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BABA effects on the behaviour of potato cultivars infected by *Phytophthora infestans* and *Fusarium solani*

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Abstract Since most plants possess resistance mechanisms which can be induced upon pre-treatment with a variety of chemical compounds, the use of β-aminobutyric acid (BABA) as a defence inducer without reported toxic effect on the environment was studied. The aim of this work was to analyse the effectiveness of BABA to induce resistance against *Phytophthora infestans* and *Fusarium solani* in potato cultivars differing in their level of resistance to late blight. The behaviour of some components of biochemical mechanisms by which BABA increases resistance against *P. infestans*, as well as the effect of BABA on the activity of a potential pathogenic factor of *F. solani*, were studied. Plants with four applications of BABA throughout the crop cycle produced tubers more resistant to *P. infestans* and *F. solani* than non-treated plants. In addition, tuber slices from treated plants, inoculated with *P. infestans,* showed an increase in phenol and phytoalexin content. The aspartyl protease *St*AP1 accumulation was also higher in tubers obtained from treated plants and inoculated with *P. infestans*. This result was observed only in the more resistant potato cv. Pampeana, early after infection. In the potato–*F. solani* interaction, infected tubers coming from BABA-treated plants showed minor fungal proteolytic activity than infected, non-treated ones. For potato cvs Pampeana and Bintje, the BABA treatment improved the yield of harvested tubers. The number of tubers per plant and total weight of harvested tubers was greater for those obtained from treated plants with two early or four applications of BABA. The results show that the BABA treatment increases the resistance of potatoes but the degree of increase depends on the original level of resistance present in each cultivar.

Keywords Aspartyl protease · β-Aminobutyric acid (BABA) · Defence-related responses · Potato · Systemic acquired resistance

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Introduction

Potato late blight (*Phytophthora infestans*) and dry rot (*Fusarium solani*) are economically important potato diseases worldwide. The most common disease control practice for late blight is fungicide applications. In spite of label recommendations, frequent excessive applications are reported in farmer's fields and this

may result in damage to the environment (Ridder et al. 1995). Genetic breeding and induced resistance become fundamental tools for disease management with minimal negative impact on the environment. As a consequence, advanced materials and cultivars have been developed, allowing a drastic reduction in the use of fungicides once these materials are adopted by farmers (Huarte et al. 1995). Polygenic or horizontal disease resistance refers to plant resistance generated via interactions between the products of multiple plant genes, in contrast to single R gene response or vertical resistance (Nelson 1978; Simmonds 1991). Horizontal resistance to late blight in potato is the primary objective of many breeding programmes (Huarte et al. 1997). This type of resistance is a less studied phenomenon that depends on timely expression of multiple gene products in the plant host. Cultural practices and cultivar resistance are the most frequently used control measures to *F. solani* infections. An oligogenic type of resistance to this disease has been suggested.

Plants possess resistance mechanisms which can be induced upon pre-treatment with a variety of chemical compounds or inducing organisms. This general phenomenon is known as induced resistance (IR). Tuzun (2001) has suggested that the constitutive accumulation of specific isozymes of hydrolytic enzymes or other defence-related gene products is an integral part of both multigenic resistance and IR.

Previous studies have shown that different gene products, such as phytoalexins and aspartyl proteases, are involved in the potato–*P. infestans* interaction (Andreu et al. 2001; Guevara et al. 2002). In addition, other hydrolytic activities (proteases and chitinases) are associated with the infection process of potato tubers by *F. solani* f. sp. *eumartii*. In particular, a *Fusarium* extracellular serin protease has been characterised and it has been suggested that this is related to fungal colonisation in the host tissue (Olivieri et al. 2002, 2004). The accumulation of these molecules may be modified by inducers applied to these interactions.

The broad spectrum protective effect of the inducer β-3-aminobutyric acid (BABA) against numerous plant diseases has been well documented (Jackab et al. 2001; Cohen 2002). In several cases, treatment with BABA induced the accumulation of pathogenesis-related (PR) proteins in treated tissue before challenge with the pathogen (Cohen 2002). Depending on the pathosystem, different defence mechanisms have been reported to be induced by BABA applications. The induction of systemic acquired resistance (SAR), a type of IR, mediated by BABA, has also been described for several cases. Reuveni et al. (2003) have reported the protective action of BABA applied to foliage in post-harvest apples against *Alternaria alternata*.

In previous work we have described the induction of SAR, mediated by the chemical activator BABA. The assay was carried out in a set of commercial potato cultivars with different levels of resistance against *P. infestans.* When BABA was applied to foliage at early stages of crop development, a protective effect against late blight was obtained. In post-harvest tuber samples, evidence for enhancement of a defence response was evaluated, and an increase in protein level of β-1,3-glucanase and aspartyl protease (*St*AP1), as well as in phenols and phytoalexins was observed (Andreu et al. 2006).

In theory, SAR may lead to the production of defence compounds that are excessive to the actual need for control, and therefore the cost of this defensive strategy may imply a yield reduction. According to Heil (1999), there are a few published studies that attempt to quantify the cost of resistance against pathogens and their results do not allow any generalisation about the possible cost of SAR. Vallad and Goodman (2004) reviewed the benefits and drawbacks of the use of chemical inducers, and compared these with the use of standard pesticides. The results contained in that review are highly variable and depend on the system under study, environmental conditions such as fertilisation regimes, etc. In plant-herbivore interactions, it has been reported that the cost of induced responses may also vary with environmental conditions (Cipollini et al. 2003).

The present work analyses the effectiveness of BABA in the protection against *P. infestans* and *F. solani* and the induction of gene products involved in the resistance to these pathogens. The effect of BABA foliar treatments on post-harvest tuber yield was also studied.

Materials and methods

P. infestans isolate

The isolate of *P. infestans* race R_2 R_3 R_6 R_7 R_9, mating type A2 was isolated from infected leaflets of the potato crop showing single lesions. Pieces of this

infected tissue surrounding the lesion were placed in potato tuber slices and incubated at 17–19°C for 5 days until new sporulation appeared. For inoculum production, small pieces of selective medium containing actively growing *P. infestans* hyphae were transferred onto tubers slices of cv. Bintje. The slices were incubated in closed plastic boxes containing wet filter paper, in the darkness, at 18°C and 90% relative humidity (RH). After 7 days, the mycelia was harvested in sterile water and stimulated to release zoospores by incubation at 4°C for 6 h. After filtration through a nylon filter cloth, the suspension of sporangia was observed under the light microscope for quantification before using as inoculum. The concentration of sporangia was adjusted to 4×10^4 sporangia ml^{-1} using a haematocytometer.

Fusarium solani isolate

The pathogenic fungus *F. solani* f. sp. *eumartii* isolate 3122 was obtained from the Instituto Nacional de Tecnología Agropecuaria (INTA) collection, Balcarce Argentina. Fungal cultures were grown on solid potato dextrose agar (PDA) for 3 weeks at 25°C.

Growing conditions of plants

Seed tubers of cvs Pampeana INTA (MPI 59.789/12× Huinkul MAG; moderately resistant to *P. infestans*) and Bintje (susceptible to *P. infestans*) were used due to their different levels of foliar and tuber blight polygenic resistance. Seed tubers were planted at 10 cm depth in steam-pasteurised greenhouse potting mix in 7 l plastic pots. During plant growth, the temperature ranged between 15–24°C and natural daylight was supplemented by high-pressure sodium lamps (400 W) in a day–night cycle 14–10 h. Plants were irrigated with a sprinkler system. Experiments were performed from 2004 to 2006 and repeated at least three times each year. In each experiment 50 plants per treatment and cultivar were used. Each set of 50 plants was randomly distributed within the greenhouse.

Foliage treatment with the inducer

Chemical induction was achieved by foliar applications of BABA. Three millilitres of BABA (40 mM) per plant were applied at a dose equivalent to 4 kg ha^{-1} at 35, 55, 78 and 95 days after emergence (four applications). As a control treatment, water was used. Early or late applications were made only at 35 and 55 or 78 and 95 days after emergence, respectively.

Tuber assays

Late blight development in tuber slices and extraction and determination of phytoalexins and phenolic compounds

After harvest, tubers from treated and control plants were stored at 10°C and 50% RH for 3 months to allow better artificial inoculation and adequate levels of biochemical markers. At this time the tubers were washed with distilled water and disinfected by immersion in 2.5 g l^{-1} sodium hypochlorite for 5 min. For tuber slice evaluation, sterile disks (4–6 cm diam, 10 mm thick) were inoculated with 50 µl of a sporangial suspension (4×10^4 sporangia ml^{-1}) and incubated at 18°C in darkness. The largest diameter of mycelium was measured on the upper surface of the slices 7 days after inoculation; 20 tubers per cultivar and per treatment were used in five independent replicates. In addition, phytoalexins and phenols were extracted from the slices according to Andreu et al. (2006). Controls also included tuber slices treated with BABA, but not infected with *P. infestans*.

Dry rot assessment in whole tuber

Whole tubers were inoculated with mycelium and spores of *Fusarium* by wounding as previously described (Olivieri et al. 1998). Briefly, a 0.8 cm diam disk of fungus grown on PDA was introduced in potato cortical tissue by the hollow punch method (Radtke and Escande 1973). Control tubers (wounded) were inoculated with a disk of sterile PDA medium. Inoculated tubers were stored at 25°C (room temperature). Cortical tissue surrounding the inoculation site was collected and analysed 12 days post-inoculation. The tubers were cut longitudinally and the index of disease severity was scored for each individual tuber using the following scale: 0=no symptoms, 1=< 2.5% of cut area with symptoms, 2=2.5–10% cut area with symptoms, 3=10–25% cut area with symptoms, 4=25–50% cut area with symptoms and 5=>50% cut area with symptoms of susceptibility. Ten tubers were used per treatment and the experiment was performed three times.

Preparation of tuber soluble extract for StAP1 detection and proteolytic activity quantification

Potato tuber tissue (1 g) from control and treated plants and infected or not infected with *P. infestans* or with *F. solani*, were homogenised in 100 mM sodium acetate pH 5.2, 0.5% (*w*/*v*) sodium metabisulphite, four pulses of 10 s with 30 s intervals using a Virtis 45 homogeniser (The Virtis Co., Gardiner, New York, NY, USA) set at speed 10. Homogenates were filtered through cheesecloth and centrifuged at 12,000×*g* for 20 min. The resulting supernatant represented the tuber soluble extract.

Gel electrophoresis and immunoblot analysis

For *St*AP1 detection, soluble extracts were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 12% (*w*/*v*) acrylamide (Laemmli 1970) and then transferred onto nitrocellulose in a semi-dry electrophoretic transfer cell (Trans-Blot, Bio Rad, Hercules, CA, USA). The nitrocellulose sheet was soaked for 2 h with a solution containing 100 mM Tris-HCl, pH 8.0 and 1% (*w*/*v*) BSA. The membrane was washed four times with 100 mM Tris-HCl, pH 8.0 containing 150 mM NaCl and 0.05% (*v*/*v*) Tween 20 (TBST) and then incubated overnight with rabbit anti-*St*AP1 (1:10,000 *v*/*v*; Guevara et al. 1999) in 100 mM Tris-HCl, pH 8.0, and 1% BSA. After four washes with TBST solution, the blot was allowed to react for 2 h with goat anti-rabbit antibody (1:10,000 *v*/*v*) labelled with alkaline phosphatase (Sigma). Bound antibody was detected using BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) according to procedures recommended by the manufacturer (Sigma). Immunoblot band intensity was estimated by densitometric analysis (TN-Image, Image Analysis Software, Compuserve, IBMAPP, Rockville, MD, USA).

Proteolytic activity quantification

For proteolytic activity quantification, soluble extracts (20 µl) from tubers inoculated or not inoculated with *F. solani*, were incubated in 50 mM Tris-HCl, pH 8.0, with 5 mg of azocasein as substrate in a final volume of 0.5 ml. The reaction mixtures were incubated at 42°C for 1 h and the reactions stopped by adding 0.5 ml of 10% trichloroacetic acid (TCA) and maintained for 30 min at 4°C. The undigested material was then removed by centrifugation at 3,000×*g* for 10 min. The proteolytic activity was estimated as the increment in absorbance at 335 nm of the TCA soluble fraction. One unit of activity represents the amount of enzyme that produces a change of 1.0 in absorbance at 335 nm during 1 h at 42°C.

Determination of fresh weight and dry matter

The tubers from ten plants per treatment were weighed in open air and in water, on a hydrostatic precision balance, for specific gravity determination. Tuber dry matter content was calculated indirectly using the specific gravity results, following the methodology of Schippers (1976).

Data analysis

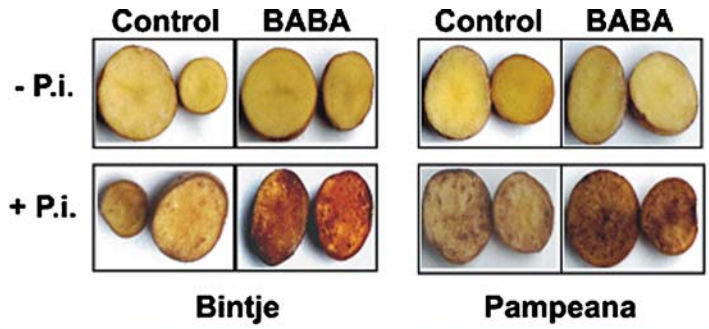
Data from tuber colonisation by *P. infestans*, phenol and phytoalexin accumulation, proteolytic activity quantification and post-harvest tuber yield of control and BABA-treated plants were analysed for significance by a two-way analysis of variance and means were compared at the $P<0.05$ level of significance by multiple range comparisons (Tukey, SigmaStat).

Results

Effect of BABA on browning and colonisation of tuber slices by *P. infestans*

Accumulation of phytoalexins and phenols in potato tuber slices

The comparison between infected tuber slices of cvs Bintje and Pampeana showed that the colonised surface was 50% smaller in the moderately resistant cv. Pampeana than in the susceptible cv. Bintje. In tubers from plants treated with four applications of BABA, these values were smaller than in the untreated control. The reductions in the diameter of the colony of *P. infestans* were 75% and 98% on the susceptible and moderately resistant cultivars, respectively (Fig. 1). In addition, a higher level of browning on the infected surface of tuber slices was observed, resulting from phytoalexin and phenol accumulation as part of the defence response. Tubers of the resistant



Treatment	Bintje Colony diam on slices (mm)	Pampeana Colony diam on slices (mm)
Untreated	55±0.5 a	24±0.3 b
BABA	15±0.3 c	0.6±0.2 d

Fig. 1 Effect of foliar applications of BABA on browning and colonisation of tuber slices inoculated with *P. infestans*. Symptoms were observed 7 days after inoculation. Twenty tuber slices were used per treatment in five independent replicates. *Values followed by the same letter* do not differ significantly at $P<0.05$ (Tukey Multiple Comparison, SigmaStat)

cultivar from both control and BABA-treated plants, showed higher phytoalexin and phenol accumulation compared with the susceptible cultivar (Fig. 2a and b). BABA treatment was able to increase the phytoalexin and phenol content in both cultivars after *P. infestans* infection. These values were two-fold higher in cv. Pampeana compared with cv. Bintje.

Effect of BABA treatment on *St*AP1 protein accumulation after *P. infestans* infection

The results obtained after the BABA treatment showed that *St*AP1 content was affected only in Pampeana tubers and at an early time of infection (40 h). This treatment increased the *St*AP1 basal content by 30% and increased it even more after wounding or infection, to 35% and 75%, with respect to their corresponding controls (Fig. 3). No differences in the *St*AP1 accumulation after the BABA treatment were observed either in Bintje at 40 h and 7 days after inoculation, or in Pampeana at 7 days after infection (data not shown).

Effect of BABA on colonisation of tubers by *F. solani*

Whole tubers of cvs Bintje and Pampeana infected with *F. solani* showed that the disease severity was reduced in tubers of both cultivars from BABA-treated plants (four applications). BABA reduced disease symptoms up to 60% compared to the control (Fig. 4).

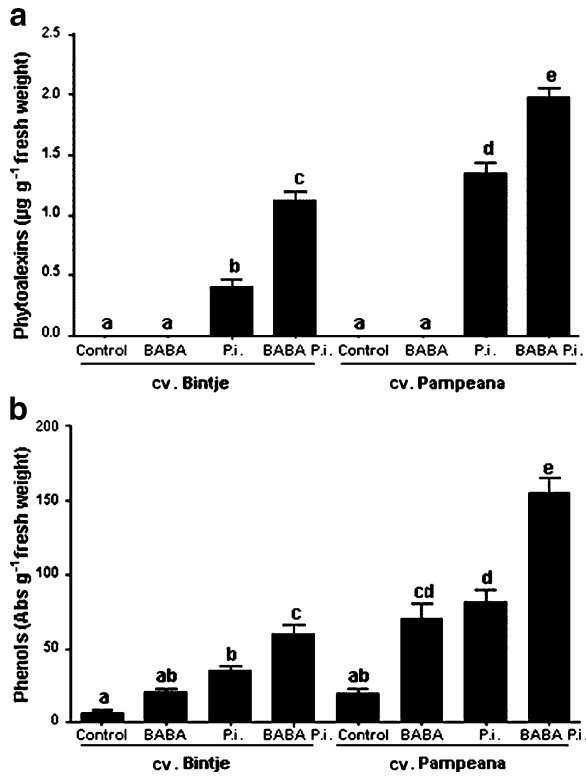


Fig. 2 Effect of foliar applications of BABA on (**a**) phytoalexin and (**b**) phenolic compound accumulation. Phytoalexins and phenols were measured in tuber slices 7 days after inoculation with *P. infestans*. Extractions were made from ten tuber slices per treatment in two independent experiments. *Bars with the same letter* do not differ significantly at $P<0.05$ (Tukey Multiple Comparison, SigmaStat)

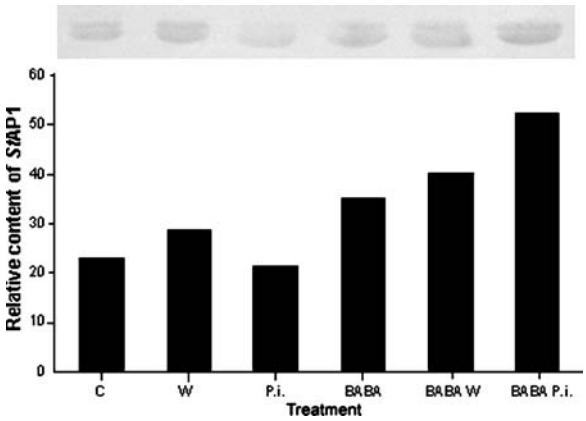


Fig. 3 Effect of BABA on *St*AP1 protein accumulation in cv. Pampeana tubers infected with *P. infestans*. The analysis of accumulation of *St*AP1 was made by western-blot. Soluble extracts were obtained from ten tuber slices per treatment, and the western-blot shown is representative of three independent experiments. *Bars* correspond to immunoblot band intensity estimated by densitometric analysis. Volumes corresponding to equal amounts of fresh weight (0.5 mg) were loaded in each *lane*. *C*: tubers from non-treated plants; *W*: tubers from non-treated plants after 40 h of wounding; *P.i*: tubers from non-treated plants 40 h after inoculation with *P. infestans*; *BABA*: tubers from BABA-treated plants; *BABA W*: tubers from BABA-treated plants after 40 h of wounding; and *BABA P.i*: tubers from BABA-treated plants 40 h after inoculation with *P. infestans*

Effect of BABA treatment on proteolytic activity after *F. solani* infection

Twelve days after *F. solani* inoculation, proteolytic activity increased in infected tubers of both cvs Bintje and Pampeana. However this increase was approximately 25% lower in tubers from BABA-treated plants than in untreated plants of both cultivars (Fig. 5).

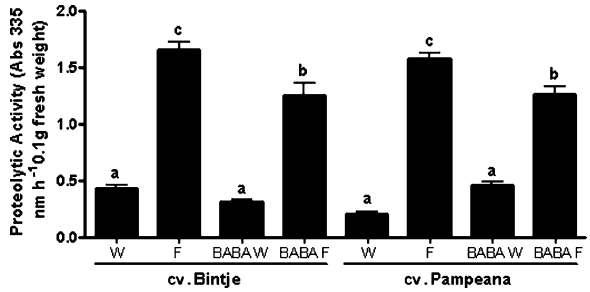
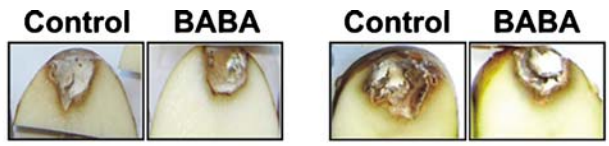


Fig. 5 Effect of foliar applications of BABA on tuber proteolytic activity after *F. solani* infection. Proteolytic activity was measured in soluble extracts obtained from tubers from untreated or BABA-treated plants, using azocasein as substrate. *W*: tubers from non-treated plants 12 days after wounding; *F*: tubers from non-treated plants 12 days after inoculation with *F. solani*; *BABA W*: tubers from BABA-treated plants 12 days after wounding; and *BABA F*: tubers from BABA-treated plants 12 days after inoculation with *F. solani*. Ten tubers were used per treatment and the experiment was repeated twice. *Bars with the same letter* do not differ significantly at $P<0.05$ (Tukey Multiple Comparison, SigmaStat)

Effect of BABA treatment on tuber yield

The results showed that four foliar applications of BABA starting at the beginning of tuberisation (35 days after emergence) resulted in a two-fold increase in the number of tubers per plant, as well as an increase in total fresh weight and percentage dry weight for both cultivars (Table 1). When the foliar treatments were made with only two early applications, 35 and 55 days after emergence, the same effect was observed but at a smaller magnitude. However, when two late applications were made, at 78 and 95 days after emergence, there was no effect of BABA on the yield parameters (Table 1).



Treatment	Bintje Grade[a]	Pampeana Grade[a]
Untreated	3	3
BABA	1	2

Fig. 4 Effect of foliar applications of BABA on colonisation of tubers by *F. solani*. [a]Twenty tubers per treatment were inoculated by hollow punch with *F. solani*, 12 days after inoculation, the *Fusarium* dry rot was measured using a index of disease severity: 0=no symptoms to 5>50% of tuber affected by symptoms (see text). The experiment was repeated twice

Table 1 Effect of BABA treatments on the amount of tubers per plant, fresh weight and dry matter in potato tubers

Cultivar	Treatment	Tubers/plant	Fresh weight/plant (g)	Dry matter (%)
Bintje	Control	6±2 a	420±20 a	18.0
	BABA *4 appl.*	13±2 bc	650±20 d	20.1
	early BABA *2 appl.*	11±2 abc	610±16 c	19.7
	late BABA *2 appl.*	7±1 a	410±10 a	18.2
Pampeana	Control	8±2 ab	540±14 b	19.2
	BABA *4 appl.*	15±2 c	740±10 f	20.6
	early BABA *2 appl.*	14±2 c	700±06 e	20.1
	late BABA *2 appl.*	8±1 ab	550±10 b	19.4

BABA treatments were applied on foliage 35, 55, 78 and 92 days after emergence (BABA 4 appl.). Two applications were made 35 and 55 (early), or 78 and 95 days after emergence (late). Values followed by the same letter do not differ significantly at $P<0.05$ (Tukey Multiple Comparison, SigmaStat).

Discussion

The non-protein amino acid aminobutyric acid (BABA) has been studied intensively in recent years as an inducer of disease resistance in plants. Cohen (2002) summarised the host-pathogen systems in which BABA induced effective resistance. In potato, it has been described as a partial protection in foliage against *P. infestans* and *Alternaria solani* and in potato tuber tissue against *Fusarium sambucinum* (Greyerbiehl and Hammerschmidt 1998). Zimmerli et al. (2000) demonstrated that BABA protected *Arabidopsis* against different virulent pathogens by enhancing pathogen-specific plant resistance mechanisms.

Results of the present work showed a significant reduction in the diameter of the colony of *P. infestans* (Fig. 1) and a higher phytoalexin and phenol accumulation in potato tuber slices from BABA-treated plants as compared to untreated control plants. This effect was higher in the moderately resistant cv. Pampeana than in the susceptible cv. Bintje (Fig. 2). Tuzun (2001) proposed that the constitutive accumulation of specific isozymes of hydrolytic enzymes or other defence-related gene products is an integral part of both multigenic resistance and induced resistance. In order to know if components involved in polygenic resistance could participate in the BABA-induced mechanisms against *P. infestans*, the accumulation of *St*AP1 was analysed. Previously, Guevara et al. (2002) reported a differential induction of *St*AP1 in tuber disks either infected with *P. infestans* or wounded, in potato cultivars with different degrees of multigenic resistance. In cv. Pampeana (moderately resistant), *St*AP1 induction was higher and faster in infected tissues than in wounded tissues. In cv. Bintje (susceptible), a lower and delayed accumulation was observed as compared to the resistant cultivar. In addition, Guevara et al. (2002) showed that *St*AP1 had a direct inhibitory effect on the germination of cysts of *P. infestans* and conidia of *F. solani*. The pattern of accumulation and in vitro activities of *St*AP1 suggest that this enzyme may play a role in the defence response of potato. The present work showed that the BABA treatment increased *St*AP1 accumulation only in cv. Pampeana tubers infected with *P. infestans* at early stages of infection (40 h; Fig. 3). Furthermore, after 7 days of either wounding or infection, the levels of *St*AP1 did not change significantly. These results suggest that *St*AP1 could be a part of the BABA-IR mechanism during initial stages of the *P. infestans*–potato interaction.

The protective effect of BABA in whole tubers against *F. solani* was also observed in both cultivars (Fig. 4). This suggests a possible broad spectrum response induced by BABA against different potato pathogens. In order to know the mechanism of BABA-IR against *F. solani*, *St*AP1 protein accumulation was measured, but no increase in this protein was observed in infected tubers 12 days after inoculation with *F. solani*. Taking into account that most of the proteolytic activity accumulated in *F. solani*-infected tubers is of fungal origin and that it is a possible pathogenic factor (Olivieri et al. 2002, 2004), the accumulation of this activity was measured in infected tubers from BABA-treated or non-treated plants. BABA treatment resulted in a decrease of proteolytic activity, suggesting that it could interfere in the *F. solani* infection process. Further studies will

be necessary to know whether BABA inhibits the proteolytic cell wall degradation by the fungus or if it has an effect on fungal viability.

Although the resistance-inducing capacity of BABA has been documented in many plant species, little is known about the factors that influence its inducing efficacy against pathogen infection, especially in susceptible plants (Jackab et al. 2001). Ge et al. (2005) showed that although wounding alone could slightly prevent TMV colonisation, simultaneous wounding attenuated the efficacy of BABA-mediated resistance to TMV. Furthermore, their studies on IR-related enzymes phenylalanine ammonia-lyase and polyphenol oxidase, revealed that the interaction between BABA and wounding was reciprocally antagonistic. It has been demonstrated that there is a greater effect of BABA on wounding-mediated responses, than of wounding on BABA-mediated responses. In the system BABA–potato–*P. infestans*, the antagonistic effect between BABA and wounding in *St*AP1 accumulation was not only detected after 40 h of infection with *P. infestans*, but a cumulative effect in *St*AP1 content induced by BABA in potato plants was observed. This would corroborate what was proposed by Tuzun (2001) who postulated that plants in which IR has been activated appear to move from a latent multigenic resistance state to another, in which a multigenic form of resistance is active. The fact that *St*AP1 does not accumulate in *F. solani*-infected tubers, suggests that a different mechanism could be acting in BABA-IR in different pathosystems. The diversity of BABA-IR possible mechanisms was previously reviewed by several authors (Jakab et al. 2001; Cohen 2002).

The phenomenological characterisation of induced plant resistance in a number of controlled and uncontrolled environments has resulted in speculation on the span of these defences and the energy costs for the plants when they deploy these defences, measured in terms of vegetative and reproductive growth. Most experiments evaluated a range of BTH (acibenzolar-*S*-methyl) application rates, and reported a trade-off between effective disease control and either phytotoxic effects or reduced plant productivity (Cole 1999; Abbasi et al. 2002; Perez et al. 2003). A conflictive theme that could be associated with several inducers is the reduction of crop yield. Often, these reductions were statistically insignificant. For example, when Louws et al. (2001) summarised yield data across 22 field experiments with tomato, plots treated with BTH yielded 11% less, on average, than plots treated with a standardised bacterisation treatment, and 2.1% less than non-treated control plots. They also observed that tomato seedlings treated with BTH were smaller than non-treated plants in greenhouse experiments. However, Shailasree et al. (2001) treated seeds of pearl millet with BABA and observed a high level of induced resistance against downy mildew. They followed the growth and the production of the BABA-protected plants for 30–60 days, respectively. At 30 days, BABA-treated plants were taller, had larger leaf areas and gave more dry weight than control plants. Van Hulten et al. (2006) evaluated the costs and benefits of priming for defence in *Arabidopsis*. They showed that the induction of direct defence only by high doses of BABA or BTH strongly affected the relative growth rate and seed production. They also proposed that the benefits of priming-mediated resistance by low doses of BABA involve fewer costs than direct defence.

In this work, results indicated that four applications of BABA starting from the beginning of tuberisation, produced enhanced resistance to phytopathogens such as *P. infestans* and *F. solani*. Another relevant finding was that the BABA treatment caused positive effects on yield parameters in potato tubers. In fact, foliar treatments with four applications of BABA increased the amount of tubers per plant, the dry matter and fresh weight of harvested tubers in both cultivars (Table 1). On the other hand, data showed that if BABA is applied fewer times, the effect on tuber yield depends on the time of application. These data suggest that the improvement in defence by BABA was not detrimental to plant yield, at least in potato in our greenhouse conditions. This is an important point to be corroborated with field conditions in order to include the use of BABA in integrated pest management strategies.

Finally, data show that BABA treatments used in this work are not able to improve the resistance level of the susceptible cv. Bintje, to a degree close to that of the resistant cv. Pampeana. This suggests that the response to BABA treatment depends on the original level of resistance of each cultivar.

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