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Ectopic expression of barley constitutively activated ROPs supports susceptibility to powdery mildew and bacterial wildfire in tobacco

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Abstract ROPs (also called RACs) are RHO-like monomeric G-proteins of plants, well-known as molecular switches in plant signal transduction processes, which are involved in plant development and a variety of biotic and abiotic stress responses. The barley (*Hordeum vulgare*) ROPs RACB, RAC1 and RAC3 have been shown to be involved in cellular growth, polarity and in susceptibility to the biotrophic barley powdery mildew fungus *Blumeria graminis* f.sp. *hordei*. We produced transgenic tobacco (*Nicotiana tabacum*) plants expressing constitutively activated (CA) mutants of the barley ROPs RACB and RAC3 to monitor the impact of heterologous ROP expression on cell polarity and disease susceptibility of tobacco. CA HvROPs influenced leaf texture, disturbed root hair polarity and induced cell expansion in tobacco. Both barley ROPs induced super-susceptibility to the compatible powdery mildew fungus *Golovinomyces cichoracearum* but only CA HvRAC3 induced super-susceptibility to the bacterial leaf pathogen *Pseudomonas syringae* pv. *tabaci*. Data suggest involvements of ROPs in tobacco cell expansion, polar growth and in response to bacterial and fungal leaf pathogens.

Keywords Cell size · G proteins · *Golovinomyces* · Hypertrophy · *Pseudomonas syringae* pv. *tabaci*

Abbreviations

Bgh	*Blumeria graminis* f.sp. *hordei*
CA	constitutively-activated
DN	dominant-negative
Pst	*Pseudomonas syringae* pv. *tabaci*

Introduction

G proteins are GTP-binding and –hydrolysing molecular switches that are essential in eukaryotic signal transduction processes. Two major classes of G proteins are known to be involved in signalling, heterotrimeric G proteins and the Ras superfamily of monomeric small GTPases (Zheng and Yang 2000). The Ras superfamily is classified into five families known as Rab, Arf, Ran, Ras and Rho. RHO family GTPases regulate signal transduction at the plasma membrane and are composed of the three subfamilies Rac, Rho and Cdc42 (Zheng and Yang 2000). Despite a high sequence similarity to animal proteins, the RHO-like GTPases of plants have no definite animal

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homologues. Instead, they build a unique subfamily called ROPs (Rho of plants) or RACs, because their primary amino acid sequences are most similar to animal RACs (Brembu et al. 2006). The ability of small G proteins to cycle between an active GTP-bound and an inactive GDP-bound state makes them an ideal 'molecular switch'. ROPs have been shown to be key regulators of a number of cellular processes (Brembu et al. 2006). RHO and ROP GTPases in animals and plants play similar regulatory roles in cellular processes such as cell polarity and production of reactive oxygen species (ROS), whereas plant ROPs also regulate plant-specific processes, such as lignin synthesis (Brembu et al. 2006; Kawasaki et al. 2006; Wong et al. 2007; Yang and Fu 2007). ROP signalling is known to be required for cell growth, hormone signalling, defence, and responses to oxygen deprivation (Yang and Fu 2007). ROPs have been suggested to modulate exocytosis, endocytosis, vesicle targeting, membrane recycling and calcium flux inside cells (Bloch et al. 2005; Yang and Fu 2007; Kost 2008; Lee and Yang 2008). A growing body of evidence also points to ROPs as key players in the generation of cell polarity in plant cell growth and morphogenesis. This has been shown in several cell systems including pollen tubes, developing root hairs, and leaf epidermal cells (Kost et al. 1999; Fu et al. 2002, 2005; Molendijk et al. 2001; Jones et al. 2002; Kost 2008; Pathuri et al. 2008). ROS and ROP-related spatial control of ROS production are also required for root hair initiation and polarity (Foreman et al. 2003; Carol et al. 2005; Jones et al. 2007).

ROPs share > 70% amino acid identity with each other and 45–64% identity with other members of the RHO family (Zheng and Yang 2000). Six different ROPs have been identified in barley and their amino acid sequences are highly conserved and similar to dicot ROPs (Schultheiss et al. 2003). Barley CA HvRACB, CA HvRAC1 and CA HvRAC3 induce enhanced susceptibility to the powdery mildew disease caused by *Blumeria graminis* f.sp. *hordei* (*Bgh*). They also induce epidermal cell expansion and disturb root hair polarity in barley (Schultheiss et al. 2003; Pathuri et al. 2008). CA HvRACB-dependent increase in susceptibility to *Bgh* was accompanied by inhibited actin polarisation in barley epidermal cells when under attack by *Bgh*, whereas knock-down of RACB promoted actin focusing and basal resistance (Schultheiss et al. 2002; Opalski et al. 2005). Over-expression of rice (*Oryza sativa*) RACB, which shares 98% identity with HvRACB at the amino acid level, was reported to enhance disease symptoms of rice blast caused by *Magnaporthe grisea* (Jung et al. 2006). In contrast, expression of CA OsRAC1 caused disease resistance against *M. grisea* by inducing ROS production and cell death through the regulation of NADPH oxidase (Ono et al. 2001; Wong et al. 2007). In barley, CA HvRAC1 supported resistance to penetration by *M. oryzae*, which appeared independent of cell death (Pathuri et al. 2008).

The use of constitutively-activated (CA) and dominant-negative (DN) ROP mutants is helpful to study their evolutionarily conserved functions in different plant species. A role of ROPs in tobacco defence reactions was suggested because ectopic expression of DN OsRAC1 was shown to decrease ROS production and hypersensitive cell death in resistant *N*-tobacco after inoculation with *tobacco mosaic virus* (Moeder et al. 2005). Heterologous expression of a *Medicago sativa* RAC1 antisense construct in tobacco resulted in suppression of hypersensitive cell death upon elicitor treatment (Schiene et al. 2000).

Here we report on the impact of monocot CA HvROPs on epidermal cell development, root hair polarity and susceptibility to leaf pathogens upon heterologous expression in dicot tobacco.

Materials and methods

Plant transformation

Stable genetic transformation of tobacco (*Nicotiana tabacum*) cv. Xanthi with *pCAMBIA-CaMV35S::HvRACB-G15V* or *pCAMBIA-CaMV35S::HvRAC3-G17V* was performed by *Agrobacterium*-mediated genetic transformation as described by Horsh et al. (1985) and Langen et al. (2006). Seeds of T_1 or T_2 generations of transgenic tobacco were selected on hygromycin-containing medium and compared to both transgenic empty vector control lines and to wild-type lines grown on hygromycin-free medium (Murashige and Skoog 1962). All experiments were performed 4–6 weeks post-selection after transfer to antibiotic-free medium or soil, respectively.

Plant material and inoculation procedures

Analysis of tobacco resistance to *Golovinomyces cichoracearum* or *Pseudomonas syringae* pv. *tabaci* (*Pst*) was performed using T_1 plants grown in standard soil ED73 under growth chamber conditions at 25°C, 60% relative humidity with 16 h of light and 8 h of darkness. The light intensity was 175–185 mol photons s^{-1} m^{-2}. Seeds of the T_1 generation with proven transgene expression in T_0 were sown on selective Murashige and Skoog (1962) solid medium containing 50 mg l^{-1} hygromycin. Intact plants were transplanted into soil and cultivated in the growth chamber.

Inoculation procedure for *Golovinomyces cichoracearum*

Detached leaves of 8 week-old tobacco plants were inoculated with conidia of the powdery mildew fungus *G. cichoracearum* to give a density of 4 conidia mm^{-2}. Leaves were kept at 22°C in closed plastic boxes on 0.5% water agar at 60 mol s^{-1} m^{-2} photon flux densities. Macroscopic evaluation was done 7 days after inoculation.

Inoculation procedure for *Pseudomonas syringae* pv. *tabaci*

Fully-expanded leaves of 7 week-old tobacco plants were used for inoculation with *Pst*, the causal agent of tobacco wildfire. Pure culture of *Pst* (strain 50312 from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was grown for 18 h in glucose yeast extract medium (20 g l^{-1} glucose, 10 g l^{-1} yeast extract, 20 g l^{-1} calcium carbonate (light precipitate)) at 28–30°C. The bacterial culture was diluted with a solution of 0.9% NaCl to obtain a final inoculum concentration of 10^6 cells ml^{-1} and used for infiltration into detached tobacco leaves, kept on 0.5% water agar in closed plastic boxes. To examine bacterial growth, leaf disks of 0.5 cm diameter were punched from the initially infiltrated area and the area directly adjacent to this with a cork borer at 4 days after inoculation. Leaf discs were surface-sterilised in 70% ethanol and then homogenised in sterile water. Bacterial populations were measured by the standard plate-dilution method, on glucose yeast extract nutrient agar (yeast extract, 20 g l^{-1} calcium carbonate (light precipitate), medium with 17 g l^{-1} agar) plates. Colony-forming units (cfu) were counted after 48 h of incubation at 28°C.

Analysis of leaf pavement cell shape and size in tobacco

Leaf discs were taken from fully expanded leaves of the same tobacco plants that were also used for powdery mildew inoculation. The leaf discs were taken from the mid-region of leaves of equal developmental stage. Leaf discs were placed in clearance solution (0.15% trichloroacetic acid [*w*/*v*] in ethylalcohol:chloroform [4:1; *v*/*v*]) and subsequently stored in 50% watery glycerol. Cleared leaves were observed with an Axioplan microscope (Zeiss, Jena, Germany). The measurement of cell sizes of pavement cells was conducted under the same magnification for all samples in all experiments**.** Each data set of a given line derived from the measurement of at least 150 cells collected from three different leaves of three individual plants in three independent experiments.

Analysis of root hair morphology in tobacco

Root hair phenotypes were observed in 2 week-old T_1 or T_2 generations of transgenic tobacco plants grown on selective hygromycin-containing medium and compared to both transgenic empty vector control lines and to wild-type lines grown on hygromycin-free medium (Murashige and Skoog 1962). Phenotypes were observed by using binoculars and light microscope (Axioplan, Zeiss, Jena, Germany).

Statistical analysis

All data were analysed by ANOVA (analysis of variance) and Tukey's multiple comparsion test at given significance levels. To compare the effects of either CA HvRACB or CA HvRAC3 on tobacco powdery mildew susceptibility to wild-type and empty vector control (Table 1), each 4 leaves from each 4 lines from the respective constructs were pooled and treated as one population for the analysis of effects of CA HvRACB (lines 11L1, 11L2, 11L3, 11L4) or CA HvRAC3 (12L1, 12L3, 12L4, 12L6).

Results

We heterologously expressed CA HvROPs (CA HvRACB and CA HvRAC3 are referred to as CA HvROPs, where both of them are meant) in the dicot plant *N. tabacum* and analysed their impact on plant development, cell polarity and pathogenesis.

CA HvROPs influence tobacco leaf texture

We expressed CA HvRACB and CA HvRAC3 in dicot tobacco under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter. Transgenic plants of the T_1 generation were selected based on their hygromycin insensitivity and then transferred to soil. Lines used were tested for presence and expression of the transgene by genomic and semi-quantitative reverse transcription PCR. Out of five hygromycin-resistant CA HvRACB tobacco lines that originally derived from independent calli, four lines equally strongly expressed CA HvRACB. For CA HvRAC3 tobacco, four out of six independent lines equally expressed CA HvRAC3. No obvious phenotypic aberrations were observed in the CA HvROP expressing transgenic tobacco plants in terms of plant height and growth rate when compared to either empty vector control or wild-type. However, CA HvROPs induced an obvious leaf phenotype. CA HvRACB tobacco leaves were often more lancet-like than wild-type or vector controls. CA HvRAC3 tobacco leaves appeared wavy, leathery and showed an irregular leaf texture (Fig. 1a). The CA HvRAC3-leaf surface was uneven, had bigger hairs, and could be clearly distinguished from wild-type (Fig. 1b). In contrast to the other genotypes, minor veins were not clearly projecting on the leaf surface of CA HvRAC3 tobacco plants, which might be due to the disproportionate growth of the leaf tissues or mesophyll hypertrophy (see below).

CA HvROPs abolish tip growth of tobacco root hairs

We inspected root hair growth phenotypes of CA HvROP-expressing tobacco to see potential effects on polar growth. Root hairs of CA HvRACB and CA HvRAC3 tobacco showed aberrant phenotypes when grown on Murashige and Skoog agar plates. Root hairs were stunted and swollen, or had balloon-like tips (Fig. 2). This was observed irrespective of whether CA HvRACB or CA HvRAC3 was expressed.

CA HvROPs induce cell expansion in tobacco

CA HvROPs affected leaf epidermal cell sizes and shapes in barley. Therefore, we checked leaf epidermal cell size and shape in CA HvROP-tobacco. Epidermal jigsaw piece-like pavement cells were increased in size by the expression of either CA HvRACB or CA HvRAC3 (Fig. 3a and b), suggesting enhanced cell expansion. Out of four independent CA HvRAC3 transgenic lines, 12L3 and 12L4 showed the highest increase in epidermal cell size (up to 97% increase relative to the wild-type), whereas in the other two lines (12L1 and 12L6) increase in cell expansion was weaker (up to 29%) in comparison to wild-type leaves. In case of CA HvRACB transgenic tobacco lines, 11L3 displayed the highest increase (up to 66%) in epidermal cell size, while the other three lines (11L1, 11L2 and 11L4) showed an increase in cell expansion of up to 31% when compared to the wild-type leaves. Taken together, in three independent experiments all independent transgenic lines expressing CA HvROPs showed a strong increase in epidermal cell size. In both CA HvRACB and CA HvRAC3 tobacco, interdigitation was less sawtooth-like when compared to wild-type but rounder (Fig. 3a). In some CA HvRACB tobacco lines, patches of pavement cells occasionally had less prominent lobes and appeared brick-like. HvROPs also affected trichome development in tobacco leaves. In particular, CA HvRAC3-expressing plants showed bigger trichomes (Fig. 1b and data not shown). To observe a potential hypertrophy of the mesophyll, mesophyll cell size was observed under the bright field microscope. This revealed that CA HvRAC3 tobacco had strongly-increased mesophyll cell sizes when compared to wild-type or vector controls, whereas CA HvRACB had no clear effect on mesophyll cell sizes (Fig. 3c).

CA HvROPs enhance susceptibility to biotrophic *G. cichoracearum* in tobacco

We inoculated tobacco leaves with spores of virulent *G. cichoracearum*. Macroscopic inspection of leaves 7 days after inoculation revealed typical powdery

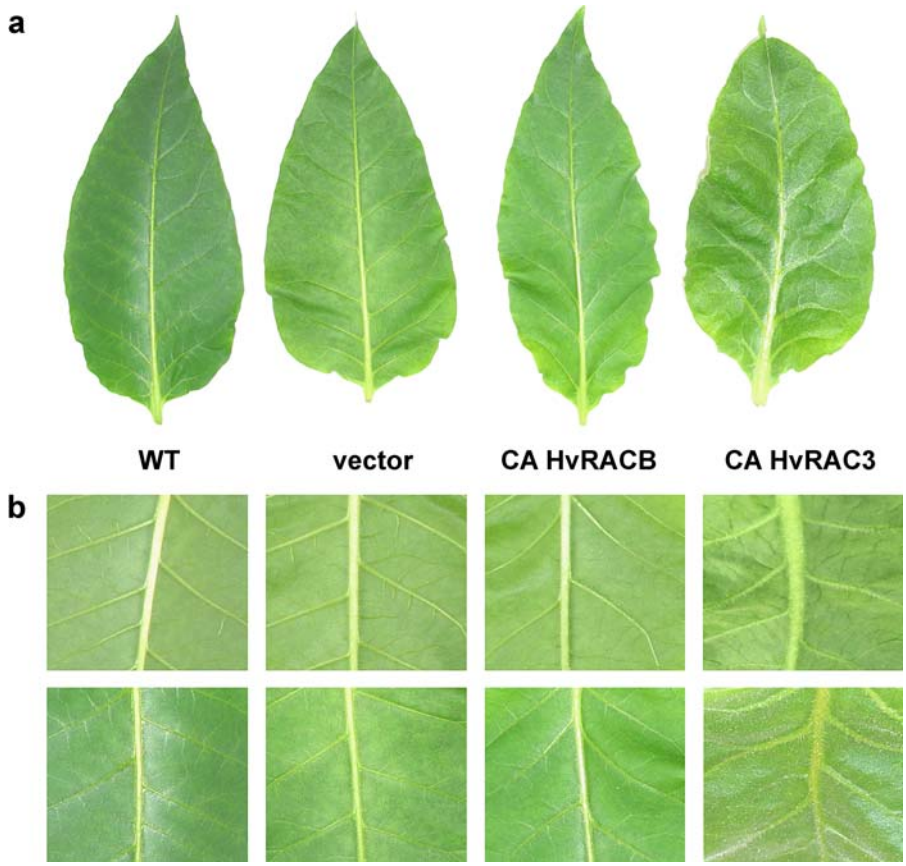

Fig. 1 Leaf phenotypes of tobacco plants expressing CA HvRACB or CA HvRAC3. (a) Fully expanded leaves of 8 week-old tobacco plants of wild-type (WT), empty vector control, *CaMV35S::CA HvRACB* (picture from line 11L1) or *CaMV35S::CA HvRAC3* (picture from line 12L3). (b) Comparison of leaf lamina morphology on both abaxial (upper row) and adaxial planes (lower row) in higher magnification. All leaves were photographed at the same age and magnification

mildew symptoms on both wild-type and empty vector control leaves (Fig. 4a). However, both CA HvRACB and CA HvRAC3 tobacco lines showed strongly enhanced disease symptoms with mycelium sometimes covering the whole leaf area. Susceptibility to *G. cichoracearum* was quantified by estimating diseased leaf areas at 7 days after inoculation. In tobacco lines expressing either of the CA HvROPs, diseased leaf area was increased by up to 200% when compared to wild-type leaves and the empty vector control (Fig. 4b). However, only single lines proved statistically different from the respective controls. To uncouple CA HvROP

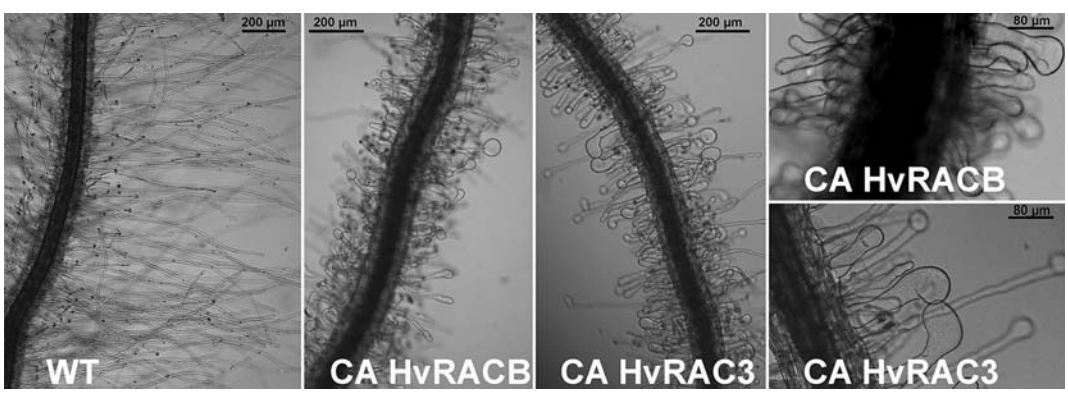

Fig. 2 Root hair phenotype of 2 week-old tobacco plantlets of wild-type (WT), *CaMV35S::CA HvRACB* (picture from line 11L3) or *CaMV35S::CA HvRAC3* (picture from line 12L6)

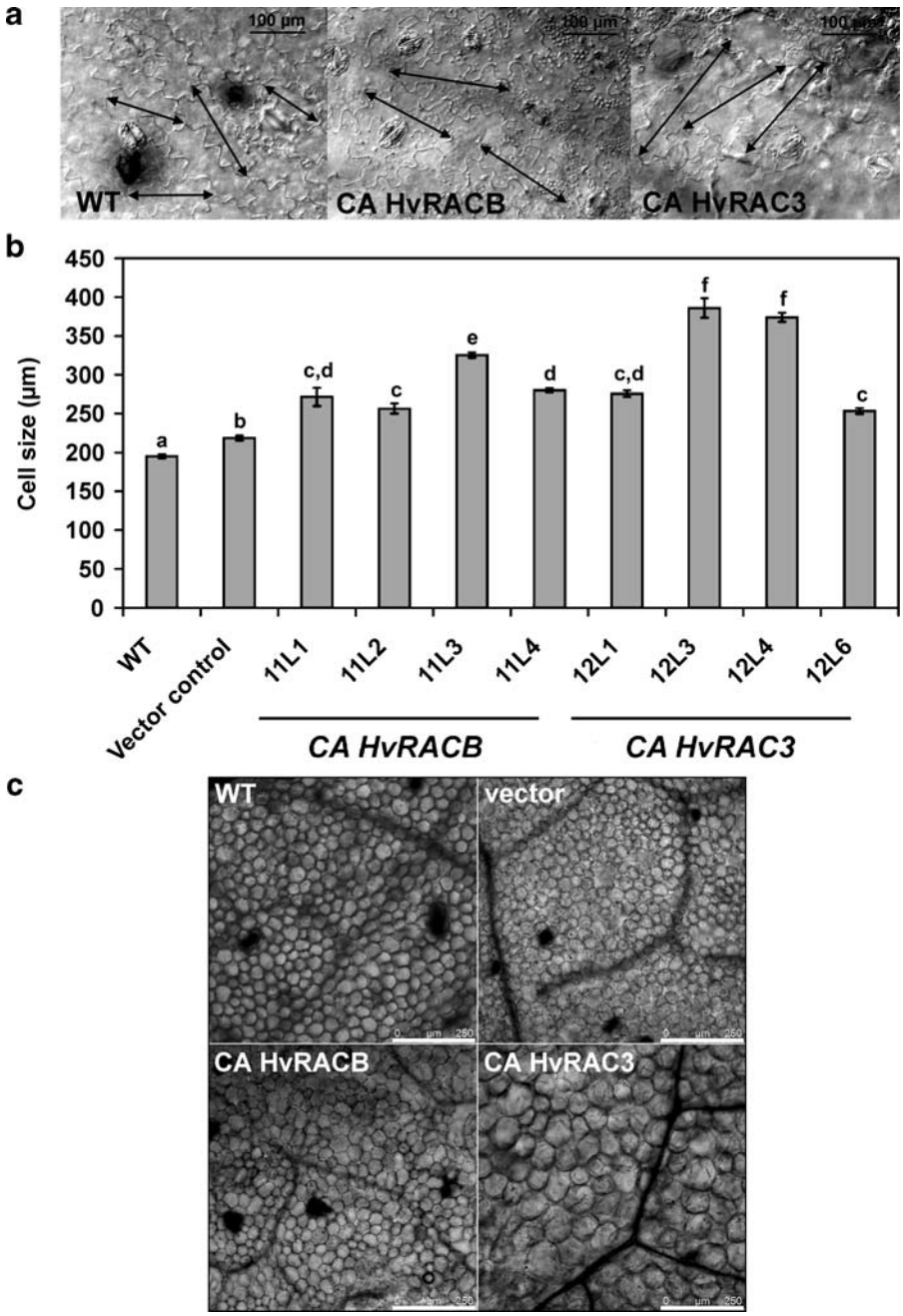

Fig. 3 Cell sizes of tobacco plants expressing CA HvRACB or CA HvRAC3. (a) Epidermal cell phenotype of fully-expanded leaves of 8 week-old tobacco plants of wild-type (WT), *CaMV35S::CA HvRACB* or *CaMV35S::CA HvRAC3*. (b) Average cell sizes of fully-expanded leaves of 8 week-old tobacco plants of wild-type, empty vector control, *CaMV35S::CA HvRACB* (4 lines transformed with binary vector construct 11) or *CaMV35S::CA HvRAC3* (4 lines transformed with binary vector construct 12). Error bars show standard errors of the means of three independent experiments. Averages highlighted with the same letter are not statistically different at $P<0.001$. (c) Mesophyll cell phenotype of fully-expanded leaves of 8 week-old tobacco plants of wild-type, vector control, *CaMV35S::CA HvRACB* (picture from line 11L2) or *CaMV35S::CA HvRAC3* (picture from line 12L3)

effects from potential tissue culture or transgene-position effects in individual lines, we averaged data from each four lines bearing either CA HvRACB or CA HvRAC3 for an independent statistical analysis. This revealed a significant impact of the CA HvROP transgenes on susceptibility when compared to either wild-type or empty vector controls (Table 1).

CA HvRAC3, but not CA HvRACB induces susceptibility to *P. syringae* pv. *tabaci*

To test the potential effect of CA HvROPs on other plant-pathogen interactions, we inoculated CA HvROP-expressing tobacco plants with a virulent strain of *P. syringae* pv. *tabaci*, the causal

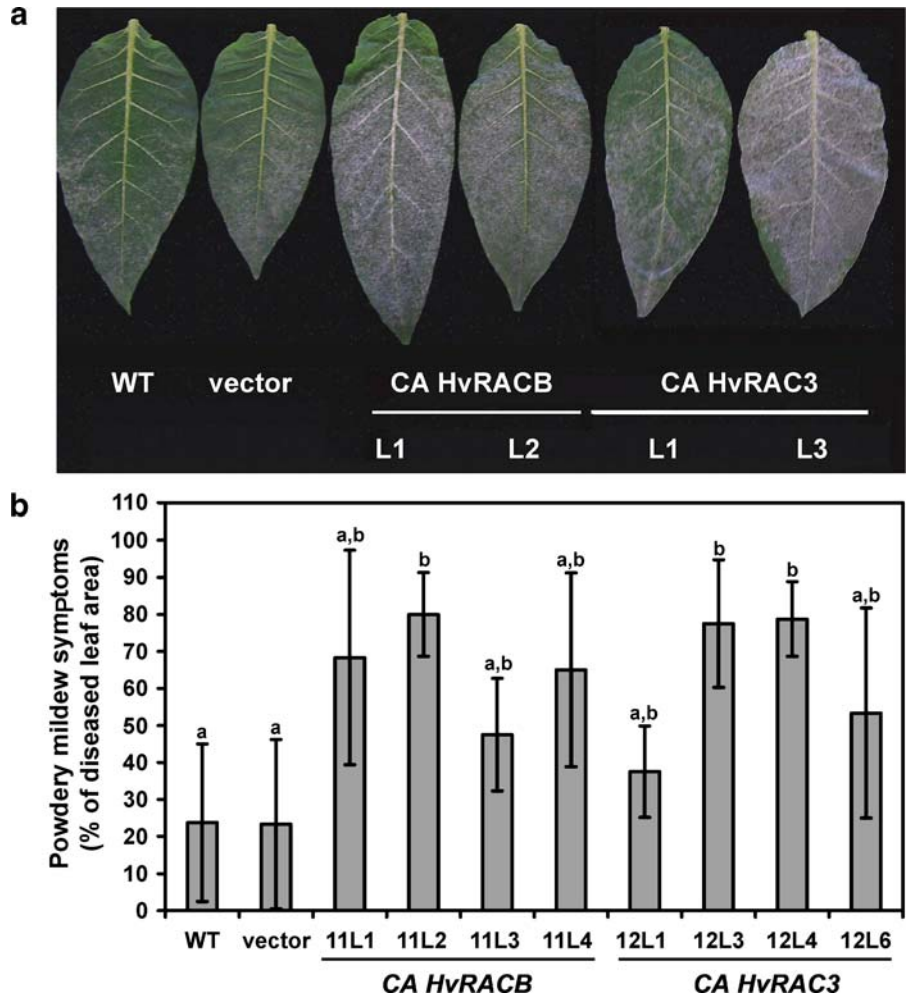

Fig. 4 Powdery mildew disease on tobacco leaves expressing CA HvRACB or CA HvRAC3. (a) Fully-expanded leaves of 8 week-old tobacco plants of wild-type (WT), empty vector control, *CaMV35S::CA HvRACB* or *CaMV35S::CA HvRAC3*. Leaves were inoculated with *Golovinomyces cichoracearum* and photographed 9 days later. (b) Powdery mildew symptom rating on fully-expanded leaves of 8 week-old tobacco plants of wild-type, empty vector control, *CaMV35S::CA HvRACB* (4 lines transformed with binary vector construct 11) or *CaMV35S::CA HvRAC3* (4 lines transformed with binary vector construct 12). Leaves were inoculated with *G. cichoracearum* and rated 7 days later. Error bars show 95% confidence intervals. Repetition of the experiment led to similar results. Averages highlighted with the same letter are not statistically different at $P<0.05$

agent of the tobacco wildfire disease. Seven days after inoculation, wild-type, empty vector control and CA HvRACB tobacco leaves appeared similar in terms of disease development after injecting a *Pst* suspension. The injected area turned necrotic and the directly adjacent tissue appeared chlorotic. In contrast, CA HvRAC3 tobacco leaves injected with bacterial suspensions developed extreme disease symptoms along with tissue maceration and liquid oozing from the infected area (Fig. 5a). Mock inoculation of CA HvROP or control genotypes did not cause any symptoms.

Four days after inoculation, bacterial populations in the infiltrated areas were measured in all tobacco lines. In tobacco lines expressing CA HvRAC3, the number of cfu g^{-1} fresh weight was doubled when compared to wild-type, empty vector control and the CA HvRACB-expressing plants (Fig. 5b). Bacterial populations were also quantified in the leaf area surrounding the infiltrated site. CA HvRAC3 leaves

Table 1 CA HvROP effects on tobacco powdery mildew symptoms

Genotype	Wild-type	Empty vector	CA HvRACB	CA HvRAC3
Average percentage of leaf area covered with powdery mildew symptoms	18.3 [a]	23.3 [a]	62.3 [b]	63.9 [b]

[a,b] Averages of each four independent lines of CA HvRACB or CA HvRAC3 tobacco versus wild-type or vector control. Averages highlighted with the same letter are not statistically different at $P<0.05$

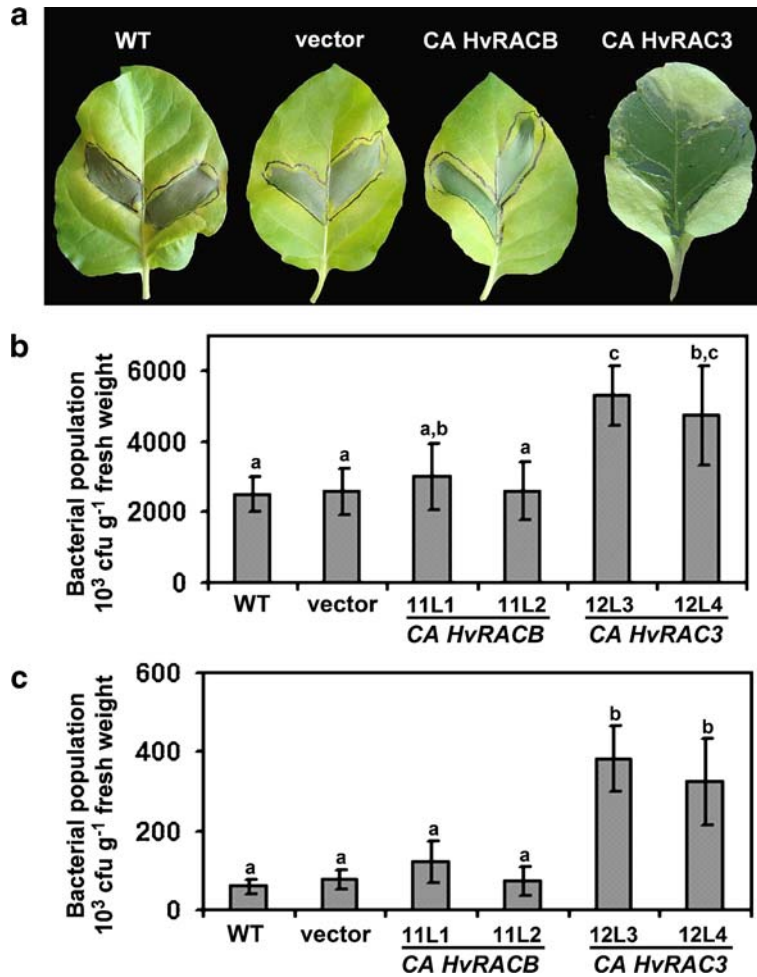

Fig. 5 Inoculation test on CA HvROP tobacco leaves with wild fire bacterium *Pseudomonas syringae* pv. *tabaci* (*Pst*). Leaves were infiltrated with bacterial suspension at a concentration of 10^6 cfu ml^{-1}. (a) Wildfire disease symptoms on leaves of seven week-old tobacco plants of wild-type (WT), empty vector control, *CaMV35S::CA HvRACB or CaMV35S::CA HvRAC3* photographed 7 days after inoculation. The infiltrated areas are indicated by black marker ink. Symptom development assay was repeated with each four lines of CA HvRACB (11L1, 11L2, 11L3, 11L4) and CA HvRAC3 (12L1, 12L3, 12L4, 12L6) and similar results were observed. (b) Bacterial growth in the infiltrated areas of tobacco leaves 4 days after inoculation. (c) Bacterial growth in the surrounding leaf area of the infiltrated zone in tobacco leaves 4 days after inoculation. Error bars show 95% confidence intervals. Repetition of the experiment led to similar results. Averages highlighted with the same letter are not statistically different at $P<0.05$

showed more than five times the number of cfu when compared to the wild-type (Fig. 5c).

Discussion

The data presented here support functions of ROP proteins in cell morphogenesis and in interaction with leaf pathogens of different life style in tobacco. Additionally, a role of cell polarity in basal disease resistance is suggested.

HvROPs control polar tip growth of tobacco root hairs

Both tip-growing plant cells and cells establishing a haustorial complex have to rapidly enlarge their membrane surface. In barley, CA HvROPs affect tip growth of root hairs (Pathuri et al. 2008). Here, we observed that CA HvRACB and CA HvRAC3 also induced shortening and ballooning of tobacco root hairs. This supports the possibility that HvROPs, similar to dicot ROPs (Bloch et al. 2005; Kost 2008; Lee and Yang 2008), operate in polar membrane

transport, which is also required for formation of the haustorial complex.

It has been demonstrated previously that ROPs need to be fine-tuned in spatial abundance and in activity for initiation and maintenance of tip growth in root hairs and pollen tubes (Molendijk et al. 2001; Tao et al. 2002; Carol et al. 2005; Jones et al. 2007; Kost 2008). Tobacco loses polarity of root hairs, when CA NtRAC1 (a homologue of HvRACB) is expressed whereas NtRAC1 RNA interference abolishes root hair growth (Tao et al. 2002). Ectopic over-activation of ROPs apparently leads to loss of spatial control and of polarity resulting in isotropic growth instead of tip growth. This may explain developmental aberrations in CA HvROP tobacco root hairs. CA HvRACB or CA HvRAC3 might have over-activated endogenous tobacco ROP effectors and dominated antagonistic processes, which are responsible for root hair polarity in the wild-type. Data further support that ROPs not only share high sequence similarity (Zheng and Yang 2000; Schultheiss et al. 2003) but also conserved functions between monocots and dicots.

Role of barley ROPs in leaf and epidermis development in tobacco

Expression of CA HvROPs in tobacco had an effect on leaf development including the shape and the texture of the leaf. In particular in CA HvRAC3 plants, the leaves were not flat but had a wavy surface with leathery texture because of irregular growth (Fig. 1a and b). This might be explained by the fact that CA HvRAC3 strongly enhanced mesophyll cell size, which was not obvious upon expression of CA HvRACB.

It is well established that synchronised action of *Arabidopsis* ROP2, ROP4, actin filaments and microtubules is crucial for pavement cell morphogenesis (Fu et al. 2002, 2005). Heterologous expression of barley CA HvROPs induced strong increases in epidermal pavement cell size in tobacco (Fig. 3a and b). This supports the notion that CA HvROPs might affect processes that are normally under control of tobacco ROPs. HvRACB is phylogenetically closely related to tobacco NtRAC1 (Tao et al. 2002) and NtRAC5 (Morel et al. 2004). Interestingly, NtRAC1 operates in auxin-dependent growth processes and in actin polymerisation (Tao et al. 2002; Chen et al. 2003), whereas NtRAC5 can negatively regulate an elicitor-activated oxidative burst in tobacco cells (Morel et al. 2004). HvRAC3 is similar to NtRAC4 (Morel et al. 2004), which has not yet been characterised. However, expression of CA HvRAC3-like CA AtRAC10 in *Arabidopsis* has an effect on membrane dynamics and on epidermal cell shape and size (Bloch et al. 2005).

CA HvROPs enhanced tobacco epidermal cell size but did not strongly affect formation of lobes and necks (Fig. 3a and b). In contrast, *Arabidopsis* CA AtROP2 affects cell shape more strongly than cell size (Fu et al. 2002; 2005). This might be explained by the fact that barley epidermal cells lack interdigitation as it is typically seen in dicot pavement cells. We speculate that barley ROPs did not target dicot effectors of cell lobe formation but rather supported cell expansion generally.

CA HvROPs support susceptibility to *G. cichoracearum* and CA HvRAC3 supports susceptibility to *Pst* in tobacco

CA HvRACB and CA HvRAC3 strongly supported susceptibility to powdery mildew in tobacco. CA HvRACB and CA HvRAC3 can negatively regulate barley basal resistance to powdery mildew (Pathuri et al. 2008). Also in tobacco, HvROPs enhanced infection by *G. cichoracearum* (Fig. 4a and b) suggesting a potential function of ROP signalling in disease susceptibility of dicot plants.

Polar actin filament orientation and local vesicle secretion are crucial for penetration resistance to powdery mildew fungi (Kobayashi et al. 1997; Opalski et al. 2005; An et al. 2006; Kwon et al. 2008). It has been reported that CA HvRACB weakens filamentous actin-based cell polarity in the interaction of barley with *Bgh* (Opalski et al. 2005). Likewise, it might be possible that CA HvROPs inhibited polarisation in epidermal cells of tobacco, thereby supporting the penetration by *G. cichoracearum*. This is supported by the regulatory function of HvRACB-like NtRAC1 on tobacco actin depolymerisation factor 1 (Chen et al. 2003). It seems also possible that CA HvRACB mimicked the closely related CA NtRAC5 in that it attenuated an elicitor-activated oxidative burst. NtRAC5 negatively regulates tobacco NADPH oxidase NtRBOHD at the transcriptional and post-transcriptional level (Morel et al. 2004).

In particular for CA HvRAC3 tobacco, there seemed to be an association of the epidermal cell size effect and induced susceptibility to powdery mildew (Figs. 3b and 4b). In stable transgenic barley, CA HvROPs induce cell size effects, which closely associate with enhanced susceptibility to powdery mildew (Pathuri et al. 2008). However, there are also indications from experiments in barley that cell expansion can be uncoupled from susceptibility to powdery mildew. For instance, RACB effects can be established after ballistic transformation of fully-expanded leaf cells (Schultheiss et al. 2002, 2003). Hence, the cell size does not directly determine susceptibility to powdery mildew. However, this does not exclude that cell growth and susceptibility to powdery mildew share common elements in ROP up– or down-stream signalling.

It was surprising that CA HvRAC3 but not CA HvRACB promoted wildfire symptoms and multiplication of *Pst* in tobacco leaves (Fig. 5a and c). Only CA HvRAC3 tobacco leaves showed an irregular leaf texture, which might have supported susceptibility to *Pst*. However, phenotypes were observed as post-invasion susceptibility, because bacteria were injected. Hence, leaf invasion via stomata was not studied here. Polar host defence is also involved in post-invasive defence against bacterial pathogens (Kwon et al. 2008). We speculate that polar defence in the mesophyll might have been particularly disturbed by CA HvRAC3. This is supported because mesophyll cell expansion was obvious only in CA HvRAC3 tobacco (Fig. 3c). The latter was reminiscent of mesophyll hypertrophy, which is induced by the bacterial type III virulence effector AVRBs3 of *Xanthomonas campestris* pv. *vesicatoria* and the pepper effector target UPA20 in *Nicotiana benthamiana* (Kay et al. 2007). This further supports the idea of common elements of cell size determination and plant disease susceptibility.

Together, the fact that ectopic expression of CA HvROPs in tobacco massively promoted disease symptoms and development of leaf pathogens suggests that the ROP proteins either supported susceptibility or hindered function of basal resistance. In context of the impact on growth processes this suggests that barley ROPs interfered with endogenous ROP signalling in tobacco. Hence, data suggest a role for tobacco ROP signalling in the outcome of interactions with different leaf pathogens. This could involve HvRACB-like NtRAC5 or NtRAC1 (Tao et al. 2002; Chen et al. 2003; Morel et al. 2004) or HvRAC3-like NtRAC4 (Morel et al. 2004). Since solanaceous plants are prominent models in plant pathology and important crops, this might encourage further investigations on ROP signalling in this plant family.

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