

M. Bliffeld · J. Mundy · I. Potrykus · J. Fütterer

## Genetic engineering of wheat for increased resistance to powdery mildew disease

Received: 20 October 1998 / Accepted: 26 October 1998

**Abstract** Fungal wheat (*Triticum aestivum*) diseases greatly affect crop productivity and require the economically and ecologically undesirable application of fungicides in wheat agriculture. We have generated transgenic wheat plants constitutively expressing an antifungal barley-seed class II chitinase. The transgene was stably expressed and the chitinase properly localized in the apoplast of the transgenic lines. The engineered wheat plants showed increased resistance to infection with the powdery mildew-causing fungus *Erysiphe graminis*.

**Key words** Antifungal proteins · Chitinase · Biolistic transformation · Wheat · Chlorophenolred · Phosphinotricin · *Erysiphe graminis*

### Introduction

Fungal wheat diseases can cause severe crop damages (Oerke et al. 1994); for example, powdery mildew is one of the most consistently damaging diseases of grain cereals in Europe. Fungicide application is economically costly and environmentally disadvantageous. Moreover, the ability of pathogens to develop resistance diminishes the effectiveness of chemical fungicides.

The most common alternative approach to chemical pesticides in modern agriculture is 'integrated pest management', including the use of plant cultivars with resistance to specific fungal races (Patterson et al. 1987). Protection by breeding for race-specific resistance genes requires the pyramiding of several such genes to achieve resistance against a large variety of fungal pathogens. As an alternative, genetic engineering could contribute to increasing the disease resistance of critical crops while reducing the need for cash inputs (Swords et al. 1997).

Plants naturally respond to fungal attack by a complex network of defense mechanisms which are activated upon perception of a pathogen and designed to limit its penetration and development. Defense responses include structural and biochemical responses like reinforcement of the plant cell wall, accumulation of phytoalexins with microbial toxicity, ribosome-inactivating proteins (RIPs) that inhibit protein synthesis, antimicrobial peptides and the synthesis of other pathogenesis-related (PR) proteins (Yang et al. 1997). Some PR proteins, such as chitinases and glucanases, have hydrolytic activities against structural components of fungal cell walls and may exhibit strong antifungal activities in vitro (Schlumbaum et al. 1987; Leah et al. 1991). In vivo, chitin oligomers released from fungal cell walls function in addition as elicitors that stimulate a general resistance response (Côte and Hahn 1994). The induction of resistance responses by chitin-derived oligosaccharides has also been described for wheat (Barber et al. 1989).

Presumably, a microbial infection is accomplished due to a delay in or a ceasing of the plant defense response rather than to an absence of defense mechanisms (Yang et al. 1997). The enforcement and maintenance of the plant response by constitutive expression of individual genes coding for antimicrobial proteins have been proposed as tools for the genetic engineering of enhanced resistance (Swords et al. 1997). Transgenic dicot species and rice plants constitutively expressing

Communicated by G. Wenzel

M. Bliffeld · I. Potrykus · J. Fütterer (✉)  
Institute of Plant Sciences, ETH Zürich, Universitätstr. 2,  
CH 8092 Zürich, Switzerland  
Fax: +41-1-632 1044  
E-mail: johannes.fuetterer@ipw.biol.ethz.ch

J. Mundy  
Department of Plant Physiology, Institute of Molecular Biology,  
Oester Farimagsgade 2A, 1353 Copenhagen K, Denmark

chitinases, alone or in combination with glucanases or RIPs, have shown enhanced resistance to certain pathogens (Broglie et al. 1991; Vierheilig et al. 1993; Jach et al. 1995; Lin et al. 1995; Swords et al. 1997). It is, however, still unclear which chitinases in which plant-cell compartment protect plants best against fungal infections. Besides chitinases, antimicrobial proteins like thionins (Epple et al. 1997), plant defensins and RIPs expressed in transgenic plants also confer enhanced resistance to pathogenic microorganisms (reviewed in Swords et al. 1997).

The production of genetically engineered wheat plants with a wide-range, durable resistance to fungal pathogens would be economically and environmentally advantageous. Wheat transformation technology has only recently become successful, and first reports have focused on selective or visual markers (Weeks et al. 1993; Nehra et al. 1994; Srivastava et al. 1995). The introduction of economically important traits is still at its beginning. First attempts to modify the bread-making quality by expression of transgenic high-molecular-weight glutenin in wheat seeds (reviewed in Vasil and Anderson 1997) and to genetically engineer male sterility (De Block et al. 1997) have been reported. Very recently, the gene for the grapevine stilbene synthase was introduced into wheat and barley, but whether it confers increased resistance to any important wheat pathogen is still open (Leckband and Lörz 1998). We have introduced a barley-seed class-II chitinase (Leah et al. 1991) into wheat. Since the causative agents of powdery mildew (*Erysiphe graminis*) is an obligate biotroph, *in vitro* testing of anti-fungal activities of chitinases against this important pathogen is not possible. It has, however, been

shown that injected chitinase confers a certain resistance to *E. graminis* in cultured barley coleoptiles (Toyoda et al. 1991) and that another obligate biotrophic fungus, *Puccinia graminis*, is also affected by a chitinase secreted by the hyperparasite *Aphanocladium album* (Srivastava et al. 1985). In addition, a correlation between powdery mildew resistance and chitinase induction in barley has been established (Boyd et al. 1994), although a direct involvement of the induced protein in the observed resistance has not been shown.

Endogenous chitinases are present in wheat, but in contrast to what is found in other cereals, chitinase upregulation during infection has not yet detected at the level of protein or enzymatic activity (Münch-Garthoff et al. 1997). Increased amounts of chitinase transcripts could be detected in wheat plants infected by *Puccinia graminis* (Liao et al. 1994; Münch-Garthoff et al. 1997).

In the course of the work described here, we generated transgenic wheat plants overexpressing a barley-seed chitinase gene under the control of the constitutive maize ubiquitin 1 promoter. These transgenic plants exhibited increased resistance to infection by the powdery mildew-causing fungus *E. graminis*.

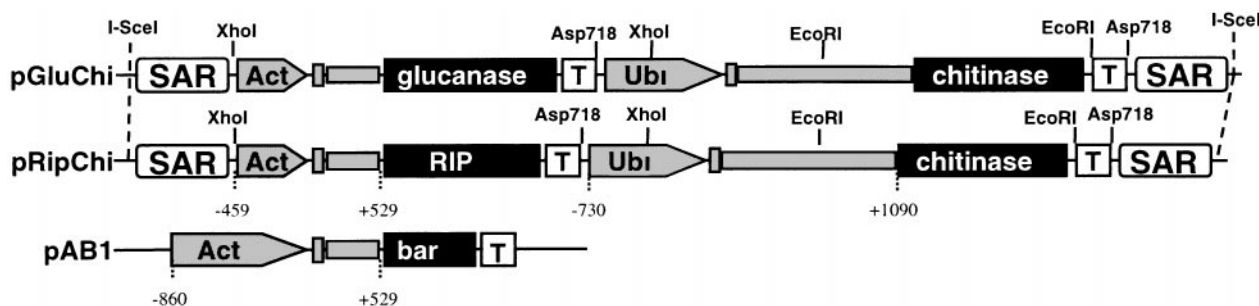
## Materials and methods

### Construction of the plasmids

A plasmid vector was constructed on the basis of pUC19. The region between the *Nde*I and the *Hind*III site was replaced by a polylinker flanked by two *I-Sce*I recognition sites. A *Bgl*III-*Eco*RI fragment from a tobacco scaffold attachment region (SAR) (Breylene et al. 1992) was inserted into that polylinker at two positions. Between these SARs we cloned engineered RIP (pRipChi) or  $\beta$ -1,3-glucanase (pGluChi) genes under the control of the rice actin 1 promoter (McElroy et al. 1990) and a class II chitinase gene under control of the maize ubiquitin 1 promoter (Christensen et al. 1990). The protein coding regions were derived from barley seed-derived cDNA clones (Leah et al. 1991), and the CaMV 35S terminator (Pietrzak et al. 1986) was used for all genes (Fig. 1). A precise description of the plasmids is available upon request.

The plasmid used for selection contains the rice actin 1 promoter linked to the phosphinotricin acetyltransferase open reading frame from *Streptomyces hygrosopicus* (Thompson et al. 1987). The cloning and characterization of plasmids were performed following

**Fig. 1** Gene constructs. The relevant regions of the plasmids used for transformation are shown schematically. Scaffold attachment regions are shown as rounded boxes, labeled SAR; promoters as shaded arrows, labeled Act for the rice actin 1 promoter or Ubi for the maize ubiquitin 1 promoter. Exons are indicated by wide boxes, introns by narrow boxes. Exons containing open reading frames are shown in black and labeled with the name of the respective gene. Restriction sites relevant for the described results are indicated, and the extension of the respective promoter fragments relative to the transcription start sites are given



standard procedures and the recommendations of the suppliers of the respective enzymes.

#### Wheat transformation

Wheat plants (*Triticum aestivum* L. cv 'Bobwhite') were grown in a green-house under an 18-h photoperiod, at 18°C, 35% humidity during the day and 15°C, 50% humidity during the night.

Scutella of immature embryos (0.5–1.0 mm in size) were aseptically isolated and cultured, abaxial (convex) surfaces in contact with the medium, on modified SMS medium (Ozias Akins and Vasil 1982), which is MS medium (Murashige and Skoog 1962) supplemented with 2 mg/l 2,4-D(2,4-dichlorophenoxyacetic acid), 20 g/l maltose and 100 mg/l casein hydrolysate and solidified with 60 g/l agarose. After 5–7 days of culture in the dark at 25°C, the explants were incubated on SMS medium supplemented with 200 g/l of maltose (osmotic treatment) for 4 h and subjected to microprojectile bombardment. A plasmid mixture of 2 µg of supercoiled pAB1 and 10 µg of I-SceI-digested pGluChi or pRipChi was precipitated on 1-to-3 mm gold particles (Aldrich) as described elsewhere (Vain et al. 1993). The scutellum-derived calli were bombarded with 400 µg of DNA-coated particles using the Particle Inflow Gun (Finer et al. 1992). The distance from the particle holder to the target was 10 cm, and the He pressure was 6 bar. Sixteen hours after bombardment, cultures were transferred to SMS medium supplemented with 1 mg/l phosphinotricin (PPT) for 6–8 weeks in the dark at 25°C. Surviving calli were transferred to modified regeneration medium (Weeks et al. 1993) [MS supplemented with 20 g/l maltose, 0.5 mg/l dicamba (Sandoz Crop Protection, Des Plaines, Ill.) and 1 mg/l PPT and solidified with 60 g/l agarose] and kept under light at 25°C. During somatic embryogenesis and regeneration, cultures were maintained by transfer to fresh medium every 2 weeks. Shoots (1.5–2.0 cm in length) were transferred to 300-ml plastic containers (Greiner, Nürtingen, Germany) with half-strength MS medium supplemented with 10 g/l maltose, 3 mg/l PPT and 60 g/l agarose. To confirm PPT tolerance of the regenerated plantlets (8–10 cm in length), we aseptically introduced shoot pieces (1 mm long) into modified chlorophenol red (CR) assay medium (SMS containing 20 mg/l PPT, 260 mg/l CR, pH 6.5) and incubated them 48 h at 25°C under light (Kramer et al. 1993). PPT-tolerant plantlets were transferred to soil, covered with a plastic lid for 1 week and grown to maturity in the greenhouse.

T1 and T2 plants were obtained by germinating rescued embryos and mature seeds, respectively, on MS medium solidified with 80 g/l agarose, and PPT tolerance was determined (CR assay).

#### Protein analysis

For protein preparation, the second and third leaves were harvested from 3-week-old greenhouse-grown wheat plants. Intercellular wash fluid (IWF) was prepared from fresh leaves by vacuum infiltration, and tissue extracts were prepared from leaf material by homogenization in extraction buffer (Jach et al. 1995). Proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, incubated with the primary antibody (Leah et al. 1987) and detected by chemiluminescence (ECL Western blotting, Amersham), according to the instructions of the manufacturer.

The protein amounts were estimated by densitometric measurements of the signal strengths of the bands on Western blots in comparison to known amounts of the purified protein.

#### Southern hybridization

Genomic DNA was isolated and blotted as described (Feuillet et al. 1997). Polymerase chain reaction (PCR) DIG-labeled (Boehringer

fragments of the coding region of the *bar*, chitinase and RIP genes were used as probes. Prehybridization, hybridization and detection were performed as described (Neuhaus-Url and Neuhaus 1993). To reconstruct a single copy of plasmid per wheat hexaploid genome we loaded 12 pg of digested pRipChi.

#### Pathogen treatment

*Erysiphe graminis* f sp *tritici* was maintained on the susceptible wheat cultivar 'Fidel'. For inoculation, freshly produced conidia from heavily infected plants were used. Infection was performed according to Limpert et al. (1987); when the fourth-formed leaf was emerging, the second and third leaves of the wheat plants were cut into 3-to-3.5-cm segments and placed, adaxial side up, on water agar (6 g/l) containing benzimidazole (0.1 g/l). Spores were blown into a settling tower, 10 min were allowed for them to settle, and then the unit was placed on the laboratory bench (20°C) for 5–6 days to produce fully sporulating colonies in control wheat plants (a stable number of colonies was reached around 5 days post-inoculation in control as well as in transgenic plants). Colonies were then counted under a stereo microscope.

## Results

### Plasmids used for transformation

The plasmids used for transformation combined the barley chitinase with either RIP or  $\beta$ -1,3-glucanase genes under the control of constitutive monocot plant promoters (Fig. 1). The integrated transgene copies was insulated from genome position effects by flanking the expression unit with about 600 bp of a scaffold attachment region (SAR) from tobacco (Breyne et al. 1992). The whole unit was flanked by recognition sites for the meganuclease I-SceI. This allowed linearization (or excision) of virtually any cloned insert. Linearization before transformation was thought to improve intact integration of the relatively long transgene unit because random nicking of a circular transformed plasmid would occur with high probability within the expression unit, which covers more than two-thirds of the transforming plasmid.

### Co-transformation of antifungal genes and *bar*

Scutellum-derived calli from immature embryos of wheat cv 'Bobwhite' were cotransformed with a mixture of a plasmid harboring antifungal genes (pGluChi or pRipChi) and pAB1 by microprojectile bombardment with a particle inflow gun (Finer et al. 1992). Plasmid pAB1 contains the herbicide-resistance gene *bar* that confers resistance to PPT (De Block et al. 1987). The overall transformation efficiency achieved (PPT resistant lines/number of scutella bombarded) was about 0.7%, and 75% of the regenerated plants reached maturity and set seeds. Fifteen independent, fertile transgenic wheat lines were generated in 14 transformation experiments. Co-transformation of the

**Table 1** Segregation of the *bar* gene in T1 progeny of transgenic wheat plants

Transgenic line	Plasmid	PPT <sup>r</sup> /PPT <sup>s</sup> <sup>a</sup>	Segregation ratio (%) <sup>b</sup>
5	PGluChi	1/1 <sup>c</sup>	50
6	PRipChi	8/1 <sup>c</sup>	89 <sup>d</sup>
7	PGluChi	10/4	71 <sup>d</sup>
8	PGluChi	0/7	0
9	PGluChi	0/3 <sup>c</sup>	0
10	PRipChi	15/15	50
11	PGluChi	12/0	100
12	PRipChi	4/7	36
14	PRipChi	25/8	76 <sup>d</sup>
18	PRipChi	10/0 <sup>c</sup>	100
19	PRipChi	6/2	75 <sup>d</sup>
21	PRipChi	8/2 <sup>c</sup>	80 <sup>d</sup>
24	PRipChi	6/12	33
29	pGluChi	3/1	75 <sup>d</sup>
30	pRipChi	14/0	100 <sup>a</sup>

<sup>a</sup> PPT<sup>r</sup>, PPT resistant, PPT<sup>s</sup>, PPT sensitive

<sup>b</sup> The observed segregation of *bar* gene expression (PPT<sup>r</sup>/PPT<sup>s</sup>) was compared to a 3:1 expected Mendelian segregation for a single insertion site ( $\chi^2_{0.05}$  test)

<sup>c</sup> All available T1 individuals

<sup>d</sup> Not significantly different

unselected chitinase gene and the *bar* gene was confirmed by Southern blot analysis (see below). Transgene-derived chitinase sequences could be detected in almost all PPT-resistant lines, indicating a very high co-transformation efficiency (not shown). However, RIP gene sequences were only rarely detected (see below), and glucanase gene sequences could be not detected at all. This indicates the selective loss of the respective parts of the expression cassettes, which were linked to the chitinase gene on the transforming plasmid.

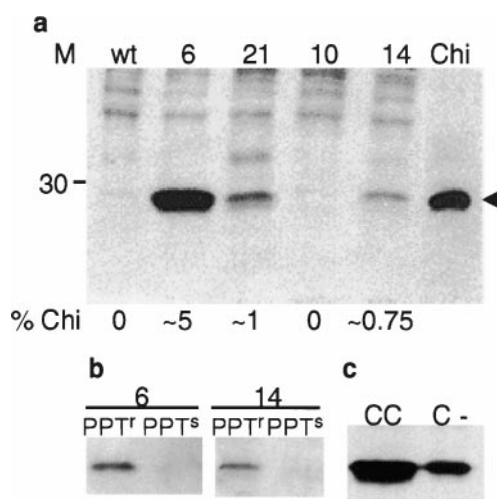
### Bar expression analysis

To study the transmission of the *bar* transgene, we analyzed progeny of primary transformants (T1 plants) for PPT tolerance in the chlorophenol red assay (Table 1). Of 15 lines, 13 transferred phenotypically active copies of the *bar* gene to the next generation, and 2 did not (lines 8 and 9). In 6 lines (6, 7, 14, 19, 21 and 29) inheritance of *bar* expression analyzed in the T1 generation did not differ significantly from Mendelian segregation for a single insertion site, and concordant patterns of segregation were proven in the T2 generation (not shown). Other lines transferred an active gene in a non-Mendelian fashion. Lines 11, 18 and 30 produced only progeny tolerant to PPT; this could be explained if more than one active copy of the *bar* gene had been integrated at different genomic sites. Only 33–50% of the T1 plants of lines 5, 10, 12 and 24 expressed the *bar* gene, indicating instability or partial inactivation of the gene.

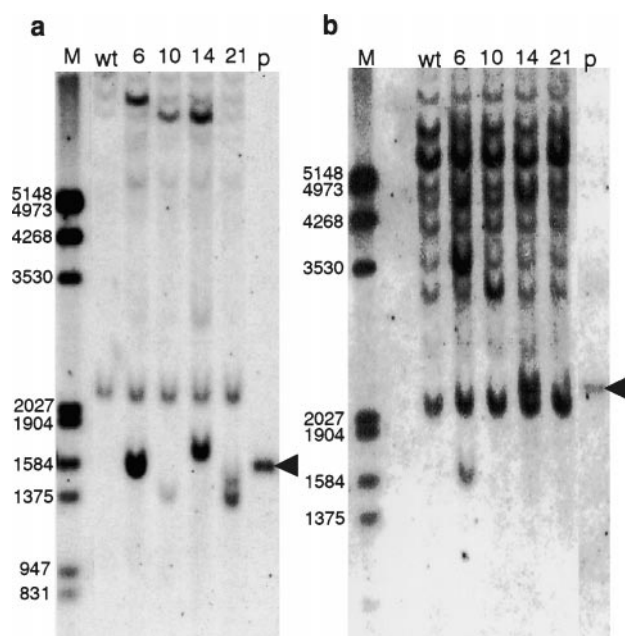
To identify T1 homozygous plants within the lines that segregated in the T1 generation according to a single transgene locus, we analyzed the segregation in the offspring of T1 plants. For each T1 plant, at least 20 T2 plantlets were assayed, and homozygosity of the T1 plant was assumed when all of the T2 plantlets tested exhibited PPT tolerance.

### Co-expression and stability of expression of the co-bombarded antifungal genes

*Bar*-expressing transgenic lines were assessed for the presence of transgene-derived antifungal proteins. Since the barley chitinase protein contains the original N-terminal transport signal that should target it out of the cell to the intercellular space, chitinase was analyzed in the intercellular washing fluid (IWF). The barley RIP should be retained inside the cell. Therefore, the total protein homogenate of leaves was used to assay for the cytoplasmic RIP. No RIP-expressing transgenic plants were found. Wheat lines 6, 14 and 21 showed transgenic chitinase expression. The amount of chitinase produced in the different lines varied (Fig. 2a). In the strongest expressing line, 5% of the total IWF protein consisted of the transgenic chitinase. Chitinase expression was stable in all of the lines for the four generations tested (T3 expression is shown in Fig. 2a) and co-segregated with PPT tolerance (Fig. 2b). The



**Fig. 2a–c** Chitinase expression in independent transgenic wheat lines: Western blot showing extracellular accumulation of immunoreactive chitinase. **a** *wt* wild type, transgenic lines 6, 14, 21 and 10 (non-expressing) (T3 generation), *Chi* 300 ng chitinase, *M* molecular-weight marker in kiloDaltons (kDa). *Arrowhead* (right) indicates the position of purified chitinase. Transgenic chitinase concentration in total IWF protein is indicated below the lanes. **b** Co-segregation of *bar* and chitinase expression in progeny of T0 transgenic lines 6 and 14. PPT<sup>r</sup> PPT-resistant plant, PPT<sup>s</sup> PPT-sensitive plant. **c** Homozygous (CC) and heterozygous (C-) progeny of T0 line 6. Lanes were loaded with 10 µg of total IWF protein

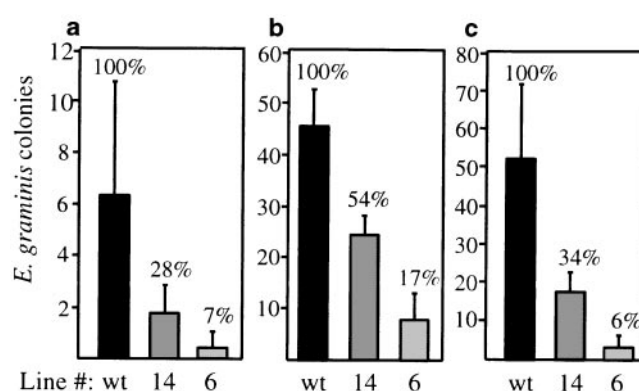


**Fig. 3a, b** Genomic Southern blot analysis of selected transformed lines. *wt* Genomic DNA from control wild type, chitinase-expressing transgenic lines 6, 14, 21 and non-expressing transgenic line 10, *p* digested pRipChi. **a** *Eco*RI digested, probed for chitinase gene, **b** *Xho*I/*Asp*718-digested, probed for RIP gene. Arrow head (right) indicates the position of plasmid originated chitinase (**a**) or RIP (**b**) fragment. For positions of restriction sites see Fig. 1

chitinase levels in successive generations of the same homozygous line were equal (not shown). Differences in chitinase accumulation due to the increase in transgene dosage of the homozygous compared with the heterozygous plant can be clearly observed for progeny of T0 line 6 (Fig. 2c).

#### DNA analysis

Genomic Southern blot analysis of transgenic wheat plants was performed. Blots for chitinase-expressing lines 6, 14 and 21 and non-expressing line 10 are shown (Fig. 3). Specific bands are present in the lanes containing DNA from the transgenic plants in addition to bands derived from endogenous wheat chitinase and RIP sequences, which were also detected by the utilized probes. Line 6 shows a band of the size expected for an intact chitinase gene fragment. Line 14 contains a larger fragment, while line 21 shows a fragment around 100 bp smaller than the expected size and an additional further truncated one (Fig. 3a). This is probably due to rearrangements. Reconstitution analysis indicated a copy number of 2 for all the lines (confirmed by restriction with other enzymes; not shown). The restriction pattern shows an intact additional RIP band in line 14, a rearranged one in line 6 and no hybridization in line 21 (Fig. 3b). The absence of intact copies of the



**Fig. 4a–c** Protection against *E. graminis* in transgenic wheat plants. *wt* Fungal infection assays of wild type, and chitinase-expressing transgenic lines 6 and 14 (T3 generation). Mean values (with standard deviations) of number of *E. graminis* colonies developed are indicated (5–14 replicates). Three independent assays (**a**, **b**, **c**) are shown. The number of colonies present on leaves of transgenic plants is expressed above the corresponding bars as a percentage of the colonies present in control wild type

RIP gene in the last 2 lines accounts for the lack of protein expression.

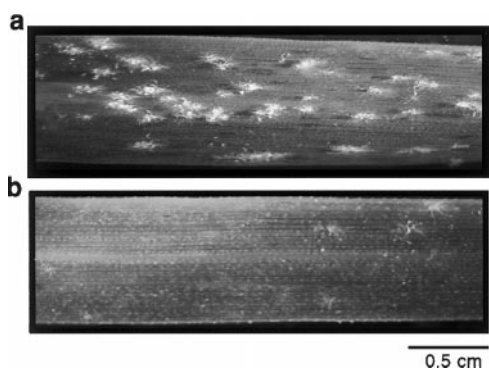
#### Protection of transgenic plants expressing chitinase against *E. graminis* infection

The chitinase-expressing, homozygous lines 6 and 14 were tested for their performance in the powdery mildew infection assay. Disease development was determined on detached leaf pieces of transgenic and wild-type plants inoculated with *E. graminis* (Backes et al. 1996). The evaluation of fungus infection was based on the direct counting of *E. graminis* colonies present on the inoculated leaves.

Significant reductions in colony formation compared to non-transformed plants was observed in transgenic lines 6 and 14 (Fig. 4). The proportion of colonies developing on the transgenic leaves compared to the number present in controls ranged from 6–17% for line 6 and 28–54% for transgenic line 14. The reduction in symptoms of the 2 lines tested correlated with their levels of immunodetectable chitinase, and line 6, which accumulated more than 6 times more chitinase than line 14 (5% of the total IWF protein composed to 0.75%), exhibited a 3- to 6-times higher resistance.

Microscopic colonies of *E. graminis* became visible about 3 days after inoculation (dai) in both control and in transgenic plants. However, by the end of the experiment (6–7 dai), the colonies produced on controls were bigger in size and highly sporulating, while the colonies on transgenic leaves were predominantly weak and produced a reduced amount of spores (Fig. 5).

Increased pathogen tolerance in both transgenic lines was confirmed in several independent infection



**Fig. 5a,b** *E. graminis* colonies in control and transgenic wheat plants. *E. graminis* colonies present in **a** control (wt) and **b** transgenic (line 6) wheat leaves 6 days after inoculation

experiments. The degree to which leaves became infected varied with the inoculation density.

These results demonstrate that the accumulation of chitinase in the apoplastic space increased the protection of transgenic wheat plants against infection by *E. graminis*.

## Discussion

The transformation of wheat with genes coding for economically useful traits is still in its infancy. We have succeeded in introducing a potentially agronomical important trait into wheat and show here that constitutive overexpression of a barley chitinase in transgenic wheat leads to increased fungus resistance.

The co-segregation of PPT tolerance, used to select the transformants, and chitinase expression is consistent with linked single insertion sites of both active genes in the transgenic wheat plants. Both of the genes are inherited in a Mendelian fashion. Southern blot analysis indicates that few copies of the chitinase gene are integrated into the lines.

The presence of the immunoreactive chitinase protein in the apoplast of transgenic wheat plants proves the correct transport and processing of the transgene product. Chitinase expression was not detrimental for wheat development or metabolism and was stable over the four generations tested so far; no loss or decline in expression levels was observed, as reported earlier for unselected transgenes in wheat (Nehra et al. 1994; Srivastava et al. 1995). The level of transgenic chitinase in the intercellular fluids of transformed wheat is similar to that previously reported for transgenic tobacco plants (Jach et al. 1995).

Chitinase-overexpressing wheat lines showed an increased resistance to infection by *E. graminis*. Infection degree, measured in terms of colonies present, was reduced on chitinase-overexpressing wheat leaves and correlated to the chitinase expression level. In addition, sporulation in the fewer colonies present on transgenic

leaves was quantitatively reduced. The apoplastic localization of the transgenic chitinase ensures the immediate contact with incoming fungal hyphae since the first stages of infection of *E. graminis* are extracellular (Boyd et al. 1994). The protection conferred by the chitinase is probably due to the activity on substrates in the mycelial cell wall causing fungal lysis by weakening the wall of growing hyphae (Benhamou et al. 1993) and/or releasing oligosaccharides that function as elicitors of wheat defense mechanisms (Pearce and Ride 1982; Barber et al. 1989).

Increased pathogen tolerance was previously reported for transgenic dicotyledonous species and rice plants expressing diverse chitinase genes (Swords et al. 1997). Previous experiments have been performed mostly for one pathogen, *Rhizoctonia solani* (Broglie et al. 1991; Vierheilig et al. 1993; Jach et al. 1995). Here we demonstrate for the first time that chitinase overexpression also increases resistance against an obligate biotrophic fungi such as *E. graminis*.

The aim of our research was to investigate the expression of antifungal genes in transgenic wheat plants. The plasmids used for transformation combined the barley chitinase either with RIP or with  $\beta$ -1,3-glucanase genes as synergistic antifungal effects have been shown for such combinations (Leah et al. 1991; Zhu et al. 1994; Jach et al. 1995). While all these antifungal proteins could be expressed in the unrelated species tobacco (Logemann et al. 1992; Jach et al. 1995), only chitinase-overexpressing transgenic wheat plants could be obtained in our work. This might suggest that the constitutive expression of barley-seed RIP or  $\beta$ -1,3-glucanase would interfere with the normal development of wheat plants. Developmental and tissue-specific expression of diverse isoforms of these enzymes has been described (Cordero et al. 1994; Hartley et al. 1996). Endogenous substrates have been identified in plants for  $\beta$ -1,3-glucanases but not for chitinases. Barley-seed glucanase may be able to degrade such endogenous substrates present in wheat tissues. Leah et al. (1991) have proposed that the barley-seed RIP is mildly cytotoxic to barley cells and that RIPs accumulated in the endosperm could be responsible for the programmed senescence of this tissue at seed maturity. Thus, this protein could have similar toxic effects toward the ribosomes of a related cereal such as wheat. Barley-seed glucanase and RIP could thus interfere with a number of processes during plant regeneration and normal development in transgenic wheat plants.

Breeding for fungus resistance in wheat is based on the selection or molecular breeding of race-specific resistance traits. The latter are usually overcome after some years. Breeding efforts are limited by the fact that the source of these genes must be amenable to crossing. In contrast to this, genetic engineering allows the expression of foreign genes coming from distant unrelated species as well as the modification of the usual pattern

of expression of an already present gene (Swords et al. 1997). Our results show that fungus resistance of wheat can be improved by genetic transformation. The introduction and expression of additional fungal resistance genes in wheat are feasible and can lead to further increased fungus resistance.

**Acknowledgement** We are very grateful to M. van Montagu, R. Wu and P. Quail for the scaffold attachment regions and promoters, to R. Bilang for plasmid pAB1 and particularly to V. A. Iglesias and to C. Sautter for advice on wheat transformation and the provision of wheat seeds. We thank R. Dudler and members of his group for advice with the fungus resistance assay. The critical reading of the manuscript by N. Hernández-Schärer is gratefully acknowledged. This work was supported by grants from the Biotechnology program of the Swiss National Foundation to M.B. and J.F. All experiments were performed in accordance with the present laws of Switzerland.

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