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Domestication affected the basal and induced disease resistance in common bean (*Phaseolus vulgaris*)

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Abstract Crop plants exhibit reduced levels of disease resistance, but little is known about the specific resistance mechanisms that are affected by breeding for increased yields. We investigated basal and chemically induced resistance of two wild accessions and four cultivars (including one landrace and three 'modern', yield-improved cultivars that have been produced by hybridisation and pedigree breeding) of common bean (*Phaseolus vulgaris*) under greenhouse and field conditions. After treatment with benzothiadiazole, a widely used inducer of systemic acquired resistance, plants were challenged with one of two bacterial pathogens (*Pseudomonas syringae* pv. *syringae* and *Enterobacter* sp. strain FCB1). Basal resistance to *Pseudomonas* in the wild accessions was significantly higher than in the cultivars. Moreover, benzothiadiazole-treatment elevated resistance to the same pathogen in a wild accession and the landrace, but not in the yield-improved cultivars. Similarly, benzothiadiazole-induced resistance to *Enterobacter* FCB1 was detected in both wild accessions and the landrace, whereas the same treatment enhanced susceptibility to *Enterobacter* FCB1 in two of the yield-improved cultivars. Basal resistance to *Pseudomonas* was highly (but negatively) correlated to induced resistance over all accessions, and basal and inducible resistance to *Enterobacter* FCB1 were negatively correlated for the cultivars, but not when considering all six accessions. Benzothiadiazole-treatment increased growth rates under pathogen pressure of the wild accessions but not the cultivars. Apparently, the yield-improved cultivars investigated here have lost a considerable part of the basal and induced broad-spectrum disease resistance that characterises their wild relatives and to some degree also the landrace. Two of the yield-improved cultivars even became highly susceptible to infection by an *Enterobacter* strain that has not yet been described as a pathogen of bean and that is likely to represent a common environmental or phyllosphere bacterium. Future studies should disentangle the effects of domestication on the various layers of plant resistance to pathogens and consider the potential of wild accessions and landraces for future breeding programmes.

Keywords Basal resistance · Biotrophic bacteria · BTH · Enterobacter · Landrace · Pseudomonas · Resistance elicitor · Systemic acquired resistance · SAR

Abbreviations

BTH	Benzothiadiazole
P. vulgaris	*Phaseolus vulgaris*
P. syringae	*Pseudomonas syringae*

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SAR Systemic acquired resistance
Ent. Enterobacter

Introduction

Many crop plants are characterised by low levels of resistance to pathogen infection and their cultivation requires the intensive use of pesticides, with all the well-known consequences for the environment, the producer and the consumer. Artificial resistance elicitors that stimulate the inherent defensive system of the plant appear to be, therefore, an attractive alternative (Heil and Walters 2009). Unfortunately, whereas it appears clear that the basal resistance of cultivated plants is dramatically reduced as compared to their wild ancestors, we know very little of the effects of plant breeding on the levels of induced resistance. One of the most intensively investigated and widely used resistance elicitors is benzothiadiazole (BTH), which is commercialised under the names Actigard® and BION®. BTH-induced resistance is characterized by the expression of genes that widely overlap with those that respond to salicylic acid (SA), is biochemically and phenotypically similar to systemic acquired resistance (SAR) and leads in general to a phenotypic resistance to biotrophic pathogens including viruses, bacteria and fungi (Oostendorp et al. 2001; Schurter et al. 1987). Reports on the successful use of BTH as a protecting agent against disease under field conditions include crops from a wide taxonomic range: for example, induced resistance in cauliflower to downy mildew caused by *Peronospora parasitica* (Godard et al. 1999) and in tobacco to *Pseudomonas syringae* (Cole 1999), a successful control of early blight (*Alternaria solani*) and of powdery mildew (*Erysiphe cichoracearum*) in potato (Bokshi et al. 2003), and an induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* (Buzi et al. 2004). In spite of these advances, the reports on the efficiency of BTH-induced resistance remained mixed. For example, some authors found low or insignificant effects of BTH-treatment on plant resistance or yield (Romero et al. 2001; Stadnik and Buchenauer 1999). Effects of BTH application also varied depending on the plant genotype used (Dann et al. 1998; Heil and Ploss 2006) or due to prior infection of the plant (Walters et al. 2011). Therefore, although SAR and BTH-induced resistance are usually considered as being active against a wide range of biotrophic pathogens, the quantitative outcome of the induction appears to depend on multiple abiotic and biotic factors.

We aimed to investigate whether basal resistance of the plants and their capacity to express SAR in response to BTH treatment depends on the domestication state of the plant, the type of pathogen, or both. One plausible explanation for the above-mentioned discrepancies is that the induction of resistance includes the reallocation of potentially limiting resources from primary metabolism towards defence and, therefore, causes significant metabolic costs (de Nardi et al. 2006; Logemann et al. 1995). Because these costs can be phenotypically manifested as reduced growth and reproduction rates and, thus, ultimately as a reduction in yield (Heil 2001), it appeared likely that this type of resistance has been lost during the domestication process. We selected two wild accessions, a landrace and three yield-improved cultivars of common bean (*Phaseolus vulgaris* L.) and compared under greenhouse and field conditions their levels of basal and induced resistance against two biotrophic bacterial pathogens: *Pseudomonas syringae* pv. *syringae* (*P. syringae*) and *Enterobacter* sp. strain FCB1 (*Ent.* FCB1), which had been isolated de novo from diseased common bean plants in the field. We also quantified the number of shoots newly produced after BTH treatment and pathogen infection, in order to estimate whether the induced resistance has a positive or negative effect on the future development of the plants growing under disease pressure.

Materials and methods

Plant and pathogen material

In this study we used two wild accessions and four cultivars of common bean. One of the cultivars represents a landrace ('Negro San Luis'), whereas the other three ('Pinto Villa', 'Flor de Junio Marcela' and 'Negro Durango') represent modern yield-improved cultivars that have been produced by hybridisation and pedigree breeding (Table 1). Landraces are cultivated lines of crop plants, which have been produced via mass selection for desirable traits from domesticated wild lines, rather than by modern breeding strategies. Therefore, landraces are commonly characterised by lower yield values but higher genetic diversity and higher resistance levels (Mavromatis et al. 2007; Rodino et al. 2009). Due to the continuous selection

Table 1 Major characteristics of the bean lines used in the present study

Code	Name	Status	Growth habit	Yield [kg ha^{-1}]	Breeding method
W1	*P. vulgaris* 4	Wild	Climber	Unknown	n.a.
W2	*P. vulgaris* 27	Wild	Climber	Unknown	n.a.
C1	Pinto Villa	Cultivated	Indeterminate prostrate	700–3000	Hybridisation and pedigree breeding
C2	Flor de Junio Marcela	Cultivated	Indeterminate prostrate	2400–3000	Hybridisation and pedigree breeding
C3	Negro Durango	Cultivated	Indeterminate prostrate	1300–2500	Hybridisation and pedigree breeding
C4	Negro San Luis	Landrace	Indeterminate prostrate	760–1300	Mass selection

Acosta-Díaz et al. 2009; Acosta-Gallegos et al. 2007

process and long-term local usage, landraces of common bean in Mexico tend to demonstrate multiple adaptations to local biotic and abiotic conditions (Acosta-Gallegos et al. 2007). All accessions are being held in the collection of INIFAP-CEBAJ in Celaya, Guanajuato. The wild accessions are climbers with an indeterminate growth habit that is characterised by a clearly dominating primary shoot. By contrast, the cultivars used here (including the landrace) exhibit a prostrate growth habit with a much earlier shift to the flowering and fruiting phases and high yields (Table 1).

Two pathogens were used. *Pseudomonas syringae* pv. *syringae* strain 61 was obtained from CM Ryu, KRIBB, S.-Korea, the same strain has already been used in an earlier study on BTH-induced resistance in bean (Yi et al. 2009). The *Enterobacter* sp. strain FCB1 had been isolated de novo from diseased common bean plants. Leaves were surface-sterilized with 70 % ethanol, rinsed three times with water and then diseased areas were cut out, homogenized in water, diluted and plated on agar plates with YDC medium (see below). Single colonies were picked randomly and formed the basis of homogenous cultures of which one was used in the present study. For identification, the 16S genes were sequenced from both bacteria. In short, bacteria were cultured in LB-broth overnight, 1 ml was centrifuged at 4,500 rpm for 3 min and the pellet was resuspended in 1 ml sterile water. Total genomic DNA was extracted using the QuickGene DNA tissue kit S (FujiFilm LifeScience). The resulting DNA was checked on an 0.8 % agarose gel and then used for PCR using 40 μl Platinum PCR super mix (Invitrogen), 1 μl primer 24F (agagtttgatcmtggct cag), 1 μl primer 1492R (tacggytaccttgttacgactt) and 1 μl DNA template. The following program was used: initial denaturation at 95 °C for 5 min, 30 cycles of 94 °C for 30 s, 48 °C for 1 min and 72 °C for 21 min, and a final extension step at 72 °C for 8 min. PCR products were purified with the PCR-purification-kit Invisorb Fragment Clean Up (Invitek, Germany) and sent for sequencing. In order to ensure that the isolated *Ent.* FCB1 strain was the causal agent of the observed disease symptoms, plants were experimentally challenged under greenhouse conditions and bacteria were re-isolated from these experimentally infected plants, in order to be subjected again to 16S sequencing.

Cultivation conditions for plants and pathogens

Two sets of experiments were conducted, one in the field and the other one under greenhouse conditions. The efficiency of chemically induced resistance has been reported to depend on the abiotic and biotic growing conditions, a phenomenon that has in part been attributed to earlier exposures of field-grown plants to pathogens or abiotic stressors (Walters et al. 2005). We carried out our experiments under both types of environment because we aimed at focussing our interpretations on stable, general effects that do not depend on the detailed growing conditions.

Field experiment The plants were cultivated individually in pots under field conditions (Campus of CINVESTAV Irapuato, central highlands of Mexico, state of Guanajuato, 2,000 m above sea level; 20 °43′13″ N; 101° 19′43″W). This experiment was carried out in May and June 2010, during the early rainy season, which represents the dominant cultivation period for common bean in the region (Acosta-Díaz et al. 2009). The pots contained 2.4 l of a standard substrate consisting of 3 parts ‘Berger BM2 germination mix’ (J.R. Thompson supply, Roseville, MN; USA), 1 part vermiculite, 1 part

'perlita mineral expandida' (Perlita de la Laguna, México DF, Mexico) and 2 parts milled leaves (leaves of *Quercus oleoides* dried and milled to <2 mm: trade name 'Tierra de Hoja', Viveros d' Silao, Silao, GTO, Mexico). The plants were watered daily with ca 300 ml water per pot and were fertilised every week with 150 ml of a solution of 3 g l^{-1} of FerViaFol 20-30-10 NPK (Agroquímicos Rivas, Celaya, GTO, México).

Greenhouse experiment Plants were cultivated under glasshouse conditions (natural light, temperatures ca. 22 °C–28 °C) in June 2011. Pots of the same size as described above were filled with the following substrate: 1 part vermiculite, 2 parts milled leaves ('Tierra de Hoja', Viveros d' Silao, Silao, GTO, Mexico), 1 part 'perlita mineral expandida' (Perlita de la Laguna, México DF, México) and 3 parts 'Sunshine mixture 3' (Sun Gro horticulture, Canada). The plants were watered daily with 200 ml water per pot and fertilized weekly, starting on week 3, with 150 ml per pot of 3 g l^{-1} of Fervia Fol 20-30-10 NPK (Agroquímicos Rivas, Celaya, GTO, México).

Cultivation of bacteria P. syringae was cultivated at 28 °C on agar plates with KB medium (20 g l^{-1} bactopeptone, 1.5 g $l^{-1}K_2HPO_4$, 15 ml l^{-1} glycerol and 50 mg l^{-1} rifampicine: King et al. 1954), *Ent.* FCB1 was cultivated at the same temperature on agar plates with YDC (yeast dextrose) medium (20 g l^{-1} $CaCO_3$, 10 g l^{-1} yeast extract, 20 g l^{-1} dextrose: Cruz-Izquierdo et al. 2001).

Treatments

In both the field experiment and the greenhouse experiment, plants were treated with BTH (BION®: active component benzo(1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester: CGA-245 704, see Schurter et al. 1987). Plants with 3–5 fully developed secondary leaves were treated by spraying them with a solution of 300 mg l^{-1} of BION in de-ionized water until the entire surface of the plants was wet. Control plants were sprayed with water only. The treatment was conducted between 8:00 and 9:00 AM. In order to avoid plant-plant signalling (Yi et al. 2009), control and BTH-treated plants in the field were placed at a minimum distance of 2.5 m. In the greenhouse, induced and control plants were separated by transparent plastic curtains to reduce airflow among plants that had been subjected to the different treatments.

On day 5 after the BTH application, plants were challenged by spraying a suspension of one of the two pathogens at a density of 1×10^7 cfu ml^{-1} (*P. syringae*: OD_{600nm}=0.065, for *Ent.* FCB1 OD_{600nm}=0.060). Phenotypic disease severity was quantified in the field on day 5 after challenging, by counting the number of necrotic lesions per leaf on 6–9 leaves per individual plant and calculating a mean of these values per plant. In the greenhouse, phenotypic disease severity was determined by counting lesions on leaves 2 and 5 on day 5 after challenging. Infection rate was quantified for greenhouse-grown plants on the same day by determining numbers of colony forming units (CFUs). We collected leaf 2 and 5 of every plant, determined the fresh weight and homogenized both leaves together in 1 ml of distilled water. From the resulting extract we prepared three different dilutions (1:10, 1:100 and 1:1000), of which 20 µl were plated on the same media as used for the cultivation of the bacteria. CFUs of *P. syringae* were counted after 2 d, those of *Ent.* FCB1 after 12 h.

Statistical analysis

Unifactorial analysis of variance (ANOVA) was used individually for each of the two experiments to test for effects of 'plant genotype', 'bacterial species' and 'BTH treatment' on the level of plant disease (quantified as either numbers of lesions or as bacterial titre) and for statistically significant ($P<0.05$) interactions among these factors. Post-hoc tests according to the LSD ('least significant difference') procedure were used to detect significant ($P<0.05$) differences among individual genotypes, whereas the efficiency of resistance induction by BTH was tested individually for every plant genotype with the non-parametric Mann–Whitney U-tests (BTH-treated versus control plants against the same level of significance: $P<0.05$). Correlation analyses were realized using Pearson correlation tests. All statistical analyses were carried out with the software package IBM SPSS 17.0.

Results

Characterisation of the bacterial strains

Initial greenhouse experiments confirmed that the cultivated *Ent.* FCB1 strain caused the same symptoms as originally observed in the field (Fig. 1) and that it could

Fig. 1 Disease symptoms caused by *Enterobacter* sp. strain FCB1 in *P. vulgaris* plants (cultivar 'Negro San Luis'). The photograph was taken on d 5 after challenging the plant

be successfully re-isolated from experimentally infected plants. All following experiments were realized with the cultivated strain. A BLASTn search (http://www.ncbi.nlm.nih.gov) of the 16S sequences placed the *Ent.* FCB1 strain close to *Enterobacter* sp. GTR15 (accession number JW157610.1, max score 2584), *Enterobacter turicensis* strain LMG 23720 (HO992947.1, max score 2582), *Enterobacter* sp. HDDMM06 (EU881982.1, max score 3532) and several uncultured *Enterobacter* strains. A search in Ribosomal Database Project (http://rdp.cme.msu.edu/) classified the sequence with 100 % within the Enterobacteriaceae and resulted in an assignment to the genus *Enterobacter* with 57 % confidence. Both sequences (*P. syringae* and *Enterobacter* strain FCB1) were deposited at GenBank (accession numbers: JQ845901 and JQ845902).

Disease severity caused by *Pseudomonas syringae* in the field

In the field experiment, the 'genotype' of the plant had a highly significant effect ($P<0.001$) on the number of lesions when the bean plants were challenged with *P. syringae* or *Ent.* FCB1 (Table 2). Among control plants challenged with *P. syringae*, the two wild accessions exhibited lower numbers of lesions than the cultivars (mean: 8 lesions per leaf for w1 and 51 lesions for w2, whereas the cultivars exhibited on average 65, 92, 155 and 186 lesions, see Fig. 2a, white bars). After the treatment with BTH, the wild accessions exhibited on average 10 and 12 lesions per leaf, whereas the cultivated accessions developed on average 40, 53, 66 and 106 lesions on each leaf (see Fig. 2a, black bars). Post-hoc tests demonstrated that the basal resistance in wild accession w1 was significantly higher than in three of the four cultivars, but not for the fourth, even though it was close ($P=0.092$, Table 3). Treatment with BTH had an overall significant effect on the numbers of lesions ($P=0.011$, see Table 2). However, individual tests between control and induced plants within the same plant genotype revealed significant effects ($P<0.05$ according to Mann–Whitney U test) only in the wild accession w2 and the landrace 'Negro San Luis' (c4: Fig. 2a). All other genotypes showed lower numbers of lesions after BTH treatment, but the differences between control and treated plants were not statistically significant (Fig. 2a).

Table 2 Factors affecting lesion numbers on leaves and growth responses of common bean cultivated under greenhouse conditions

Effect (*P*)		
Source	Lesion number	Plant growth
Genotype	<0.001	<0.001
Treatment	0.011	<0.001
Bacterial species	0.175	0.537
Genotype * Treatment	0.029	0.001
Genotype * Bacterial sp.	0.160	0.012
Treatment * Bacterial sp.	0.042	0.066
Genotype * Treatment * Bacterial sp.	0.418	0.620

Results of ANOVA for effects of genotype, BTH treatment and bacterial species on lesion numbers on leaves and on growth rate (quantified as newly produced shoots on day 26 after challenging) of common bean plant ($n=5$ plants per combination treatment × genotype × bacterial sp.)

Disease severity caused by *Enterobacter* FCB1 in the field

Resistance assays with *Ent.* FCB1 revealed a different pattern than the *P. syringae* assays. The two wild

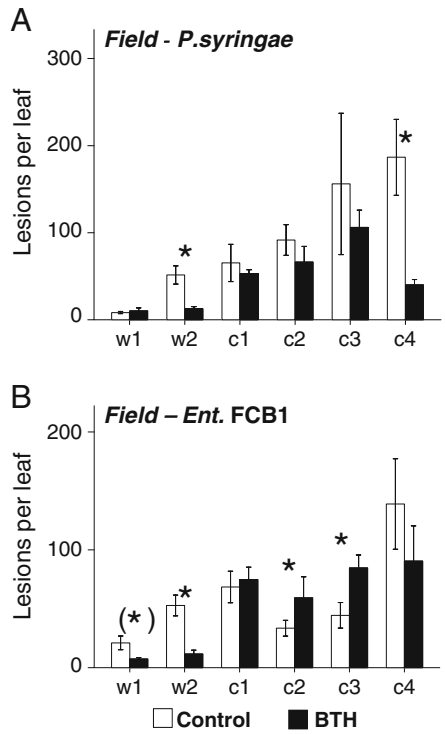

Fig. 2 Phenotypic disease rates of wild and cultivated accessions of common bean in the field. Lesion numbers on field-grown *P. vulgaris* plants (w: wild accessions, c: cultivated accessions) after infection with *Pseudomonas syringae* pv. *syringae* (**a**) or *Enterobacter* sp. strain FCB1 (**b**) were counted separately for control plants (*white bars*) and BTH-treated plants (*black bars*) on day 5 after challenging. Bars indicate means, error bars indicate standard errors. Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Significant differences among control and treated plants of the same cultivar are indicated: * $P<0.05$, (*) $0.05<P<0.10$; P according to Mann–Whitney U test, $n=5$

accessions did not show significantly higher basal resistance to *Ent.* FCB1 than the cultivars, even though they showed the lowest infection levels in the control stage (w1: 18 lesions, w2: 47 lesions; averages for cultivars: 72, 45, 55 and 128; see Fig. 2b, white bars). However, the wild accessions were much less infected than the cultivars when the plants had been subjected to BTH treatment before challenging them with *Ent.* FCB1 (10 and 12 lesions, respectively, for the wild accessions, versus 75, 60, 93 and 94 lesions on average for the cultivars, see Fig. 1b, black bars). Thus, one of the wild accessions exhibited a significant ($P<0.05$) induction of resistance by BTH. By contrast, cultivars c2 and c3 ('Flor de Junio Marcela' and 'Negro Durango') suffered from significantly enhanced disease symptoms in response to BTH treatment, whereas cultivar c1 ('Pinto Villa') showed no significant response to BTH treatment. Again, the level of resistance in accession w1 was significantly different from three of the four cultivars, but not from the fourth, even though it was close ($P=0.061$, Table 4).

Relationship between resistance and plant growth in the field

'Treatment' and 'genotype' had highly significant effects on the number of shoots produced ($P<0.001$) by plants that were treated with BTH and then challenged with pathogens. Furthermore, the 'treatment × genotype'-interaction was highly significant (Table 2), indicating that different accessions respond differently to BTH. Upon disease pressure by either of both pathogens, BTH-treated plants of both wild accessions exhibited a significantly higher number of newly produced shoots than the controls, whereas none of the

Table 3 Differences among six bean genotypes in their resistance to *P. syringae*

	W2	C1	C2	C3	C4
W1	0.436	0.092	0.020	< 0.001	0.001
W2		0.356	0.113	0.001	0.007
C1			0.500	0.017	0.068
C2				0.080	0.242
C3					0.548

The table shows results (P values) of post-hoc tests for 'genotype' realized after an ANOVA of data obtained only for plants infected with *P. syringae* pv. *syringae*

Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Major ANOVA results were $P=0.010$ for 'treatment' and $P<0.001$ for genotype. Degrees of grey illustrate levels of significance: $P>0.10$; $0.05<P<0.010$; $P<0.05$

Table 4 Differences among six bean genotypes in their resistance to *Enterobacter* sp. strain FCB1

	W2	C1	C2	C3	C4
W1	0.288	0.001	0.061	0.004	< 0.001
W2		0.023	0.401	0.060	< 0.001
C1			0.139	0.674	0.013
C2				0.285	< 0.001
C3					0.004

The table shows results (*P* values) of post-hoc tests for 'genotype' realized after an ANOVA of data obtained only for plants infected with *Enterobacter* sp. strain FCB1

Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Major ANOVA results were $P=0.600$ for 'treatment' and $P<0.001$ for genotype. See legend to Table 3 for grey scales

cultivars benefited from BTH treatment under pressure by either of the two pathogens (Fig. 3). Post-hoc tests revealed that almost all accessions differed from each other in their growth response to BTH under disease pressure (data not shown). After challenge with *P. syringae*, 11 out of 17 possible comparisons revealed differences that were significant ($P<0.05$). The same pairs of accessions showed significantly different growth rates after challenge with *Ent.* FCB1. Thus, in general, every different bean genotypes exhibited a specific behaviour in terms of its growth response to BTH treatment under pathogen pressure.

Greenhouse trials

In general, the greenhouse experiment confirmed the behaviour of the different bean genotypes under field conditions, although the absolute levels of disease severity were in part different (Figs. 4 and 5, Table 5). For both pathogens tested, lesion number (Fig. 4) was a good predictor of bacterial titres as determined by counting CFUs (Fig. 5). As observed in the field, the wild accessions exhibited higher basal resistance to *P. syringae* than the cultivated lines. For example, control plants of the two wild accessions developed on average 2 and 10 lesions per leaf after challenge with *P. syringae*, whereas the cultivated accessions had on average 122, 230, 232 and 108 lesions (Fig. 4a). Treatment with BTH before challenging resulted in averages of 2 and 4 lesions on leaves of the wild accessions and 43, 223, 65 and 4 lesions per leaf for the cultivated genotypes (Fig. 4a). Thus, wild accession w1 exhibited the highest levels of basal resistance, which barely could be enhanced by BTH-treatment, even though it was close for the number of CFUs of *Ent.* FCB1 ($P=0.058$, Fig. 5b). By contrast,

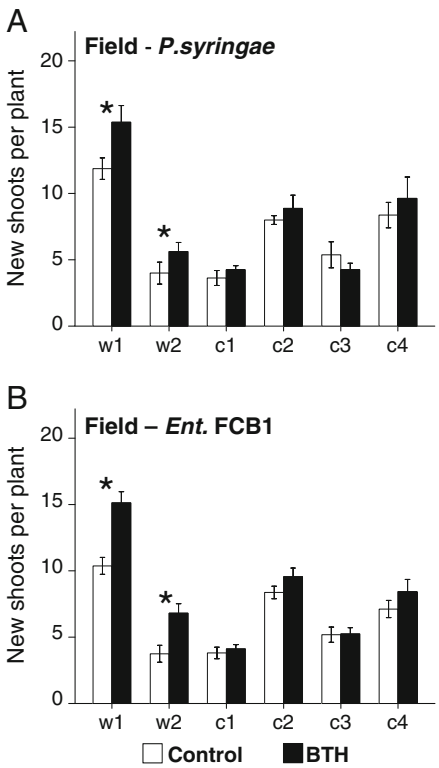

Fig. 3 Effects of resistance induction on the production of new shoots by wild and cultivated accessions of common bean under disease pressure in the greenhouse. Shoots that were newly produced by *P. vulgaris* plants (w: wild accessions, c: cultivated accessions) after infection with *Pseudomonas syringae* pv. *syringae* (**a**) or *Enterobacter* sp. strain FCB1 (**b**) were counted separately for control plants (*white bars*) and BTH-treated plants (*black bars*) on day 26 after challenging all plants with bacteria. Bars indicate means, error bars indicate standard errors. Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Significant differences among control and treated plants of the same cultivar are indicated: * $P<0.05$ according to Mann–Whitney U test, $n=8$

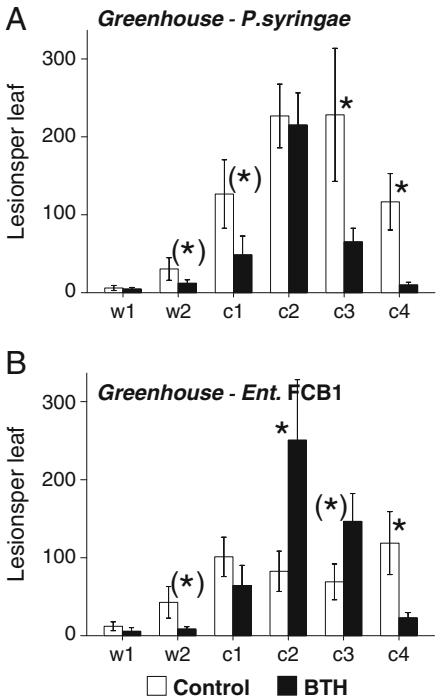

Fig. 4 Phenotypic disease rates of wild and cultivated accessions of common bean in the greenhouse. Lesion numbers on greenhouse-grown *P. vulgaris* plants (w: wild accessions, c: cultivated accessions) after infection with *Pseudomonas syringae* pv. *syringae* (**a**) or *Enterobacter* sp. strain FCB1 (**b**) were counted separately for control plants (*white bars*) and BTH-treated plants (*black bars*) on day 5 after challenging. Bars indicate means, error bars indicate standard errors. Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Significant differences among control and treated plants are indicated: * $P<0.05$, (*) $0.05<P<0.10$; P according to Mann–Whitney U test, $n=10$

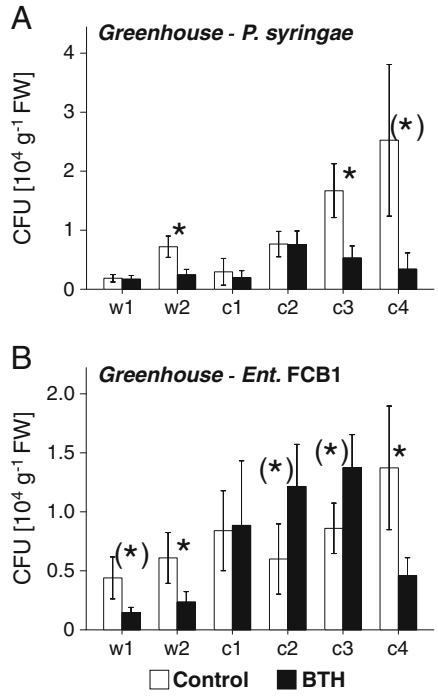

Fig. 5 Infection rates of wild and cultivated accessions of common bean in the greenhouse. Bacterial titres (CFU) in leaves of greenhouse-cultivated *P. vulgaris* plants (w: wild accessions, c: cultivated accessions) after infection with *Pseudomonas syringae* pv. *syringae* (**a**) or *Enterobacter* sp. strain FCB1 (**b**) were determined separately for control plants (*white bars*) and BTH-treated plants (*black bars*) on day 5 after challenging. Bars indicate means, error bars indicate standard errors. Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'. Significant differences among control and treated plants are indicated: * $P<0.05$, (*) $0.05<P<0.10$; P according to Mann–Whitney U test, $n=10$

accession 2 exhibited pronounced levels of BTH-induced resistance against both pathogens. As observed in the field, cultivars 2 and 3 ('Flor de Junio Marcela' and 'Negro Durango') exhibited induced susceptibility to *Ent.* FCB1 after BTH treatment, whereas the landrace (c4: 'Negro San Luis') was the only cultivated genotype that exhibited BTH-induced resistance to both pathogens. In the overall analysis of variance, plant 'genotype' had a significant effect on both lesion numbers and bacterial titres (Table 5). Furthermore, the 'genotype × treatment'-interaction was significant for both disease parameters, confirming that the bean genotypes respond differently to BTH. 'Treatment' had a significant effect on the bacterial titres, whereas it did not affect the lesion numbers significantly, even though it was close ($P=0.063$,Table 5). By contrast, the 'treatment × bacterium'-interaction was significant for lesion numbers, but not for bacterial titres, even though it was close ($P=0.054$, Table 5). These differences among the two disease parameters suggest that the different genotypes differ not only in their levels of resistance (defined as the negative effect on bacterial growth rates) but also in their levels of tolerance (defined as the phenotypic disease severity that is caused by a defined bacterial titre).

Discussion

Systemic acquired resistance is generally considered as a broad-spectrum disease resistance and treatment

Table 5 Factors affecting lesion numbers and infection rates in leaves of greenhouse-grown plants

Source	Lesion number	Bacterial titre (CFUs)
Genotype	<0.001	0.001
Treatment	0.063	0.018
Bacterial species	0.313	0.724
Genotype * Treatment	0.008	0.015
Genotype * Bacterial sp.	0.689	0.440
Treatment * Bacterial sp.	0.006	0.054
Genotype * Treatment * Bacterial sp.	0.026	0.409

Results of ANOVA for effects of genotype, BTH treatment and bacterial species on lesion numbers and bacterial titres in leaves of greenhouse-grown common bean (n=10 plants per combination treatment × genotype × bacterial sp.)

with benzothiadiazole is believed to cause a very similar phenotype: resistance against a wide range of biotrophic pathogens. BTH at a concentration as applied in our study has no direct effect on the development of bacteria (Oostendorp et al. 2001; Schurter et al. 1987; and our own observations). However, the responses of different accessions of the same plant species (*Phaseolus vulgaris*) to two biotrophic bacterial pathogens under study differed, both among untreated control plants and when plants were treated with BTH before being challenged. All plants were challenged on the same day by using the same bacterial suspension and therefore, we can reasonably assume that all plants had been exposed to the same pathogen pressure. Different disease severities observed among the control plants therefore represent differences in the basal resistance of the plants, which can be attributed to morphological, anatomical and constitutively expressed molecular traits as well as molecular traits that are rapidly induced after pathogen challenge (Ahmad et al. 2010; Jones and Dangl 2006). By contrast, all effects seen after BTH treatment can be attributed to inducible changes in the resistance phenotype of the plant depending on plant gene expression or metabolic processes.

As predicted, the wild accessions were generally characterised by a higher basal resistance than the cultivated genotypes. Interestingly, BTH-treated plants of both wild accessions exhibited high levels of resistance to both *Pseudomonas syringae* pv. *syringae* and *Enterobacter* sp. strain FCB1. In fact, BTH-treated plants of the two wild accessions exhibited the lowest infection rates seen in all experiments (black bars in Figs. 2, 4 and 5). The same plants also showed significantly improved growth responses to BTH treatment under disease pressure (Fig. 3). By contrast, among the cultivars, only the landrace ('Negro San Luis') exhibited significant levels of BTH-induced resistance to both pathogens. Whereas the other cultivars ('Pinto Villa', 'Flor de Junio Marcela' and 'Negro Durango') showed slightly lower infection levels by *P. syringae* after BTH treatment (although not significantly so, P>0.10), two of them ('Flor de Junio Marcela' and 'Negro Durango') exhibited BTH-induced susceptibility to infection by *Ent.* FCB1. *Enterobacter* species are commonly reported in environmental samples (Hunter et al. 2010; Schreiner et al. 2009) and can represent important plant-growth promoting or nitrogen-fixing strains (Schreiner et al. 2009). Other *Enterobacter* strains have been reported as saprophytes colonizing lesions caused by other bacteria (Volksch et al. 1992) or as asymptomatic endophytes (Quadt-Hallmann et al. 1997; Seo et al. 2010). By contrast, only four species of *Enterobacter* have been described as plant pathogens so far (Wang et al. 2010), although recent studies continue reporting new cases of truly phytopathogenic *Enterobacter* strains (Wang et al. 2010; Wu et al. 2011; Zhu et al. 2011). The 16S sequence of *Ent.* strain FCB1 showed closest similarities to strains isolated from environmental samples (according to entries in NCBI) but was only distantly related to the described phytopathogens (Wang et al. 2010). Thus, pending future studies we hypothesise that *Ent.* FCB1 represents an environmental bacterium to which the bean cultivars used in our study are particularly susceptible. Induced susceptibility to this or other opportunistic pathogens as discovered in our study is likely to represent an important mechanism that contributes to the low phenotypic resistance levels that were exhibited by most of the high-yield cultivars. The differences that we observed among the genotypes tested were stable under both field and greenhouse conditions and, thus, did not depend on the growing conditions. We conclude that plant genotype and type of pathogen are important factors in deciding whether or not an application of BTH causes an induced resistance phenotype or rather increases the danger that plants become infected by opportunistic environmental bacteria.

In order to examine if there was a correlation between levels of basal and BTH-inducible resistance, we calculated the means for each genotype and for each of the

two disease parameters (numbers of lesions and CFUs) and plotted values of untreated plants against the mean relative change in the disease parameters after BTH treatment. Both bacterial titres and lesion numbers in the control stage were significantly and positively correlated with the effect of BTH treatment for *P. syringae* (Fig. 6a and b), whereas no correlation was found for plants infected by *Ent.* FCB1 (Fig. 6c and d). Interestingly, the correlation turned out to be significant for plants challenged with *Ent.* FCB1 when we considered only the four cultivars ($P_{(les,\ cult)}<0.05$, Fig. 6). Because low disease values indicate a high level of resistance, these positive correlations imply that the basal resistance was negatively correlated with the potential to further improve resistance in response to BTH treatment in most accessions. In fact, the levels of basal resistance in the two wild accessions (in particular to *P. syringae*) were so high that they barely could be further improved by BTH-dependent responses. By contrast, the modern cultivars exhibited a high level of susceptibility before treatment but still did not gain a strongly improved resistance in response to BTH treatment. In summary, the yield-improved bean cultivars tested in our study are characterised by a decreased basal resistance and have also lost their capacity to respond to BTH treatment with a reliable induced resistance to bacterial infections. Only the landrace ('Negro San Luis'), which exhibited high basal susceptibility to both pathogens, was capable of enhancing its resistance level significantly after treatment with BTH under both field and greenhouse conditions.

Resistance to *Ent.* FCB1 in both wild accessions was boosted by BTH, whereas two of the cultivars showed inverse responses to BTH with respect to *Ent.* FCB1 resistance. Similarly, the growth response of BTH-treated plants after pathogen challenge was positive for the wild accessions, whereas no significant effect on this fitness parameter could be detected in any of the cultivars. We conclude that the capacity to develop an induced resistance response has been lost in most of the bean cultivars tested. Any recommendation for the use of BTH as an agent of crop protection will therefore have to depend on prior knowledge about the responsiveness of the plant genotype in question in mounting resistance.

Since this study has been conducted at the phenotypic level, mechanistic interpretations about these phenotypes remain speculative. As to our knowledge, BTH-induced changes in the composition or structure

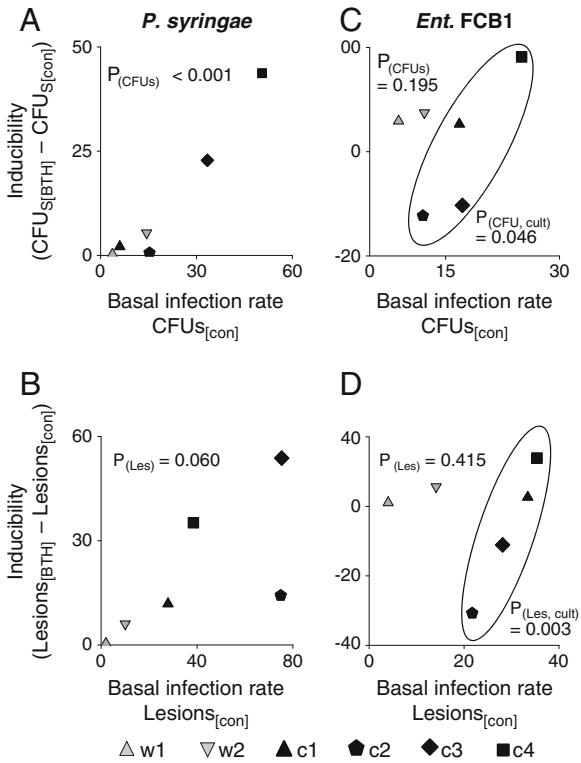

Fig. 6 Relations among basal and induced resistance in the greenhouse. Disease parameters (bacterial titres in the panels **a** and **c**, numbers of lesions in panels **b** and **d**) were averaged for all equally treated 10 plants per accession and means of untreated plants are plotted against the mean inducibility of the resistance (calculated as the mean of the BTH-treated plants minus the mean of the control plants). *P*-values (according to Pearson correlation test) are depicted for a correlation of basal infection rate vs. inducibility for all lines and separately for the cultivars in case of challenge with *Enterobacter* sp. strain FCB1 (right panels: $P_{(les,\ cult)}$). Note that basal infection rates are a negative indicator of basal resistance: positive correlations mean therefore a negative correlation between the basal resistance and the relative inducibility of the resistance. Codes for accessions: w1 and w2 are wild accessions; c1: high-yield cultivar 'Pinto Villa'; c2: high-yield cultivar 'Flor de Junio Marcela'; c3: high-yield cultivar 'Negro Durango', c4: landrace 'Negro San Luis'

of the cuticle or other structural surface elements have not been reported, which makes it likely that all changes in the resistance phenotype that we observed after BTH treatment can be attributed to changes in defence response parameters such as phytoalexins and pathogenesis-related (PR) proteins (Oostendorp et al. 2001). Other studies have pointed to the importance of nutrient supply for an efficient resistance induction by BTH (Heil et al. 2000; Walters et al. 2005). For example, Arabidopsis plants treated with BTH expressed

lower activities of the PR enzymes chitinase and peroxidase when grown under low-nitrogen conditions (Dietrich et al. 2004). SAR depends on the de novo synthesis of multiple compounds, including proteins, and therefore represents a very demanding process in term of resources, particularly nitrogen (Heil et al. 2000; Walters et al. 2005). Shortage in nutrient supply is therefore a possible explanation for a lack of resistance in response to a treatment that usually elicits phenotypic resistance. However, in the present study, all plants were cultivated in pots filled with very similar substrates and had been fertilised equally. The examined cultivars are characterized by higher growth rates than the wild accessions and thus perhaps higher soil nutrient demands. However, we observed no decreased growth rates in response to BTH treatment in any of the genotypes tested (Fig. 2), although such a reduction is commonly observed in response to resistance induction under limiting conditions (Heil et al. 2000; Walters et al. 2005). Nutrient limitation alone is therefore not likely to explain our results. We conclude that the physiological and genetic differences between cultivated and wild accessions are more likely to be the explanation for the lower resistance levels and the lowered capacities to express induced resistance of the cultivars as compared to the plants of the wild accessions.

It may not be surprising that the landrace was the only cultivar in which at least some disease resistance could be induced under all conditions tested. The landrace is characterised by lower yield levels than the other cultivars and showed high susceptibility in the control stage, meaning a high potential for an improvement of its resistance after BTH application. Along the same line, the lack of an induced resistance response in accession w1 was obviously caused by the extremely high level of basal resistance in this genotype, which simply could not be improved any more. For a similar reason, soybean cultivars expressing high basal resistance against *Sclerotinia sclerotiorum* were less responsive to BTH than highly susceptible cultivars (Dann et al. 1998) and barley that had already been induced by natural infection lacked the capacity to respond to BTH-treatment with increased defence responses (Walters et al. 2011). However, it remains to be investigated whether the high phenotypic resistance of w1 is caused by a high basal level of priming (Ahmad et al. 2010) or by a constitutively expressed resistance trait. Thus, the overall differences among cultivated and wild accessions can be explained by the trade-off between increased yields and the costs of basal and induced resistance.

In conclusion, both basal and induced resistance have been affected during the selection for elite cultivars of common bean and the responses of both resistance mechanisms to the breeding process vary among the different genotypes. Two cultivars exhibited induced susceptibility to infection by *Ent*. FCB1, which is likely to be an opportunistic environmental bacterium. This phenomenon might enhance the possibility of the development of new diseases. More research is needed to understand which components/genes in the complex regulation of basal and induced resistance were affected during the domestication process. We also conclude that care must be taken before making generalised recommendations concerning the use of chemical elicitors, such as BTH, in crop protection. Both the degree of resistance achieved and the net costs that result from its induction depend on the abiotic conditions (Cole 1999; Heil et al. 2000; Romero et al. 2001; Stadnik and Buchenauer 1999). Our study provides additional evidence for the notion that yield improvement in cultivated crops is achieved at the expense of biotic stress resistance. In addition, we demonstrate that this trade-off can also affect the plant's capacity to express induced resistance after treatment with chemical plant defence activators. Future breeding strategies should consider the genetic potential of landraces and wild forms.

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