Eur J Plant Pathol (2013) 136:261–271
DOI 10.1007/s10658-012-0160-9

Sugar beet extract induces defence against *Phytophthora infestans* in potato plants

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Accepted: 13 December 2012 / Published online: 11 January 2013

Abstract The aim of this study was to find a natural and cheap agent that could induce defence responses in potato plants to combat *Phytophthora infestans*, which causes late blight disease that is one of the most devastating plant pathogens in agriculture. We tested whether a sugar beet extract (SBE), derived through a simple extraction procedure from a large-scale plant waste product, induced resistance under green-house conditions. In three potato genotypes differing in their level of resistance to *P. infestans* (two susceptible genotypes: Desiree and Bintje and one partially resistant: Ovatio), treatment with SBE resulted in significant reduction of the size of the infection lesions in a pattern similar to that seen with application of a known defence-inducing compound, β-aminobutyric acid (BABA). Lower sporangial production was also observed on SBE-treated leaves, but the reduction in sporangial production was more pronounced after BABA treatment. SBE had no apparent toxic effect on the hyphal growth of the pathogen or on the germination of sporangia. Instead, SBE triggered pathogenesis-related protein (PR-1 and PR-2) induction which suggests that the protection conferred by SBE could be via induced resistance. An array of phenolic metabolites was found in the SBE that may contribute to the defence response.

Keywords BABA · Defence-inducing agent · Induced resistance · Plant extract · Potato late blight · PR-1

Abbreviations

BABA	β-aminobutyric acid
Dpi	Days post inoculation
IPM	Integrated pest management
PAMP	Pathogen-associated molecular patterns
PR-1 protein	Pathogenesis-related protein 1
SBE	Sugar beet extract
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Introduction

Although plant disease management policies are continuously developed all over the world, modern agriculture still faces devastating plant diseases. One of

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these diseases is potato late blight, caused by the oomycete *Phytophthora infestans*, which causes billion-dollar losses annually (Fry 2008). The current control strategies of the disease are mainly fungicide application and breeding of cultivars with dominant resistance genes. The pathogen is notorious for its ability to overcome resistance and this is why durable control strategies must be explored. Furthermore, the excessive use of agrochemicals can result in negative effects on humans and ecosystems. Some agrochemicals, for example, have been linked to detrimental effects to health as represented by their potential implication in endocrine disruption and hormonal imbalances (Kashiwagi et al. 2008; Langer et al. 2009). In many countries, these hazards have forced the legislative and governmental bodies to enforce tight regulations concerning the application of agrochemicals, as recently exemplified by a new EU directive including IPM (integrated pest management) strategies (Directive 2009/128/EC).

Due to the factors mentioned above, there is a need to increase our toolbox of disease management strategies to combat plant diseases. In this context, natural antimicrobial metabolites from plants having an effect on the growth of phytopathogens might be one alternative method to explore. For example, plant extracts containing caffeic and rosmarinic acids inhibited the germination of *Phytophthora* spp. (Widmer and Laurent 2006). Suppression of late blight development on tomato plants was achieved with ethanol plant extracts of *Paeonia suffruticosa* and *Hedera helix*, both of which inhibited *P. infestans* zoospore release and germination (Röhner et al. 2004). Plant extracts of *Rheum rhabarbarum* and *Solidago canadensis* reduced the growth of *P. infestans* on potato leaves (Stephan et al. 2005). Essential oils from aromatic plants like oregano, thyme, lavender, rosemary, fennel, and laurel were reported to inhibit the growth of *P. infestans* (Soylu et al. 2006). Moreover, antibacterial, antifungal, and anti-oomycete activity of garlic juice has been found to reduce the severity of late blight in potato plants (Curtis et al. 2004; Slusarenko et al. 2008). Still, only a few of these antimicrobial compounds have been successfully commercialized or applied on a large scale in agriculture, perhaps due to concerns about their toxicity, consistency, cost, or mode of application. However, one example of a commercial product that shows an efficacy against *P. infestans* on potatoes is Elot-Vis®, which is based on an ethanol plant extract and is being used in Germany (Stephan et al. 2005). Elot-Vis® has been shown to be directly toxic to the pathogen.

Promising results achieved with the application of some defence-inducing agents to plants suggest that the principle of induced resistance might be a means to employ in integrated disease management programs creating less human and environmental concerns (Walters et al. 2005; Beckers and Conrath 2007; Walters and Fountaine 2009). Defence-inducing agents can be biotic agents (e.g., microorganisms), abiotic stresses, or chemicals (Walters et al. 2005). Several synthetic compounds have been shown to result in induced resistance in plants (Kuć 2001). One of the most widely studied compounds is the non-essential amino acid β-aminobutyric acid (BABA). These studies have shown that BABA application results in enhanced resistance to different types of stresses (biotic and abiotic) (Jakab et al. 2005). More recently, we have shown (Liljeroth et al. 2010) that BABA reduced late blight in potato under Swedish field conditions and it was possible to reduce the dose of fungicide to 20–25 % if BABA was combined with the fungicide Shirlan® which has fluazinam as the active ingredient. No reduction in potato yield was found. However, Wu et al. (2010) recently suggested that BABA induces a stress-induced morphogenic response which results in reduced vegetative growth in *Arabidopsis*.

Different microbe-derived molecules are released during plant-microbe interactions. These elicitor molecules, which are known as pathogen-associated molecular patterns (PAMPs), can activate defence responses in plants and induce resistance to stress (Boller and Felix 2009; Postel and Kemmerling 2009). Carbohydrate polymers (e.g., lipopolysaccharides) and glycoproteins are examples of these PAMPs. Some of these PAMPs have already been used for practical applications in the agricultural sector to enhance plant resistance to pathogens. For example, chitosan which is a de-acetylated form of N-acetylchitooligosaccharide is currently used as a commercial product (Elexa®) in plant protection (Walters and Fountaine 2009) as is also the case for algal-derived glucans (Natural Protect®; Gust et al. 2010).

The aim of our study was to find a new, natural and cheap plant-derived agent that could trigger the plant's own defence responses. The final extract should preferably also be able to combine with other control agents and have a consistent content. As a potential raw material, we focused on the use of an ethanol extract from a plant-based waste product from a local

sugar refinery which is available in large volumes. Our results demonstrated that when applied to the plants, the sugar beet extract (SBE) could induce resistance in potato plants against *P. infestans* without being directly toxic to the pathogen.

Materials and methods

Plant cultivation

Three potato (*Solanum tuberosum*) cultivars were used throughout this study: Desiree and Bintje, which are susceptible to *P. infestans*, and Ovatio, which shows partial resistance. The tubers were planted in 5-l plastic pots with peat-based soil (Weibull Horto, Sweden), supplemented with 3 kgm^{-3} dolomite lime, 3 kgm^{-3} limestone, 0.7 kgm^{-3} PG-mix™ fertilizer (NPK + micronutrients) and 120 kgm^{-3} sand (1–3 mm). The pots were placed in a greenhouse chamber with controlled conditions. The temperature was 20 °C during the day and 15 °C during the night with 16 h exposure to natural light supplemented with high-pressure sodium lamps. The plants were used after four weeks of cultivation. For protein analysis, plants were grown for 4 weeks in a chamber with 16 h artificial light (300 $\mu molm^{-2}s^{-1}$) from fluorescent lamps at 20 °C.

Phytophthora infestans growth and inoculation

The *P. infestans* isolate SE030558 (mating type A1, virulent on plants carrying R1, R3, R4, R7, R10, R11, where R denotes a resistance gene) used is highly virulent and was kindly provided by Björn Andersson, Department of Forest Mycology and Pathology, SLU, Sweden. Cultures were maintained on pea agar medium that was prepared by boiling 120 g of frozen peas in 1 litre of water. After removing the peas by filtering through cheesecloth, 15 g/l agar was added and the medium was autoclaved. The plates were stored at 20 °C in the dark and were sub-cultured every 3 weeks. In order to obtain infectious sporangia, *P. infestans* was grown and maintained on detached leaves of the potato cultivar Desiree or Bintje in sealed plastic boxes containing moist filter papers. The boxes were kept for a week at 15 °C with the following light regime: 16 h light and 8 h darkness. Sporangia were released by shaking an infected leaf in 10–15 ml distilled water. The concentration of the sporangia was set to 15,000 sporangia/ml after counting with a Fuchs-Rosenthal haemocytometer. The sporangial suspension was kept at 4 °C for 2 h before the inoculation experiment in order to induce zoospore release. Twelve fully expanded leaves of 4-week-old plants from each treatment were detached and placed in boxes and *P. infestans* was drop-inoculated in two spots on the leaves by adding 20 μl of the sporangial suspension and thereafter the boxes were kept as mentioned earlier. The diameter of the circular growth of the lesion was measured 6 days post inoculation (dpi) with a ruler. The experiment was repeated twice.

Sugar beet extract (SBE) preparation and application

A solid fraction from carbonated sugar beet root juice that is produced as waste by-product during sugar production was used to prepare the sugar beet extract in this study. The sugar beet extract (SBE) was obtained by mixing 0.5 l of 99.9 % ethanol to 1,000 g of the fraction from the processed sugar beet biomass that was stored at 8 °C until use. The mixture was thoroughly blended and left for 2 h to settle in a cold room. After settling, a layer of yellowish supernatant was formed and carefully separated from the slurry and filtered with two layers of cheesecloth to remove the suspended particles present in the liquid. The filtrate was then centrifuged (3,000 rpm, 3 min at 5 °C). After centrifugation, the supernatant was stored at 8 °C until use. Just before the experiments started, the SBE was diluted five-fold with distilled water. Each potato genotype was exposed to four different treatments. Three replicate plants from each of the three genotypes (in total 12 plants from each different genotype) were sprayed with one of the following agents: SBE (diluted to 20 %), 0.3 g/l BABA (DL-β-aminobutyric acid, catalogue number A4, 420 Sigma-Aldrich), 20 % ethanol or distilled water. For each potato plant, 100–200 ml was sprayed with a hand pressure sprayer until run-off. Spraying was done two times intervened by 1 day and leaf sampling was done 1 day after the final spraying treatment. Leaves were inoculated with *P. infestans* as previously described and the diameter of the growth of the lesion was measured 6 days post inoculation (dpi) with a ruler.

Direct toxicity bioassays

Assay for hyphal growth was done as agar disc diffusion bioassays (Röhner et al. 2004). *P. infestans* was

grown on pea agar plates after transfer of mycelial plugs, which had been growing on pea agar plates for 3 weeks. Seven days after initiation of the hyphal growth, sterile paper discs (12 mm diameter) were placed 5 mm outside the opposite edge of a hyphal colony of *P. infestans* growing on pea agar medium. An aliquot of 80 μl of 20 % SBE or distilled water were placed on the paper disc and the plates were sealed with parafilm after evaporation of the liquid. After 2 and 3 days of incubation in the dark at 18 °C, the sites surrounding the impregnated paper discs were visually inspected to evaluate the hyphal growth on the paper discs. The experiment was repeated twice.

In order to measure inhibition of sporangial germination, Petri dishes containing *P. infestans* grown for 17–20 days on pea agar medium were flooded with sterile deionized water and the hyphal growth was gently scraped with a plastic scraper in order to release the sporangia. The solution was first filtered with two layers of cheese cloth to remove the hyphal material, the density of sporangia counted with a Fuchs-Rosenthal chamber and the solution was filtered again through a cell strainer with 100 μm nylon mesh (BD Falcon, USA). A more concentrated suspension of sporangia was made by centrifugation of the sporangial solution at 3,000 rpm for 3 min. In order to test the effect of SBE (final concentration 20 %) on the direct germination of sporangia and to exclude any possible effect from ethanol, the SBE solution was first concentrated. One ml of undiluted SBE was run in a rotary evaporator at room temperature in order to concentrate the SBE to 50 μl and a stock solution of 30 % SBE with low ethanol concentration was made by adding sterile distilled water. To test the effect of SBE on direct germination of sporangia (i.e., germ tube formation), 1 ml of solution was prepared in Eppendorf tubes (three replications for each treatment) so that the final concentrations of SBE and sporangia were 20 % and 40,000 sporangia/ml, respectively. The final concentration of ethanol was maximum 1 % and therefore 1 % ethanol was used as a control treatment. The samples were placed in a box and wrapped with wet paper. Then the box was wrapped in aluminium foil and placed in an incubator at 21 °C for 1 day. The percentage of sporangial germination was calculated by counting germinated and non-germinated sporangia in four randomly chosen fields from three replications of each treatment. The whole experiment was repeated twice.

Sporangia counting in the infected leaves

The day after the final spraying of plants with water, SBE or BABA, fully expanded leaves were detached and inoculated with *P. infestans* (15,000 sporangia/ml) in the detached leaf assay as described earlier. Ten days after inoculation, each of the infected leaves (12 in total) was placed in a 50 ml tube containing 15 ml distilled water, and the samples were shaken (3,000 rpm for 10 min) to release sporangia. The number of sporangia was counted using a Fuchs-Rosenthal chamber. The experiment was repeated twice.

Extraction of apoplastic fluid

Leaf sampling was done by taking seven fully expanded leaves from four-week old plants that were sprayed with SBE, BABA, and water. The leaves were dipped in 1 % Tween 20 solution for 10 s and then briefly dried on paper. The leaves were then placed in Petri dishes with their abaxial side up and immersed in phosphate buffered saline (150 mM sodium phosphate, 50 mM sodium chloride). Metal nets were placed on the leaves to better submerge them in the buffer and the Petri dishes were placed in a vacuum chamber for 10 min. Subsequently, the leaves were briefly blotted on paper, rolled and inserted carefully into 15-ml Falcon tubes on ice, to which 3.5 μl of a protease inhibitor cocktail (AEBSF, P9599 Sigma-Aldrich) and a hollow metal ring were added at the bottom of each tube. The AEBSF protease inhibitor cocktail consists of 4-(2-Aminoethyl) benzenesulphonyl fluoride hydrochloride, bestatin hydrochloride, pepstatin A, E-64[N-(transepoxysuccinyl)-L-leucine 4-guanidinobutylamid], leupeptin hemisulphate salt, and 1,10-phenanthroline. The metal rings were used to separate the leaves from the apoplastic fluid accumulating at the bottom of the tube after centrifugation at 3,000 rpm for 3 min at 4 °C. The apoplastic fluid was stored at -80 °C. The experiment was repeated twice.

SDS-PAGE separation

Thirty μl of each apoplastic sample (10 % of the total secretome sample obtained from seven leaves) were denatured with 6X sample buffer (0.5 M Tris, 30 % glycerol, 10 % SDS, 0.012 % bromophenol blue, 0.1 M dithiothreitol) by heating at 65 °C for 10 min.

The whole volume of these samples was loaded into single wells of 12 % SDS-PAGE gels. Five μl of a protein standard (SM0661, Fermentas) were used as a molecular marker. The gels were run until the bromophenol blue reached the bottom of the gel at 80 V for 20 min and then at 110 V. Gels were stained overnight in a staining solution containing 45 % methanol, 10 % acetic acid, and 0.25 % Coomassie Brilliant blue, and de-stained in a solution containing 30 % methanol and 10 % acetic acid.

Mass spectrometry and protein identification

Selected gel bands were cut out and each piece was subjected to in-gel tryptic digestion. In brief, the gel pieces were de-stained and washed and after dithiothreitol reduction and iodoacetamide alkylation, the proteins were digested with trypsin (modified sequencing grade; Promega) overnight at 37 °C. The gel pieces were shaken vigorously at room temperature for 15 min and 0.5 μl of each sample was spotted directly onto a stainless steel MALDI target and were left to dry. A volume of 0.5 μl of a matrix solution containing 5 mg/ml α-cyano-4-hydroxy cinnamic acid, 50 % acetonitrile, 0.1 % TFA and 50 mM citric acid was added and allowed to dry. MALDI-TOF MS and MS/MS spectra were recorded automatically using a 4,700 Proteomics Analyzer (Applied Biosystems, Framingham, CA, USA). Protein identification was performed using the GPS Explorer software, with an in-house Mascot search engine (Matrix Science, London, UK). The following parameters were applied for database-search: database (Uniprot); taxonomy (*Solanum tuberosum*); max missed cleavage (1). A protein was considered as identified when the level of confidence for the total ion score exceeded 99.95 %.

HPLC analysis of SBE

In order to identify compounds present in the SBE, it was subjected to HPLC analysis using a Merck Hitachi LaChrom HPLC system consisting of a D-7100 pump, D-7200 autosampler, D-7300 column oven (set at 40 °C) and a D-7455 DAD detector scanning the absorbance between 220 and 400 nm. Five hundred μl of the extract was evaporated to dryness and dissolved in 100 μl of methanol and 50 μl water. Separation on an Aquasil C18 column (Thermo Scientific, Waltham, MA, USA) was achieved using a gradient of water (acidified with *o*-phosphoric acid to pH 3; A) and methanol (B) as follows: 0 % B (0–1 min); 5–45 % B (1–15 min); 45–85 % B (15–20 min); 85 % B (20–23 min) and 85–100 % B (23–25 min), 100 % B (25–27 min), followed by equilibration to initial conditions. The flow rate was 0.8 ml/min and the injection volume was 40 μl. The peak area data were collected at 280 nm, allowing quantification of simple phenolics, phenolic acids and flavonoids. The UV-spectrum collected at 200 to 400 nm were compared to spectral data in a standard compound library and the compounds were tentatively identified if their spectrum exhibited a match of 97 % or better against that of an authentic standard.

Statistical data analysis

In order to compare the effects of different treatments, the results from lesion measurements and sporangia experiments were analysed with a multiple comparison test (Tukey's multiple range test, $P < 0.05$) using the SAS software package (version 9.1). Sporangial counts were log-transformed before analysis. Each experiment was repeated twice and gave similar results. Data from individual experiments are represented as means in the figures and error bars represent the standard error of the means.

Results

Application of SBE decreased the size of the infection lesions

SBE application decreased the macroscopic growth of the infection compared to the control leaves sprayed with water only in assays on detached leaves of Desiree plants (Fig. 1a). After quantification, significant reductions of lesion size (approximately 20 %) were found in all three potato genotypes after the SBE application (Fig. 1b,c, and d). The controls with 20 % ethanol or water did not differ, and the effect of SBE was not significantly different from the result from BABA-treated plants (Fig. 1b,c, and d). Lower concentrations of SBE were also tested, but did not give a significant effect (data not shown). Small necrotic dots or lesions, characteristic of BABA treatments, were visible two days after BABA treatment on Desiree plants and to a lesser extent on Ovatio, whereas no such lesions were observed on Bintje plants. SBE-treated leaves did not exhibit necrotic lesions.

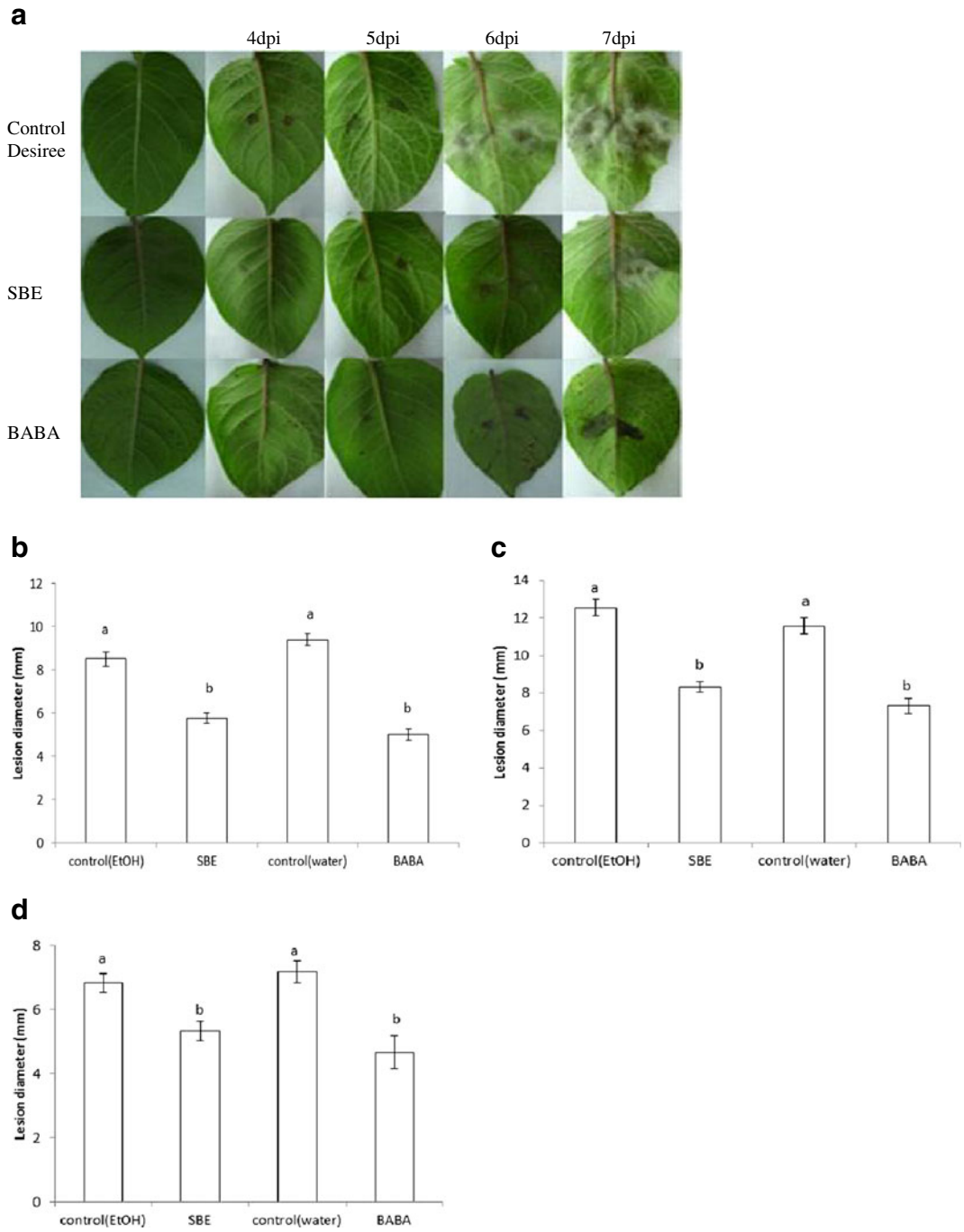

Fig. 1 **a** Lesion appearance 4–7 days post inoculation (dpi) for Desiree plants sprayed with water (control), SBE or BABA (0.3 g/l) and inoculated with *P. infestans* (15,000 sporangia/ml) in a detached leaf assay. The small necrotic dots on the BABA-treated leaves were only detected in that treatment. **b–d** Lesion size measurements 6 dpi for potato plants. Leaves were from Desiree **(b)**, Bintje **(c)**, and Ovatio **(d).** Data show mean values of lesion measurements in mm from 12 leaves from an individual experiment and the error bars represent the standard error of the mean. Different letters represent data that are statistically different according to Tukey's multiple range test ($P<0.05$)

SBE decreased sporangia production

We hypothesized that the reduced lesion size that was seen after the SBE treatment could be caused by reduced sporangial production in the inoculated leaves. In order to test this hypothesis, sporangial production was estimated in the inoculated leaves taken from different treatments. A significant reduction in sporangial production was observed in the leaves from the SBE-treated Desiree plants compared to the control leaves from the water-treated plants (Fig. 2). However, the reduction in sporangial production was larger in BABA-treated plants (Fig. 2).

SBE had no direct toxic effect on P. infestans

It is possible that the reduced lesion development and sporangial production observed after SBE treatment were caused by direct toxic effect of the extract on germination of sporangia or hyphal growth. Inhibition of hyphal growth was tested in two independent experiments, each containing three plates with control and SBE-impregnated paper discs. No direct toxic effect of SBE on the hyphal growth of *P. infestans* was observed (data not shown). Moreover, sporangia germinated normally (i.e., formed germ tubes) in the presence of SBE as revealed by microscopy of germinated sporangia. No significant difference in germination was observed between the different treatments. Thus, in experiment 1 (sum of three replications): for the SBE treatment, the germination percentage was 81 % (196 sporangia counted) whereas it was 82 % for the control (250 sporangia counted). In experiment 2: 74 % for the SBE treatment (507 sporangia counted) whereas it was 76 % for the control (460 sporangia counted).

PR-protein induction after SBE treatment

Since no direct effect on the pathogen was detected by SBE treatment, we tested if induced defence responses in the host were activated after SBE treatment by studying the proteins secreted into the apoplast using SDS-PAGE. During defence activation, the so called pathogenesis-related (PR)-proteins are known to accumulate in such high amounts as to influence the total protein concentration in the apoplast. Thus, we used these proteins as a marker for the induction of defence responses. An equal fraction of apoplastic liquid from seven leaves from each treatment or genotype was loaded on the gels. Comparison of secreted protein patterns showed that a number of proteins were induced after BABA and/or SBE treatment. From the lanes with apoplastic liquid from SBE-treated Desiree leaves, two different gel-bands (designated with arrowhead and asterisk in Fig. 3), that showed induction after SBE application, were cut out, trypsin digested, and analysed by mass spectrometry. From the band designated with an arrowhead in the Fig. 3, we

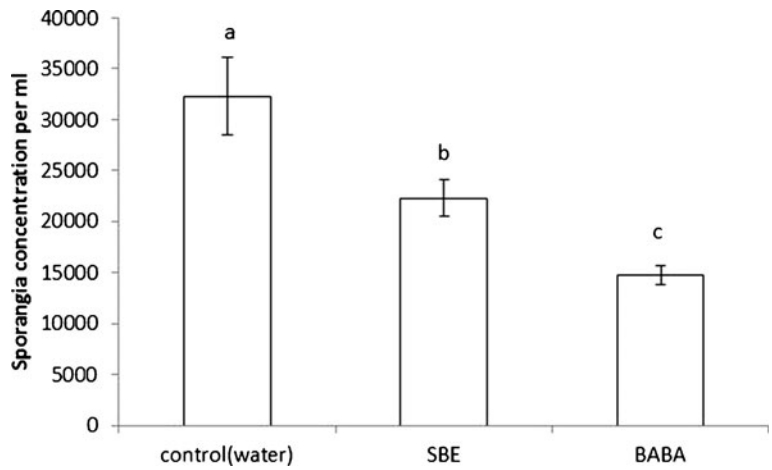

Fig. 2 Sporangial production 10 days post inoculation (10 dpi) in Desiree. Leaves from Desiree plants treated with water, SBE, and BABA (0.3 g/l) were inoculated with *P. infestans* (15,000 sporangia/ml) in a detached leaf assay. Data show mean values of sporangial concentration from 12 leaves from an individual experiment and the error bars represent the standard error of the mean. Different letters represent data that are statistically different according to Tukey's multiple range test ($P<0.05$)

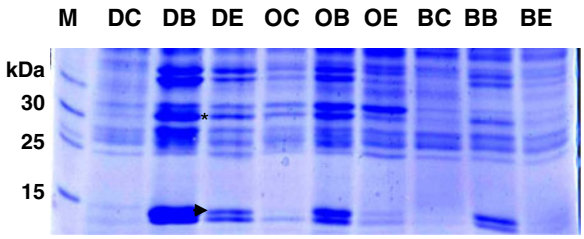

Fig. 3 SDS-PAGE analysis of the apoplastic proteins. Apoplastic fluids from leaves of the three different potato genotypes treated with water, BABA (0.3 g/l) or SBE were analyzed for PR-1 (arrowhead) and PR-2 (asterisk) protein induction. From left to right M: Marker; DC: Desiree Control (water); DB: Desiree BABA (0.3 g/l); DE: Desiree Extract (SBE); OC: Ovatio Control; OB: Ovatio BABA; OE: Ovatio Extract (SBE); BC: Bintje Control; BB: Bintje BABA; BE: Bintje Extract (SBE). An equal volume (30 µl) of the apoplastic fluid (10 % of the total secretome sample obtained from seven leaves) was loaded into each well. The arrow head and the asterisk (*) denote bands that were confirmed with mass spectrometry to contain PR1 and PR2 respectively

identified the peptide AQVGVGPMSWDAGLASR that was found in two different PR-1 proteins; Q9SC15 and Q941G6 whereas in the band designated with an asterisk, two peptides (SPLLVNIYPYFAK and NLFDALLDATYSALEK) that were found in the PR-2 protein endo-1,3-β-glucanase Q2HPL1, were identified. One day after the last spraying treatment with SBE, PR-1 and PR-2 protein induction was found to be strongest in the leaves of Desiree, less strong in Ovatio and weakest in Bintje. A similar pattern was also evident after BABA treatment although the overall induction was stronger after BABA application (Fig. 3).

Presence of phenolic compounds in SBE

The observation of PR-1 protein induction after SBE treatment led us to perform a screen for compounds present in the SBE that might play a role in triggering the induced resistance observed in the SBE-treated plants. Several compounds absorbing in the UV-range were detected in the extract. HPLC analysis of the SBE resulted in the identification of a number of phenolic compounds. The main peaks separated under the analytic conditions were tentatively identified as derivatives of cinnamic acid, and flavone compounds (Fig. 4). In addition, simple phenolics with spectral resemblance to p-hydroxybenzoic acid and vanillic acid were detected (Fig. 4).

Discussion

Our results demonstrated that an extract from a plant waste product obtained from sugar beet processing is a new promising agent for control of *P. infestans* in potatoes. Spraying three potato genotypes differing in their level of resistance to *P. infestans* with SBE resulted in significant reduction of the lesion size. This positive effect was similar to that seen with the application of BABA. Despite the fact that the *P. infestans* strain used in this study is highly aggressive, the SBE displayed a noticeably promising effect, thus warranting further consideration in field trials. Trials should also be initiated to investigate whether the SBE effect would be even stronger in plant-pathogen systems involving less aggressive pathogens such as *Phytophthora fragariae*. Moreover, future studies should also focus on optimization of the extraction method and concentration to use in order to improve the effectiveness of the product.

A direct toxic effect is not a likely mechanism behind the protective effect of SBE application as we could not detect any inhibitory effect, neither on the growth of hyphae nor on the germination of sporangia. Instead, the analysis of the apoplastic proteins in potato indicates that induction of plant defence responses is the likely explanation for the decreased disease symptoms. PR-1 and PR-2 proteins were induced after SBE application and both are well known pathogenesis-related proteins that have been shown to be induced during plant defence (Van loon et al. 2006). The induction of PR-1 protein varied among the cultivars and was more pronounced in Desiree, intermediate in Ovatio and weakest in Bintje (Fig. 3). This difference in the response was similar as after BABA treatment, indicating that both these agents may act via induced resistance.

Because plant phenolic compounds have well documented roles in plant-pathogen interactions, we speculate that the phenolics found in the SBE may have contributed to the induced defence to *P. infestans*. One of the phenolic compounds in our HPLC analysis was identified as p-hydroxybenzoic acid (Fig. 4). This compound, which is a derivative of benzoic acid, has been found to induce systemic resistance in cucumber against *Colletotrichum lagenarum* (Fought and Kuć 1996). Although some indications were recently reported that refer to the possible involvement of phenolics in induction of defence responses (see Yang et al. 2010; Martín et al. 2010), apart from salicylic

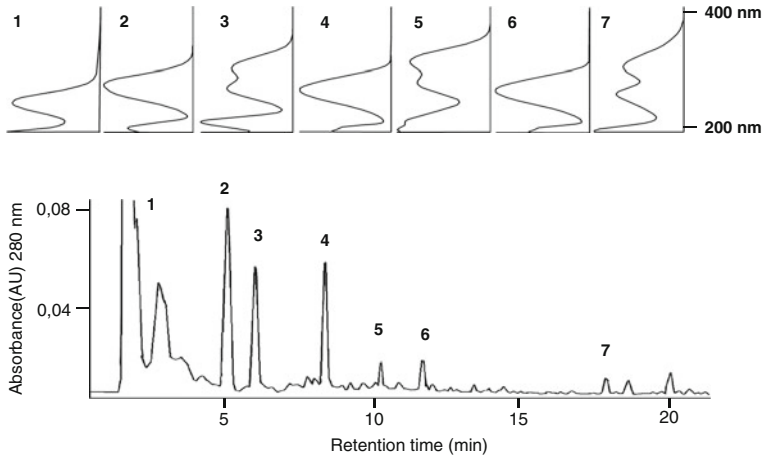

Fig. 4 HPLC profile of SBE at 280 nm and examples of UV-spectra for the detected phenolic compounds (numbers above the peaks correspond to numbers by the UV-spectra scanned at wavelengths of 200–400 nm). Peak 1 show the high spectral match with an authentic p-hydroxybenzoic acid standard

acid, little is known until now about the possible defence-inducing effect of exogenously applied phenolic compounds on plant resistance. Therefore, while our study does not provide direct evidence for the role of phenolics as inducers of resistance, detailed studies to clarify their possible role as potential inducers of resistance in potato should be performed. Future studies should, in the first place, focus on clarifying whether the HPLC-purified extracts have the same effect as total SBE on the inhibition of growth of *P. infestans* or on lesion formation by *P. infestans*.

Sporangia are the infectious propagules that start the disease cycle in nature. Thus, the rate of sporangial production can have important implications for the overall epidemiology of the late blight disease. In our study, SBE application significantly reduced sporangial production although not as efficiently as the BABA treatment. Previous studies have shown that disease severity and sporulation capacity of *P. infestans* were reduced in BABA-treated tomato accessions (Cohen 1994; Jeun et al. 2000; Sharma et al. 2010). However, this is apparently the first demonstration that BABA can reduce sporangial production in the potato-*P. infestans* pathosystem and which may not be directly explained by the reduction in lesion size.

Introducing non-toxic defence inducing agents or plant derived compounds could reduce concerns about environmental and biodiversity conservation issues. Agents like thiamine (Boubakri et al. 2012), cerebrosides (Deepak et al. 2003) and leaf extracts of *Datura metel* (Devaiah et al. 2009) were reported to induce resistance against oomycetes causing downy mildew of grapevine and pearl millet. Likewise, Elot-Vis® is a commercial product based on a plant extract that had no defence-inducing ability, but had a direct inhibitory effect on *P. infestans* (Stephan et al. 2005). If applied one day before *P. infestans* inoculation, this product could reduce disease in potato. Whereas SBE had no apparent toxicity on the mycelial or the sporangial stages of the pathogen, it reduces sporangial production in infected leaves, suggesting a potential for this agent as a resistance inducer. Using the tomato-*P. infestans* pathosystem, a water extract of the mycelium of *Penicillium chrysogenum* (named 'Pen') was also reported to be effective against late blight in tomato, but was found to be ineffective in potato (Thuerig et al. 2006; Unger et al. 2006). Although it could induce resistance in tomato plants, phytotoxic side effects were observed on the tomato plants sprayed with Pen such as accelerated senescence of leaves, leaf bending, and speckling on the leaves and stems (Thuerig et al. 2006). No such effect was observed with the SBE. Thus, it is tempting to speculate that SBE could be a promising product with potential to be used as a control agent in organic farming as it is derived from natural compounds that are cycled through the agricultural system. Alternatively, the concept of induced resistance may be employed in an integrated pest management (IPM) strategy in modern agriculture where plant-defence inducers can be combined with fungicides to achieve an efficient control strategy with an aim to reduce fungicide doses. This strategy has been found to be functional, for example, in the case of BABA (Cohen 2002; Liljeroth et al. 2010). SBE has the potential since it is a cheap product and is made in large quantities with consistent quality that is devoid of plant debris or mud particles. Therefore, SBE is an interesting candidate to be mixed with

fungicides. Plant defence inducers are still not widely applied or accepted by farmers for a number of reasons. They rarely provide full protection for plants since their performance is variable and not as efficient as conventional fungicides. However, due to the fact that the number of agrochemicals available for disease control is expected to be reduced in the future due to new legislation in Europe, high costs and public concern, additional approaches to control plant diseases need to be explored.

Acknowledgements We thank the Swedish Foundation for Strategic Research (SSF) and the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning (FORMAS) for financial support, Mia Mogren for excellent technical help and William Walker for manuscript review.

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