03 mL 1 M potassium phosphate buffer (pH 7.1) to hydrolyse the chitin oligomers (Cabib and Bowers, 1971). The resulting GlcNAc was determined according to Reissig et al. (1955).

A standard curve relating the amount of GlcNAc equivalents to absorbance (A585) was employed for determination of enzyme activity (results not shown). The formation of GlcNAc was a linear function of enzyme concentration. Chitinase activity was expressed as $\mu\text{mol GlcNAc h}^{-1} \text{mg protein}^{-1}$. Means \pm SE of six assays were determined.

Protein Determination

Protein was determined according to Bradford (1976) using the Bio-Rad protein assay reagent with gamma globulin as a standard.

Preparation of Glycol Chitin

Glycol chitin was obtained by acetylation of glycol chitosan using a modification of the method of Molano et al. (1979). Five grams of glycol chitosan was dissolved in 100 mL of 10% acetic acid by grinding in a mortar, and

the viscous solution was allowed to stand overnight at 22°C. Methanol was added, and the solution was vacuum filtrated through a Whatman No. 4 filter paper. The filtrate was transferred into a beaker and 7.5 mL of acetic anhydride was added. The gel was covered with methanol and homogenized. The suspension was centrifuged at 27,000 g for 15 min at 4°C. The gelatinous pellet was resuspended in 1 volume of methanol, homogenized, and centrifuged as in the preceding step. The pellet was resuspended in 500 mL distilled water containing 0.02% (m/v) sodium azide (Trudel and Asselin, 1989).

Non-Denaturing Isoelectric Focusing Electrophoresis (IEF) and Overlay Gel

To detect the isoforms of chitinase, non-denaturing IEF gels (7.5% acrylamide, 0.75 mm thick), were prepared as described by Ried and Collmer (1985), using Sigma broad range ampholines (pH 3.5–10). Sample preparations and staining for chitinase were performed as described by Pan et al. (1991). Following electrophoresis, the gels attached to supporting glass plates were incubated in 0.1 M sodium acetate buffer (pH 5) for 5 min. They were then covered with a 7.5% polyacrylamide (ratio of acrylamide to N,N'-methylene-bisacrylamide 100 : 2.6) overlay gel containing 0.04% (m/v) glycol chitin in 0.1 M sodium acetate buffer (pH 5.0). The gels were incubated under moist conditions at 40°C for 2 h. The overlay gels were then incubated in freshly prepared 0.01% (m/v) fluorescent brightener 28 (Calcofluor white M2R) in 0.5 M Tris-HCl (pH 8.9) at room temperature for 5 min. The brightener solution was discarded and the overlay gels were incubated overnight in distilled water at 4°C or at room temperature in the dark for 2–4 h. Chitinase isoenzymes were visualized as cleared zones by placing the overlay gels on a UV light source (Pan et al., 1991). The IEF gels were cut into 10 pieces and incubated overnight in 500 μL distilled water, after which time the pH of each piece was determined.

Determination of Isoelectric Point (pI)

Isoelectric focusing was carried out on a Bio-Rad Rotorfor cell at 4°C as described by the manufacturer. Chitinase samples from the IWF of control, aphid infested, ethylene treated, and mechanically wounded plants were mixed with 6% ampholines (pH 3.5–10) and loaded into the Rotorfor cell. After 3 h of focusing, the proteins were harvested in fractions of 2 mL, and the pH of each sample was measured. Three replicates were done for each treatment.

Results

Isoelectric Focusing Gel Electrophoresis

Non-denaturing IEF gels revealed the existence of multiple intercellular isoenzymes of chitinase in wheat plants. Seven bands with chitinolytic activity were observed in control plants (Figure 1A and B). The pI values of these bands were approximately 9.5, 9.2, 8.8, 8.5, 8.4, 7.8, and

5.0. The pI values of the bands were determined according to their position on the non-denaturing IEF gel. Plants infested with RWA had an additional band (pI 5.5) (Figure 1A). Susceptible plants exposed to ethylene had two additional isoenzyme bands (pI 6.8 and 4.1) (Figure 1A and B). Mechanically wounded susceptible plants had one additional band (pI 4.1) (Figure 1A).

Determination of Isoelectric Point (pI)

Isoelectric focusing in liquid medium confirmed the multiple isoenzymes of chitinase obtained with non-denaturing IEF gels. Five different peaks with chitinase activity were detected in the control susceptible plants, with estimated pI values of 9.2, 7.8, 5.0, 4.1, and 3.6 (Figure 2A). Six activity peaks were present in the control resistant plants with estimated values of 9.2, 8.4, 7.8,

5.0, 4.4, and 3.6 (Figure 2A). RWA infestation of susceptible and resistant plants resulted in different IWF isoenzyme profiles consisting of seven isoenzyme peaks (Figure 2B). Isoenzymes with estimated pI values of 9.5, 8.8, 7.8, 6.6, 5.0, 4.1, and 3.6 were present in the susceptible plants while isoenzymes with pI values of 9.5, 8.8, 7.8, 5.5, 5.0, 4.1, and 3.6 were expressed in the resistant plants. They, therefore, differed from each other only by the presence of an isoenzyme (pI 6.6) in the susceptible plants and an isoenzyme (pI 5.5) in the resistant plants.

Different isoenzyme profiles were apparent after ethylene application. Seven isoenzyme peaks were present in the susceptible plants (pI values of 9.2, 8.4, 7.8, 5.0, 4.1, 3.7, and 3.5), while eight peaks were observed in the resistant plants (pI values of 9.7, 8.8, 7.8, 6.6, 5.5, 5.0, 4.4, and 3.7) (Figure 2C). Isoenzymes with pI's 9.2, 5.0, 4.1,

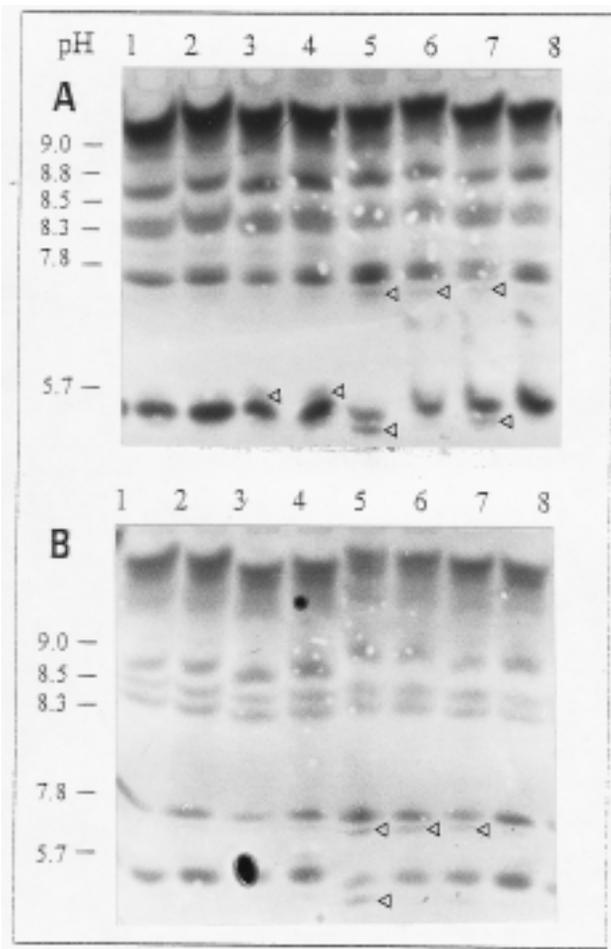


Figure 1. Chitinase isoform banding profiles in wheat after different treatments. (A) Non-denaturing IEF gel after 1 h and (B) 1.5 h separation. Bands were visualised on an overlay gel. Lanes 1 and 2 represent the control, susceptible and resistant plants; lanes 3 and 4 the aphid infested, susceptible, and resistant plants; lanes 5 and 6, ethylene treated susceptible and resistant plants, and lanes 7 and 8, mechanically wounded, susceptible, and resistant plants. All lanes were loaded with 15 μ g proteins. New bands are indicated by triangles.

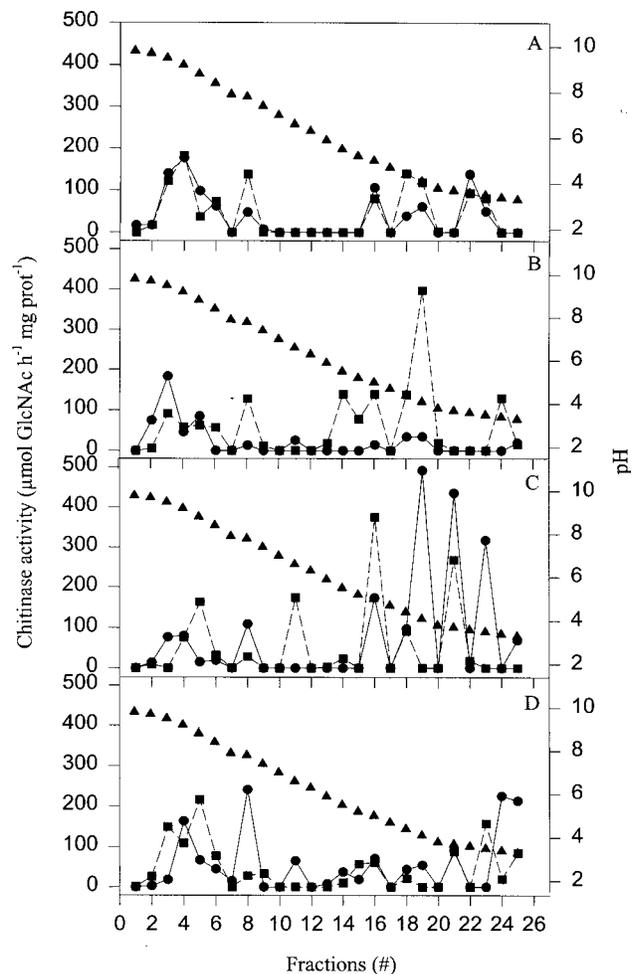


Figure 2. IEF of chitinase isolated from wheat leaves of (A) control plants (B) aphid infested, (C) ethylene treated, and (D) mechanical wounded plants. The susceptible (—) and resistant (---) plants are represented by solid (—) and dotted (---) lines, respectively; while (▲) represents the pH gradient during the IEF. The samples were subjected to IEF in a pH range 3.5–10 as described in Materials and Methods.

and 3.5 were only present in the susceptible plants, whereas isoenzymes with pI's 9.7, 8.8, 6.6, 5.5, and 4.4 were only present in the resistant plants. Isoenzyme pI 9.7 occurred only in resistant plants after ethylene treatment.

Eight peaks were present in the susceptible and seven peaks in the resistant plants after mechanical wounding (Figure 2D). The isoenzymes of susceptible plants had the following pI values: 9.2, 7.8, 6.6, 5.5, 5.0, 4.1, 3.7, and 3.4. Isoenzymes with estimated pI values of 9.5, 8.8, 7.4, 5.0, 4.4, 3.7, and 3.5 were present in the resistant plants. The isoenzyme pI 7.4 was only present in resistant plants after mechanical wounding.

One isoenzyme (pI 6.6) was common in RWA infested susceptible plants, ethylene treated resistant plants, and mechanically wounded susceptible plants (Figure 2B–D). The activity measured in this fraction after RWA infestation was very low, which explains the absence of this band in the IEF gel. RWA infestation induced an isozyme (pI 5.5) in resistant plants (Figure 2B). One isoenzyme (pI 4.1) was induced by RWA infestation in resistant plants (Figure 2B) and by ethylene in the susceptible plants (Figure 2C). Ethylene treatment and mechanical wounding commonly induced one isoenzyme (pI 3.7) in resistant and susceptible plants (Figure 2C and D), while another isoenzyme (pI 7.8) was induced in susceptible plants after mechanical wounding.

IEF allowed separation of chitinase into two different groups, designated as basic and acidic. In wheat, 50% of the chitinase isoenzymes was found in the basic fractions while the other 50% was located in the acidic fractions. RWA infestation and ethylene treatment resulted in an increase of activity in the acidic fractions. The different treatments did not significantly induce the basic chitinase isoforms

Discussion

Chitinase activity was increased in the symplastic tissue and the IWF of wheat over the 14-day investigation period. RWA infestation resulted in a large induction in chitinase activity in the IWF of the RWA resistant plants. Ethylene, however, only induced chitinase activity in the susceptible plants while mechanical wounding had little effect on chitinase activity in any of the tissues (Botha et al., 1995).

Plant chitinases are expressed constitutively and enzyme activity is the result of physiological changes (Boller, 1985). Conventional assays of chitinase activities, therefore, have limited value in studying the role of these enzymes since such assays do not distinguish between isoenzymes which might be involved in diverse roles of the enzyme. In this study, we have established by using IEF gels that seven chitinase isoforms were expressed constitutively and three new isoforms were induced after different treatments (Figures 1 and 2). In tobacco, seven chitinase isoforms were identified (Pan et al., 1992). In cucumber (Zhang and Punja, 1994) and celery (Krebs and

Grumet, 1991), four chitinase isoforms were constitutively expressed. In tobacco, one new isoform was induced after fungal infection (Pan et al., 1992), while four chitinases were induced in peanut by elicitors, a yeast extract, and UV irradiation—whereas one chitinase was differentially induced after inoculation of peanut by pathogens (Herget et al., 1990). In our study, a new chitinase isoenzyme (pI 5.5) was induced in resistant wheat after RWA infestation (Figure 1). Ethylene treatment induced two new isoenzymes (pI 6.8 and pI 4.1), while mechanical wounding induced an isoform with pI value of 4.1. These results are in accordance with the results of Zhang and Punja (1994), who found that wounding induced new chitinase isoenzymes in cucumber. In contrast, Wu et al. (1994) speculated that wounding did not induce any chitinase genes.

When comparing susceptible and resistant untreated plants, it was found that there was no correlation between isoform banding patterns on IEF gels and the genetic resistance of the plants to *D. noxia* (Figure 1). Chitinase isoforms were induced in both susceptible and resistant plants. It appeared, however, that the induction was at higher levels in resistant than in susceptible plants. It has been suggested that chitinase activity may accumulate faster, and to a higher level, in resistant plants than in susceptible plants (Botha et al., 1995; Daugrois et al., 1990). After ethylene treatment and mechanical wounding, a chitinase isoform (pI 4.1) was present in the susceptible Tugela plants, but was absent or undetectable in resistant plants (Figure 1). The differences observed between the induction of chitinases under different stress conditions suggest that different sets of genes may be involved in plant defence related reactions.

In wheat, chitinases are present in multiple isoenzymes (Figure 2). Beerhues and Kombrink (1994) reported a chitinase with a pI 6.84 that accumulated after infection of potato leaves with *Phytophthora infestans*. According to their results, this isoenzyme was also induced by ethylene treatment or by wounding.

IEF separated the chitinases into two different groups, designated as basic and acidic (Figure 2). In tobacco, PR proteins are known to be strictly compartmentalised (Stintzi et al., 1993): acidic chitinases accumulate extracellularly in the apoplastic fluid while the basic chitinases are found in the vacuole (Stintzi et al., 1993). Kombrink et al. (1988) detected basic chitinase and β -1,3-glucanase isoforms in the extracellular space of potato. Sock et al. (1990) found that the β -1,3-glucanase isoforms in the apoplastic fluid of wheat leaves infected with stem rust exhibited isoelectric points ranging from 3.6 to 9.6, providing clear evidence that the classification system for the PR proteins in tobacco as presented by Stintzi et al. (1993) does not fully apply to the PR proteins in wheat. In our study, 50% of the chitinase isoenzymes isolated from the IWF were found in the basic fractions, with the rest located in the acidic fractions.

Results obtained with total activity measured in the IWF (Botha et al., 1995) correlated positively with the activities of the isoenzymes (Figure 2). We speculate, there-

fore, that the increase in total chitinase activity in the IWF is related to the presence of the pI 4.1 chitinase. The specific induction of a new acidic chitinase isoform was also shown in tobacco roots infected with mycorrhizal fungi (Dumas-Gaudot et al., 1992). No significant induction of the basic chitinase isoforms occurred (Figure 2). Total endochitinase activity of the symplastic tissue revealed similar results (Botha et al., 1995).

It has been reported for tobacco (Stintzi et al., 1993), bean (Mauch and Staehelin, 1989), and maize (Nasser et al., 1990) that acidic chitinases accumulate extracellularly. After seven days of RWA infestation, a major induction in endochitinase activity was measured in the IWF of the resistant plants (Botha et al., 1995), and IEF revealed the presence of two additional peaks with chitinolytic activity in the resistant plants after RWA infestation (Figure 2B). These isoenzymes had pI values of 9.5 and 5.5, respectively, and were not present in the control plants. More important was the massive accumulation of chitinase isoform pI 4.1. A fourfold higher chitinase activity was measured for this isoenzyme after RWA infestation. Therefore, it can be argued that the higher activity in the IWF of resistant plants is the result of either the two new bands or the presence of the pI 4.1 chitinase. Western blot analysis has showed that the levels of the constitutively expressed 36 kDa, 34 kDa, and 22 kDa chitinases are significantly higher in the resistant plants after RWA infestation (unpublished results). Thus, the increase in total endochitinase activity is due to enhanced constitutive and newly induced chitinase isoforms. A similar observation was made in cucumber (Zhang and Punja, 1994).

Isoforms of chitinases are known to be differentially induced by several types of stress (Bowles, 1990; Dixon and Lamb, 1990). It has further been reported that ethylene induced the expression of genes encoding for basic chitinase (Boller, 1985). These ethylene induced chitinases (usually class I) are located in the vacuoles (Boller and Vögeli, 1984). Botha et al. (1995) reported induction of chitinase in susceptible plants in both the symplastic tissue and in the IWF after ethylene treatment. No significant induction of the basic chitinase isoforms occurred in the susceptible ethylene treated plants (Figure 2C). A possible reason is that IWF was used for IEF; therefore, the symplastic chitinases were not present.

To conclude, three groups of chitinase isoforms with isoelectric points ranging from 9.7 (basic) to 3.4 (acidic) were observed in this study. Each group may have different functions in plant defence. Group I isoforms were produced constitutively at a low level. Increased stress or novel signals that could activate the host defence system can result in increased expression of chitinase isoforms (group II chitinases) and also activation of newly inducible isoforms (group III chitinases). These chitinases may play an important role in induced resistance to the RWA in wheat. Moreover, the induction of chitinase after infestation was not directly the result of wounding, as exogenously applied ethylene mainly induced chitinase in the susceptible plants, and no significant induction was observed after mechanical wounding.

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