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Efficiency of biological and chemical inducers for controlling Septoria tritici leaf blotch (STB) on wheat (*Triticum aestivum* L.)

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Abstract The hemibiotrophic fungus *Zymoseptoria tritici* is the causative agent of Septoria tritici leaf blotch (STB) disease of wheat (*Triticum aestivum* L.), the economically most damaging disease of wheat in Europe. Today, ecofriendly plant protection methods compatible with sustainable agriculture are strongly desirable. Here, we tested two chemical inducers β-aminobutyric acid (BABA) and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and the two biotic inducers *Pseudomonas protegens* CHA0 (CHA0) and *P. chlororaphis* PCL1391 (PCL) for their ability to induce resistance against STB in wheat seedlings. At 21 days after inoculation, only plants treated with BABA showed a smaller area covered by lesions and less pycnidia compared to the untreated control plants. We evaluated spore germination and fungal development on inoculated wheat leaves at early infection stages using calcofluor white staining. Overall, spores of *Z. tritici* germinated less on plants soil-drenched with BABA and BTH and their hyphal growth was significantly delayed. On the contrary, CHA0 and PCL seed treatments did not affect fungal growth in wheat leaves. In conclusion, BABA efficiently enhanced plant resistance to *Z. tritici*, BTH delayed fungal development at early stages while the two biotic inducers did not influence the resistance against STB disease.

Keywords Induced resistance · β-Aminobutyric acid · Benzothiadiazole · *Pseudomonas protegens* CHA0 · *Pseudomonas chlororaphis* PCL1391

Introduction

The fungus *Zymoseptoria tritici* causes Septoria tritici blotch (STB) on wheat. *Z. tritici* is considered the most damaging wheat pathogen in Europe, mainly because of the conducive climatic conditions (Jørgensen et al. 2014). During severe epidemics, up to 50% of losses have been registered in a field planted with wheat cultivars susceptible to STB (Fones and Gurr 2015). Currently, no genetic resistance source in wheat cultivars is known to confer full resistance against STB. For control, farmers rely on the use of cultivars with partial resistance and on conventional fungicides (Schaad et al. 2019; Torriani et al. 2015). Yet, the fungus displays high capacity of adaptation by developing fungicide resistance and by overcoming plant resistances (Cowger et al. 2000; Cheval et al. 2017). As an alternative to chemical fungicides that may have a negative impact on the environment, on human and animal health (Aktar et al. 2009; Berny 2007), we decided to evaluate

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a more sustainable approach to combat this disease in the form of induced resistance.

Plant-growth promoting rhizobacteria (PGPR) can improve plant nutrition and/or help plants overcome biotic or abiotic stresses (Chaudhary and Shukla 2019; Vurukonda et al. 2016; Dimkpa et al. 2009). These studies mainly involved the genus *Pseudomonas* commonly found among the predominant genera present in the rhizosphere and also in the root system of wheat plants (Weller et al. 2007). Many of these Pseudomonads are well-characterized PGPR and are able to exert plant-beneficial functions, including the suppression of plant diseases and stimulation of plant defences (Vacheron et al. 2013; Mauchline and Malone 2017). A subgroup including the species *P. protegens* and *P. chlororaphis* has been widely studied (Haas and Défago 2005; Mercado-Blanco and Bakker 2007; Hol et al. 2013; Vacheron et al. 2013). The strain *P. protegens* CHA0 (CHA0) is naturally suppressive to black root rot in tobacco (Stutz et al. 1986). It has been reported as a potential bacterial antagonist to control plant diseases (Hase et al. 2000; Ramette et al. 2011), and also for its capacity to induce resistance in dicotyledonous plants (Maurhofer et al. 1994; Iavicoli et al. 2003), as well as in monocots (Henkes et al. 2011; Sari et al. 2008). The strain *P. chlororaphis* PCL1391 (PCL), isolated from the rhizosphere of tomato, shows a high antagonistic activity against *Fusarium oxysporum*, the causal agent of tomato root rot (Chin-A-Woeng et al. 1998). The capacity of PCL to protect plants against different other attackers has also been documented (Imperiali et al. 2017; Bardas et al. 2009; Flury et al. 2017).

Specific chemicals also have the capacity to enhance plant disease resistance. For instance, synthetic compounds such as benzo[1,2,3]thiadiazole-7-carbothionic acid-*S*-methyl ester (BTH, also called acibenzolar-S-methyl) and β-aminobutyric acid (BABA) have been reported to induce resistance in plants against a wide range of microbial pathogens without possessing direct antimicrobial activity (Görlach et al. 1996; Jakab et al. 2001; Karthikeyan and Gnanamanickam 2011). The plant defense activator BTH is a functional analogue of salicylic acid and was one of the first chemical compounds shown to enhance activation of several defence responses against major fungal and bacterial pathogens in various crops including wheat (Iriti et al. 2004; Karthikeyan and Gnanamanickam 2011; Soleimani and Kirk 2012; Görlach et al. 1996; Vallad and Goodman 2004). Previous studies have shown that BTH enhances plant resistance to fungal pathogens by activating the systemic acquired resistance signal transduction pathway (Benhamou and Bélanger 1998; Liu et al. 2005; Azami-Sardooei et al. 2013; Abdel-Monaim et al. 2011). In wheat, BTH can induce resistance to powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*) and Septoria leaf spot. The increase in resistance is accompanied by the induction of a number of wheat chemically induced (WCI) genes (Görlach et al. 1996). However, BTH failed to provide resistance to Fusarium head blight in wheat (Yu and Muehlbauer 2001). BABA induces resistance in a wide range of economically important crop species and against a broad spectrum of pathogens including nematodes, virus, bacteria, oomycetes and fungi (Jakab et al. 2001; Barilli et al. 2010; Porat et al. 2003; Amzalek and Cohen 2007). Expression of BABA-induced resistance coincides with a faster and stronger defense response following pathogen attack, a phenomenon that has been termed priming (Cohen et al. 2016; Balmer et al. 2015). Even though BABA, BTH, CHA0 and PCL have been tested in many different pathosystems, to our knowledge, they have never been tested in wheat against STB.

Z. tritici is a filamentous fungal pathogen having the particularity of being hemibiotrophic, with two distinct phases of infection (Ponomarenko et al. 2011). Once the leaf stays moist for at least 20 h after the contact, spores germinate by forming germ tubes that enter inside the leaf through the stomata 24–48 h after the infection (Kema et al. 1996; Ponomarenko et al. 2011). During this biotrophic phase that lasts until day 9–11, no visual symptoms are present on leaves (Kema et al. 1996; Shetty et al. 2003). Rudd (2015) suggests that during the first phase, the pathogen may secrete effectors preventing its recognition and thus suppressing host defences. Meanwhile, the mycelium grows extracellularly within the mesophyll, extracting nutrients from the apoplast (Shetty et al. 2003; Keon et al. 2007; O'Driscoll et al. 2014. After 12–20 days, the pathogen switches to the necrotrophic phase, during which mesophyllous cells die (Palmer and Skinner 2002; Shetty et al. 2003) and the first chlorotic and nectrotic spots appear. Furtheron, the lesions become brown and develop darker coloured fruiting bodies (pycnidia) (Ponomarenko et al. 2011).

In this study we aimed to assess the efficacy of the two chemical inducers (BABA and BTH) and the two biological inducers (CHA0 and PCL) as a preventative

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treatment on wheat seedlings against *Z. tritici*. The effect of the plant resistance inducers on fungal development was investigated at an early stage of infection. To exclude any direct inhibitory effect, we also assessed the antifungal activity of the chemical inducers on *Z. tritici* growth by in vitro assays.

Materials and methods

Plant material and growth conditions

Experiments were carried out with the STB susceptible wheat variety Spluga (Agroscope/DSP). Prior to seeding, seeds were surface sterilized by rinsing with 70% ethanol and incubating for 5 min in 5% bleach (sodium hypochlorite solution, Fisher Chemical, U.K.). Subsequently, the seeds were rinsed three times in sterile distilled water. The seeds were then pre-germinated for 3–4 days on humid filter paper (Filterkrepp Papier braun, E. Weber & Cie AG, 8157 Dielsdorf, Switzerland). We selected the seedlings with similar growth state and morphology to plant in 120 mL polypropylene tubes (Semadeni, 3072 Ostermundigen, Switzerland) filled with a standard potting mixture (peat:sand, 3:1, vol:vol). The plants grew in a growth chamber with the following conditions: 8 h night at 18 °C 16 h day at 22 °C, and an irradiance of 300 μmol m^{-2} s^{-1}. The plants were watered as needed.

Treatment with biological inducers

The biological inducers used in the following trials were the rifampicin-resistant mutants CHA0-RIF (Natsch et al. 1994), and PCL-RIF of strain (Chin-A-Woeng et al. 1998). The strains were routinely grown on solid King's Medium B (KMB; *Pseudomonas* agar F, Merck KGaA, 64,271 Darmstadt, Germany) supplemented with rifampicin 100 μg/mL at 24 °C for 4 days. From this culture, a single colony was transferred to 100 mL of King's liquid medium B (3 g protease-peptone, 1.5 g K_2HPO_4, 2.46 g $MgSO_4$, 1.5 g glycerol in 1 L distilled water) supplemented with 50 μg mL^{-1} rifampicin and incubated overnight at 28 °C with continuous shaking at 150 rpm. The resulting bacterial culture was centrifuged at 3700 rpm and washed twice with sterile 10 mM $MgSO_4$ solution. The final pellets were re-suspended in sterile distilled water and adjusted to 10^6 cfu/mL (OD_{600} of 0.1) and used for seed inoculation. To this end, the wheat seeds were immerged into the bacterial suspension for 6 h with shaking at 35–40 rpm at room temperature. Control seeds were soaked in distilled water for the same duration. Inoculated seeds underwent the pre-germination procedure as described above.

Chemical inducer treatment

BTH formulated as BION® 50 WG (50% active ingredient) was obtained from Syngenta (Basel, Switzerland) and the racemic mixture of BABA from Sigma-Aldrich (Buchs, Switzerland). BTH (2 mM) and BABA (15 mM), respectively, were dissolved in distilled water and 10 mL per growing tube were used as soil-drench 2 days before *Z. tritici* inoculation. Control plants were just watered with distilled water. The concentration of BTH used in this study was chosen according to Görlach et al. (1996). While, BABA at 15 mM was chosen as ideal concentration to induce resistance against leaf rust without any effect on plant growth (unpublished data).

Fungal cultures and plant inoculation

Z. tritici isolate 3D7 (Zhan et al. 2002) was provided by Prof. Daniel Croll (University of Neuchâtel, Switzerland). The isolate was stored at −80 °C in 50% glycerol. Stock cultures were cultivated on Yeast-Sucrose Agar (10 g L^{-1} yeast extract, 10 g L^{-1} sucrose, 1.2% agar) supplemented with kanamycin (50 μg/mL). For inoculum preparation, the strain was cultured in Yeast-Sucrose Broth (YSB) amended with 50 μg/mL kanamycin and incubated for 8 days at 18 °C under continuous shaking at 150 rpm. After incubation, the suspension was filtered through a sterile cheese cloth and rinsed with sterile distilled water. Prior to infection, the spore concentration was adjusted to 10^5 spores/mL in distilled water using a haemocytometer. After adding 0.1% tween 20 to the spore suspension at the 3-leaf stage, each plant was spray-inoculated until run-off. The plants were then maintained at 100% relative humidity for 48 h. After this, the plants were placed in a growth chamber as described above.

Infection quantification

At 21 days after inoculation (dai), symptoms on wheat plants were quantified as described by Stewart et al. (2016). Briefly, the third leaf of each plant was excised,

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fixed on a sheet of paper and immediately scanned at 1.200 dpi (Epson perfection, V370 PHOTO). The leaf surface covered with pycnidia, lesions or leaf necrosis was measured using an automated image analyses macro for the software ImageJ version 1.x (Schneider et al. 2012). The disease severity was the expressed as percentage of leaf area covered by lesions (PLACL).

In planta fungal growth

Monitoring of spore germination and hyphal growth of *Z. tritici* on the leaf surface was performed as described by Mejri et al. (2018) using Calcofluor White (Sigma-Aldrich, Germany) staining according to Siah et al. (2010). Briefly, third leaf segments (4 cm) from three randomly selected replicates of each treatment were harvested at 24, 48 and 120 h after inoculation (hai) and immersed for 5 min in 0.1% (*w/v*) Calcofluor White solution prepared in 0.1 M Tris-HCl buffer pH 8.5. After washing with sterile distilled water, the leaf segments were dried in darkness at room temperature. After covering with a cover slip, the preparations were examined under the epifluorescence microscope (Model E800; Nikon Instruments Europe, Badhoevedorp, The Netherlands) using excitation at 365 nm in combination with a 450 nm barrier filter and a dichroic mirror at 400 nm.

In vitro antifungal assay

A potential antimicrobial effect of BTH and BABA on growth of *Z. tritici* was spectrophotometrically assessed in liquid YSB supplemented with kanamycin 50 μg/mL. Since BION contains additional ingredients that can influence the absorbance measurement, the active molecule Acibenzolar-S-methyl (Sigma-Aldrich, Germany) was used. BABA and BTH were first dissolved in distilled water and filter-sterilized with a 0.22-μm syringe filter (Millex GP, Millipore). Concentrations of 0, 0.02, 0.2 and 2 mM of BTH and 0, 0.15, 1.5 and 15 mM of BABA were tested. Aliquots of 40 mL culture medium were inoculated with 50 μL of fresh fungal spore suspension (10^5 spores/mL) and placed at 18 °C in the dark under continuous shaking at 150 rpm. Fungal growth was assessed daily by measuring the optical density at 405 nm.

Statistical analyses

All experiments were repeated twice in time. Data were collected and stored in spreadsheets (Microsoft® Excel 2013, Redmond USA). Statistical analysis was conducted in R (R Core Team 2018). In all trials, significant differences were considered at $p < 0.05$.

Infection quantification, was carried out in eight biological replicates and disease severity was scored as the percentage of leaf area covered by lesions (PLACL) and the density of pycnidia. Differences of PLACL in response to the treatments was analysed with the Chi-squared test. Data of pycnidia density were analysed by a Student's *t* test in comparison to the control.

The germination of conidia *in planta* was observed in at least 50 spores on three independent replicates for each treatment. Pairwise comparisons within each class between the treatments were carried out using Wilcoxon signed rank test.

For the growth inhibition assay, the area under the growth curve was calculated for each BABA and BTH concentration in three independent replicates. Significant difference in response to dose-treatment were analysed by a Student's *t* test in comparison to the control (0 mM BABA or BTH) using the R-package "Growthcurver" (Sprouffske and Wagner 2016).

Results

Disease severity evaluation after treatment with resistance inducers

Symptoms on leaves were assessed on the third leaf, at 21 days after infection (Fig. 1a). Infected leaf tissue initially became chlorotic and later turned necrotic. In the untreated control, in the bacteria-treated plants and in the BTH treatment a large proportion of the leaves was necrotic and only a small part was alive (green). On the contrary, plants treated with 15 mM BABA presented less symptoms compared to the untreated control and leaves were green. The extension of the lesions (PLACL) was significantly lower in plants treated with BABA in comparison with the untreated control and the other pre-treatments (Fig. 1b). Similarly, the density of pycnidia was significantly reduced in BABA-treated leaves but not in the other treatments (Table 1).

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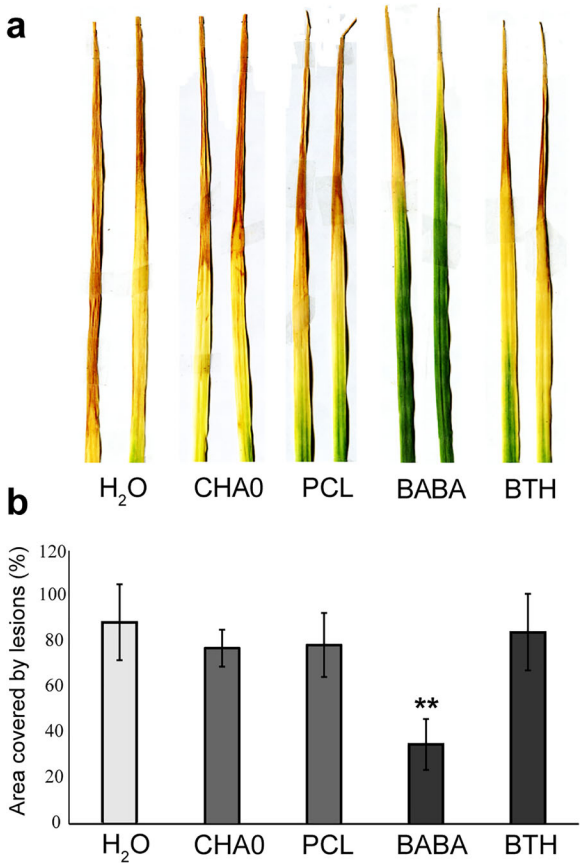

Fig. 1 Response of wheat seedlings cv. Spluga to infection with *Z. tritici* after pre-treatment with H_2O (control), CHA0, PCL, BABA and BTH, respectively. Symptoms were observed at 21 dai (**a**) and percentage of total leaf area covered by lesions (**b**) was obtained from scanned images analysed with an ImageJ macro. Error bars indicate the standard error for the average values of 8 replicates. Asterisks indicate significant differences in area under curves in response to treatment determined by Chi-squared test: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

Early effect of plant resistance inducers on *in planta* spore development

Spore germination and hyphal growth of *Z. tritici* on the leaf surface of wheat plants (cv. Spluga) and growth structures were determined at 24, 48 and 120 hai. To quantify the effect of resistance inducers during this observation period, four distinct developmental classes have been defined (Fig. 2a): class 1, spore non-germinated; class 2, geminated spore with a short germ tube; class 3, geminated spore with a well-developed germ tube; class 4, germinated spore with branched hyphae. Figure 2b shows the percentage of these classes in each treatment. At 24 hai, fungal development displayed similar proportions of class 1 and class 2. Only in the control and in the CHA0 treatment a small proportion of class 3 structures (about 3%) were present. At 48 hai, the control presented about 45% of class 1, 42% of class 2 and about 13% of class 3 structures. The proportions in the bacterial treatments were similar to the control and statistically not different. With chemical resistance induction, significantly more spores were in class 1 and 2 compared to the control. While the proportions were statistically not different between BTH treatment and the bacterial treatments, the BABA treatment presented a significantly higher number of class 1 structures compared to the bacterial CHA0 treatment.

Table 1 Pycnidia density per leaf (pycnidia/cm^2) in H_2O-treated control plants and CHA0-, PCL-, BABA- or BTH-treated plants. Pycnidia density was assessed on the third leaf of each biological replicate ($n = 8$)

Treatment	Pycnidia/cm^2
H_2O	97.79 ± 39.78 [a]
CHA0	109.94 ± 25.04[a]
PCL	112.39 ± 13.76[a]
BABA	14.29 ± 19.81[b]
BTH	88.48 ± 36.29[ab]

Values with the same letter are significantly not different at $P < 0.05$.

At 120 hai, a small proportion of hyphae with branching (class 4) was present in the control, in both bacterial treatments and in the BTH treatment but not in the BABA-treated plants. The BABA-treated group displayed also differences in other parameters. About 70% of spores were in growth class 1. This was by 25% higher than in the BTH-and CHA0-treated plants. The number of spores in class 1 was not different in the CHA0, PCL, BTH and the control treatment. For class 2, the number of spores that produced a small germ tube did not differ between all treatments. Yet, the number of spores with a well-developed germ tube (class 3) was not different between the bacterial treatments and the control and between the bacterial treatments and the BTH-treated plants. The proportion of class 3 spore3s was very small in BABA-treated and did not differ between the BABA-and the BTH-treated plants. However, the proportion was significantly different between BABA / BTH and the control.

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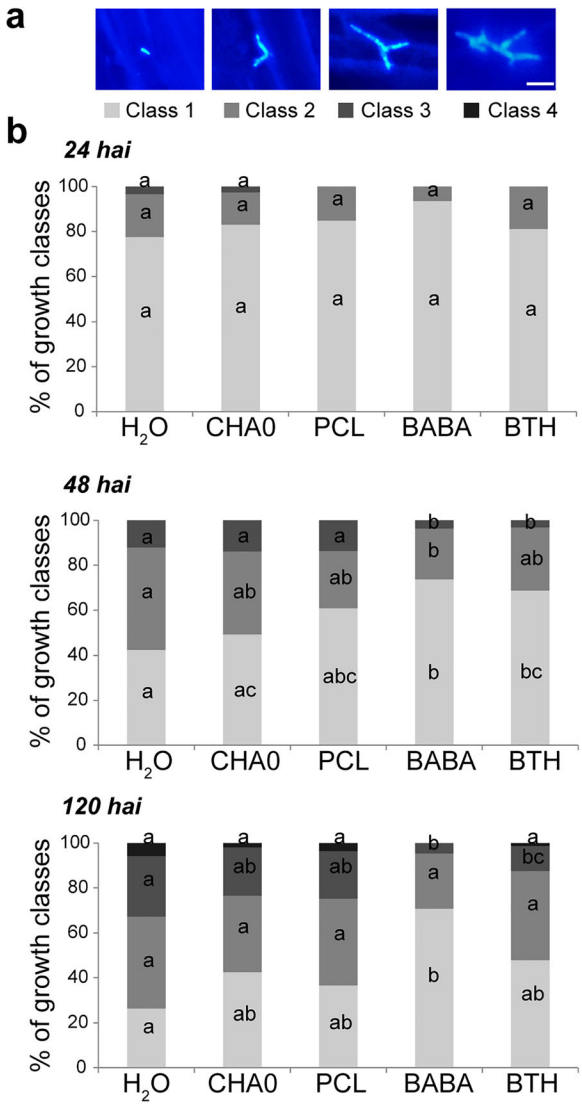

Fig. 2 Effect of different plant resistance inducers on spore germination and hyphal growth of *Z. tritici* on leaves of wheat cv. Spluga. Four types of fungal developmental classes were defined (**a**): class 1, spore non-germinated; class 2, geminated spore with a short germ tube; class 3, geminated spore with a well-developed germ tube; class 4, germinated spore with branched hyphae. Scale bar = 10 μm. On 3 biological replicates, 50 spores were observed. The percentage of each class was assessed in each treatment at 24, 48 and 120 hai (**b**). Bars with the same letter are significantly not different at $p < 0.05$ according to Wilcoxon signed rank test

In vitro antifungal activity of BABA and BTH on *Z. tritici* growth

In order to test whether BABA or BTH have any direct inhibitory effect on fungal growth, *Z. tritici* was grown in YSB liquid medium amended with the two inducers (Fig. 3). No antifungal activity was observed for all tested BTH concentrations. At the highest concentration (BTH 2 mM), *Z. tritici* growth was slightly inhibited (Fig. 3a) but no significant differences were observed. When BABA was added to the medium only the highest tested concentration (15 mM) led to a significant delay in fungal growth compared to the control without BABA (Fig. 3b).

Discussion

Plant resistance inducers are a promising alternative to control fungal disease (Wang and Zhou 2018; Chaudhary and Shukla 2019). Here, we report on the possibility to reduce the severity of Septoria leaf blotch, caused by *Z. tritici* with BABA as a preventive treatment in wheat.

General leaf symptoms of Septoria leaf blotch, such as chlorosis and necrosis, were assessed during 21 dai. Severe symptoms were observed in the untreated control as well as in BTH-, PCL- and CHA0-treated plants. Only BABA-treated plants showed a significantly lower percentage of leaf area covered by lesions (PLACL) and a significantly lower number of pycnidia.

To understand the response to infection with *Z. tritici* in wheat treated with resistance inducers, at the early stage, the fungal development was tracked by microscopy observations at 24, 48 and 120 h post-infection. As expected, in BABA-treated plants, pathogen growth was significantly delayed. Therefore, BABA as a well-known priming agent, presumably activated a fast and robust response to fungal attack in the host plants (Zimmerli et al. 2000; Ton and Mauch-Mani 2004; van Hulten et al. 2006).

Exogenous application of BABA can inhibit development of disease directly by antimicrobial effect or indirectly via BABA-IR (Cohen et al. 2016). Since BABA is highly systemic, readily taken up by roots and transported to the leaves (Cohen and Gisi 1994), it was not possible to conclude whether the observed resistance was direct or not. A potential direct fungicidal action by BABA on the growth of *Z. tritici* could be excluded in the in vitro growth assay. Only a high concentration of BABA (15 mM) reduced fungal growth. Similar results showing that a high concentration of BABA exhibited a toxic effect on pathogen in vitro growth have been reported. Porat et al. (2003) observed that a very high concentration of BABA

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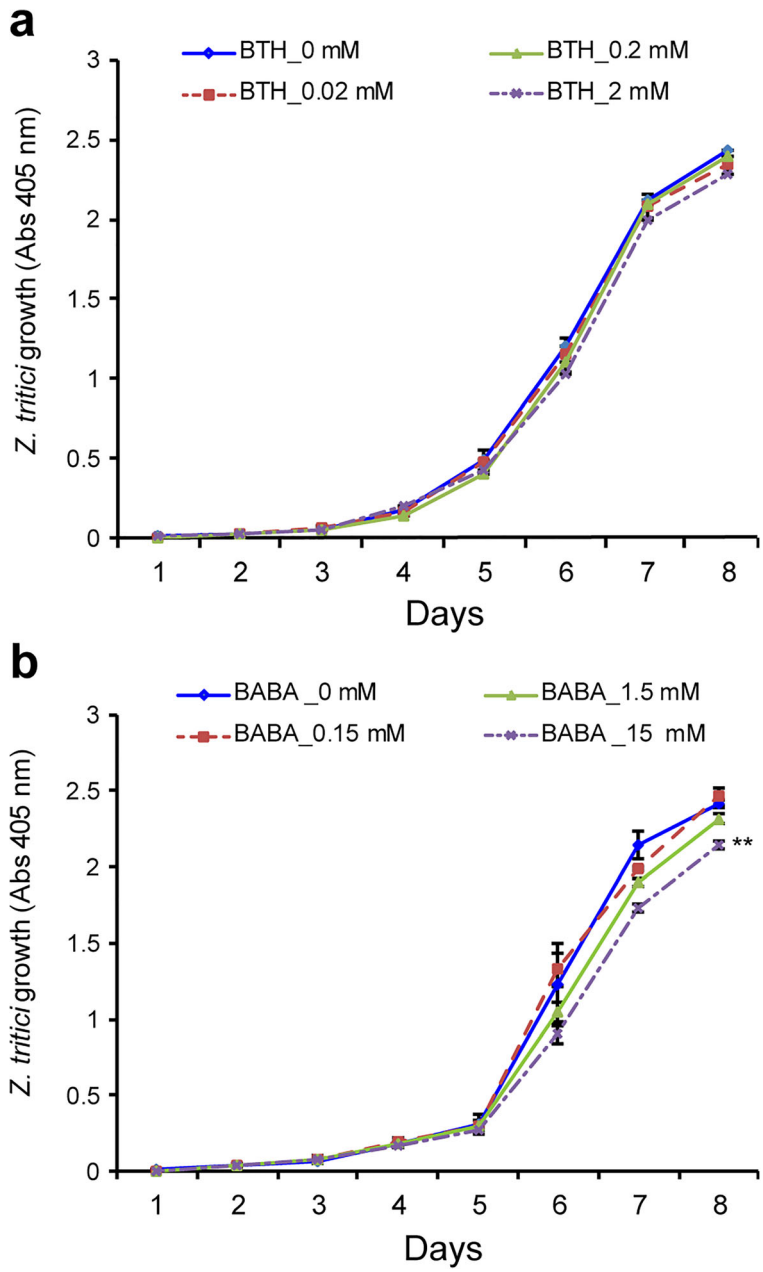

Fig. 3 In vitro dose–response curves of *Z. tritici* to BABA (**a**) and BTH (**b**). Fungal growth was spectrophotometrically measured at 405 nm during 8 days in YSB amended with BABA 0.15, 1.5 and 15 mM or BTH 0.02, 0.2 and 2 mM. Error bars indicate the standard error for the average values of 3 replicates. Asterisks indicate significant differences in area under curves in response to dose-treatment determined by Student's *t* test: *p < 0.05; **$p < 0.01$; ***$p < 0.001$

(100 mM) completely inhibited spore germination and mycelial growth of *Penicillium digitatum*. Similarly, the addition of BABA (50 to 200 mM) to the suspension culture of *Penicillium italicum* inhibited spore germination and germ tube elongation in vitro (Torriani et al. 2015). In another study, Fischer et al. (2009) showed that BABA inhibited mycelial growth and germination of *Botrytis cinerea* in a concentration-dependent manner, suggesting that direct antifungal effects of BABA may be associated with its concentration. In our study, low concentrations of BABA (0.15 and 1.5 mM) did not limit *Z. tritici* growth. It is important to note that the concentration of BABA inside wheat leaves at 2 and 6 days post application of 15 mM BABA to the roots were 15 and 5 μM respectively (Table S1), this is far below the in vitro inhibition concentration. Therefore, for our *in planta* assays, we postulate that BABA primes resistance mechanisms of the plant that inhibit the germination of *Z. tritici* in the wheat leaves.

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As mentioned before, at 21 dai, BABA-treated plants showed less PLCAL and a lower number of pycnidia. This could be explained by results observed in the fungal growth assessment, where BABA treatment significantly limited *Z. tritici* growth. During the transition to the necrotrophic phase, the fungus releases cell wall-degrading enzymes such as β-1,4-endoxylanase, which have been shown to be correlated with symptom and sporulation levels of *Z. tritici* (Siah et al. 2010; Douaiher et al. 2007). Hence, the limitation in fungal growth may decrease the production of cell wall-degrading enzymes resulting in less PLCAL and a lower number of pycnidia.

In the initial infection phase (48 hai), BTH limited fungal development. However, the effect of BTH did not persist during the whole infection process. One hundred and twenty hours after inoculation, hyphal development on BTH-treated plants hardly differed from the non-treated controls. In addition, 21 dai, symptoms on plant leaves were similar to untreated plants. This suggests that BTH did not enhance resistance against Z. *tritici* in wheat seedlings. We suppose that the delay of spore germination observed during the initial infection phase may be due to an indirect effect since none of the tested concentrations of BTH delayed or inhibited *Z. tritici* growth in vitro. Recently, Mejri et al. (2019) studied the protection efficacy of several salicylic acid conjugated derivatives on wheat against *Z. tritici*, and observed no correlation between direct fungicidal activity in vitro and protection of wheat plant.

Neither CHA0 nor PCL induced plant resistance to *Z. tritici* infection in wheat. Seed treatment by both rhizobacteria did not affect spore germination and hyphal growth in the early infection phase of *Z. tritici*. Moreover, symptoms on bacteria-treated plants were the same as in control plants. Following seed inoculation, both bacteria colonized the wheat roots to more than 10^5 CFU/g of root fresh weight (Table S2). This degree of colonization provided effective plant protection in soils suppressive to take-all of wheat and barley caused by *Gaeumannomyces graminis* var. *tritici* (Weller et al. 2007), Fusarium wilt of pea mediated by *Fusarium oxysporum* f. sp. *pisi* (Landa et al. 2002) and black root rot of tobacco (Stutz et al. 1986). Previous work conducted in our laboratory showed the effectiveness of seed treatment with CHA0 to induce resistance against leaf rust caused by *Puccinia triticina* (unpublished). Also, in other studies, *P. fluorescens* species, including CHA0 and PCL, were reported to be efficient suppressive agents of fungal pathogens by inducing systemic resistance (Defago et al. 1990; Tziros et al. 2007; Bardas et al. 2009). The control of *Z. tritici* by beneficial *P. fluorescens* was attributed to a direct inhibition in situ of the fungus by hydrogen cyanide and antimicrobial compounds (Flaishman et al. 1996; Levy et al. 1992). In this study, inoculation of the STB-susceptible wheat cv. Spluga was performed using *Z. tritici* isolate 3D7, which was collected in a Swiss wheat field in 1999 and was found to be highly aggressive on several wheat cultivars (Zhan et al. 2002; Zhan et al. 2005). This high virulence might be the reason for the observed lack of resistance induction by the tested bacteria.

The present study shows that BABA applied as a soil-drench was effective in protecting wheat seedlings from *Z. tritici* infection, whereas in plants soil-drenched with BTH, fungal growth was only delayed during the early germination phase. In this case, foliar application may be more effective, since BTH displayed a direct antifungal activity already at very low concentrations. Unexpectedly, wheat seed treatment with CHA0 or PCL did not enhance resistance to STB disease in wheat. Recently, Imperiali et al. (2017) demonstrated the possibility to combine CHA0 and PCL without affecting their capacity to colonize wheat roots. Hence, a combination of these two strains could result in a synergistic effect that may help to control STB disease, as was reported in other case of biological control in wheat (El-Sharkawy et al. 2018; Pierson and Weller 1994). Our results suggest to the possibility of developing effective protective measures against *Z. tritici* infection of wheat based on chemical inducer application. However, a histochemical analysis of plant reactions during the infection process should be performed. This will provide a better understanding of the defence mechanism involved in resistance and providing a conscious choice between the disease resistance inducers.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

Refrences

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