Research Article

Management of Late Blight and Sucking Insect Pests of Potato with Application of Salicylic Acid and β-aminobutyric Acid under Greenhouse Conditions

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Abstract | Pre-treatment of plants with chemical elicitors can induce systemic resistance against plant pathogens and insect herbivores. The plan of the study was to study induction of defense responses and protective effects against *Phytophthora infestans* (Mont.) de Bary and sucking insect pests of potato (*Solanum tuberosum* L.) with application of salicylic acid (SA) and β-aminobutyric acid (BABA). Concentration of SA and BABA (2, 4 and 5 mM) were applied to foliage. Application of both resistance inducers suppressed disease development by 54.1% and 67.4%, for SA and BABA, respectively, 5 days after pathogen inoculation. Numbers and size of lesions per plant was reduced by application of SA and BABA. Plants treated with BABA exhibited increased resistance against herbivory by aphids (*Myzus persicae* Sulzer) (Homoptera: Aphidiae), thrips (*Thrips tabaci* Lindeman) (Thysanoptera: Thripidae) and whitefly (*Bemisia tabaci* Gennadius) (Hemiptera: Aleyrodidae) compared to SA treated plants. Potato plants treated with SA and BABA had higher peroxidase and polyphenol oxidase activity than water treated plants. Enzymes produced as result of treatment with elicitors are responsible for protection against late blight and sucking insect pests. The inducers can be used for disease and insect pest's management.

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Introduction

Potato late blight caused by the fungus *Phytophthora infestans* (Mont.) de Bary is probably the most destructive disease of potato crop and is accountable for estimated crop losses of $6.7 billion worldwide (Haverkort et al., 2008; Shahbazi et al., 2011). The pathogen infects directly through sporangia and affects on leaves, stems, and tubers (Judelson and Blanco, 2005). In Pakistan, late blight is the major yield limiting factor resulting in 70% reduction in yield (Ghazanfar et al., 2010).

The most appropriate, and practical, approach to manage late blight is the use of resistant varieties (Kröner et al., 2011). However, developing potato varieties with established resistance to potato blight is complex, because some specific known resistance genes can be swiftly conquer by mutants in local populations (Fry and Goodwin, 1997). Integrated management of late blight includes use of healthy seed, sanitation and cull clean-up, crop rotation, scouting, forecasting techniques, application of fungicides, storage monitoring and use of moderately resistant varieties (Ghazanfar et al., 2010). However, none of these practices give complete control of potato late blight. Use of fungicides is effective (Evenhuis et al., 2006) but is expensive and many virulent strains of *P. infestans* have developed fungicide resistance, causing a worldwide recurrence of the potato late blight disease (Fry and Goodwin, 1997). The frequent exercise of fungicides reduces both yield and lower quality of tubers.

One novel method that can minimize potato blight infection without having any negative impact on the environment may be resistance induction; that is application of non-hazardous compounds that stimulate or commence plant defense (Olivieri et al., 2009). Among resistance inducers, BABA known as DL-ß-aminobutyric acid, non protein amino acid (Hodge et al., 2011), has the capacity to boost defense in a wide range of plant species (Cohen, 2001; Silue et al., 2002; Xie et al., 2002; Liljeroth et al., 2010; Sharma et al., 2012). It is an effective plant defense initiator against fungi, bacteria, viruses (Jakab et al., 2001; Conrath et al., 2002; Cohen et al., 2011), nematodes (Oka and Cohen, 2001) and abiotic stress. BABA (β-aminobutyric acid) and salicylic acid (SA) have been reported as effective plant defense initiators in potato and other crops (Halim et al., 2006; Hussain et al., 2014). In numerous cases, BABA treatment induced accretion of pathogenesis-related (PR) proteins in treated tissues before challenge with pathogen (Cohen, 2002).

Potato production in Pakistan is hampered by sucking insect pests (Ali et al., 2010). The green peach potato *Myzus persicae* (Sulzer) (Aphid), associated with many diseases including potato virus Y (PVY), potato leaf roll virus (PLRV) and potato virus X (PVX) (Yardımci et al., 2014) and sweet potato whitefly, *Bemisia tabaci* (Gennadius) are the most destructive (Roditakis et al., 2005) pests of numerous crops (Perring, 2001). Bemisia tabaci biotype A (Gennadius) has the ability to transmit 111 economically important viruses (Jones, 2003). *Thrips tabaci* Lindeman, worldwide in distribution are also rated as potential threat to potato (Allen et al., 2005). Conventionally, these sucking insect pests are managed by application of insecticides but due to injudicious use of chemicals, insecticide resistance has developed (Ahmad et al., 2002; Allen et al., 2005; Van Toor and Teulon, 2006) in these insect pests. Induced resistance is a new approach that could be augmented in the successful integrated pest management for sucking insect pests of potato.

Among most successful reported inducing agents is BABA, non protein amino acid (Jakab et al., 2001; Cohen, 2002; Kim and Jeun, 2007; Altamiranda et al., 2008; Olivieri et al., 2009). BABA has been reported very effective in field conditions in Israel (Cohen, 2002). Depend on pathosystem, different defense mechanisms are induced by BABA application. The initiation of systemic acquired resistance, a type of IR mediated by BABA has been described in literature. Exogenous applications of defense inducers intensify plant resistance to various herbivores and induce expression of defensive proteins. Drenching of BABA resulted in increased aphid mortality (Hodge et al., 2005; Hodge et al., 2011; Hodge and Powell, 2012).

The purpose of current study was to examine induction of defense responses against *Phytophthora infestans* (Mont.) de Bary and sucking insects by application of salicylic acid (SA) and β-aminobutyric acid (BABA) to formulate a common strategy to manage the disease and sucking insect pests.

Materials and Methods

Research experiments were conducted in the Plant Pathology laboratory, College of Agriculture, University of Sargodha, Punjab, Pakistan during 2017-18 on the highly susceptible potato cv. Desiree. Plants were grown in 12-cm diameter pots (one eye per pot) from tuber eyes in a loam sand mixture under greenhouse conditions of 22°C and 16:8 h light dark. Plants were fertilized one week once after planting with 5% solution of N:P:K (20:20:20) and maintained for a further 5 weeks until plants developed 8 to 11 leaves.

Leaves showing typical sporulating lesions collected from the field were washed in 0.5% sodium hypochlorite solution and placed in humid boxes with the leaf's abaxial side up (inverted petri dishes with agar media). The inoculated petri plates were incubated at 15-18°C until fresh sporulation appeared. Small pieces of infected tissues from the sporulating border of the lesion were cut and placed under potato slices in empty Petri dishes. These dishes were incubated at 15-18°C until abundant sporulation appeared. Tissues were washed in distilled water. The sporangial solution was passed through a 30 micron mesh filter to remove mycelium and other debris. The final concentration was adjusted to 1.2×10^6 spores /ml using a Neubauer haemocytometer.

Aphids, thrips, and whiteflies were reared on potato leaves maintained at 16:8 h light dark, 22°C in an insect rearing room until used in bioassays. The concentrations 2, 4 and 5 mM of β-aminobutyric acid (BABA) and salicylic acid (SA) purchased from Sigma (Lahore, Pakistan) were prepared. Each concentration was applied to the upper (adaxial) leaf surface of plants using a glass sprayer until the plant was uniformly covered. Control plants were treated with distilled water.

Induced plants were challenge inoculated with *P. infestans*, 5 days post treatment. Plants were treated on the adaxial surfaces with sporangial suspension (10 ml/plant) containing 7500 sporangia·mL^{-1} with the hand sprayer. Plants were kept in the dark at 90% relative humidity and then moved to a green house with 22°C under 16:8 h light dark regime. Late blight development was recorded 5 days after inoculation. Lesion size and numbers on leaves of each plant were observed. Percent reduction in disease was calculated according to Cohen et al. (1991).

Excised leaves from control and induced plants were placed on wet cotton in Petri dishes (140 mm diameter × 25 mm height). Twenty-five adults of potato aphids, thrips and whitefly were added separately to each Petri dish and the plates maintained in incubator at 22°C under a 16:8 h light dark regime. Numbers of dead aphids, thrips and whitefly were counted on the 5th day after inoculation.

Samples (0.5 g) of potato leaflets were ground using a mortar and pestle and the extracts filtered and centrifuged with the supernatants used for enzyme assay. The polyphenol oxidase activity was measured spectrophotometrically at 400 nm by using chlorogenic acid as a substrate. The enzyme activity unit was distinct as the change in absorbance·min^{-1}·μg^{-1} protein. Peroxidase activity was observed spectrophotometrically by oxidation of guaiacol (@420 nm) by using extract described above. The enzyme activity unit was expressed in mkatal·min^{-1}·μg^{-1} protein

The experiments were performed 3 times independently for disease reduction and insect herbivory. Data were evaluated by Analysis of Variance (ANOVA) and treatment means separated by Tukey's HSD through Statistix® (version 8.1) (Gomez and Gomez, 1984). Corrected mortality % for *in vitro* study was computed using Abbott's formula (Abbott, 1925).

Results and Discussion

Application of BABA and SA to potato leaf surfaces protected these against *P. infestans*. BABA was more effective ($F= 3884.23$; $df= 3, 11$; $P<0.05$) followed by SA ($F= 1777.43$; $df= 3, 11$; $P<0.05$) (Figure 1). Application of resistance elicitors reduced the number of the lesions ($F= 4244$; $df= 3, 11$; $P<0.05$) in case of SA and BABA ($F= 1963.67$; $df= 3, 11$; $P<0.05$) and their size of ($F= 6.67$; $df= 3, 11$; $P<0.05$) in case of BABA while in case of SA it was $F= 21$; $df= 3, 11$; $P<0.05$. Protection was obvious as reduction in lesion size and number (Figure 2). The minimum number of lesions was for BABA at the maximum conc. for SA numbers of lesions was higher with the same conc. (Figure 3).

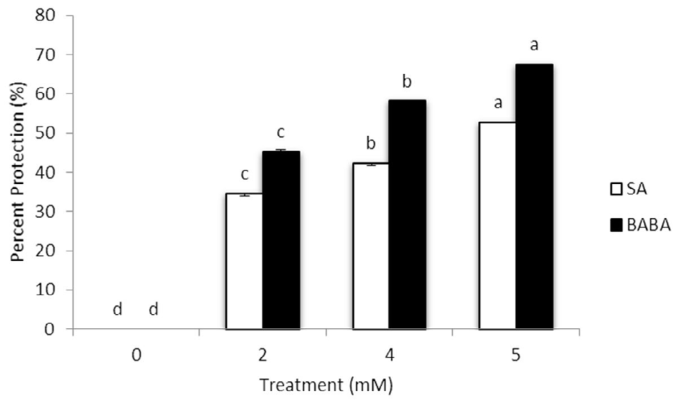

Figure 1: *Effect of SA and BABA dose on percent protection against Phytophthora infestans on potato. Bars with the same letter are not significantly different, P = 0.05, Tukey's HSD test.*

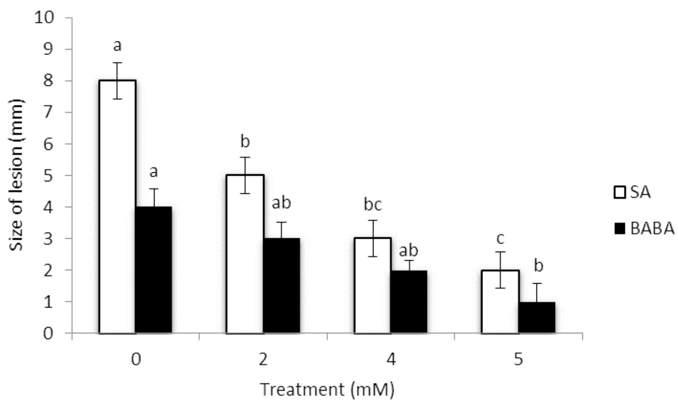

Figure 2: *Effect of SA and BABA dose on lesion size against Phytophthora infestans on potato. Bars with the same letter are not significantly different, P = 0.05, Tukey's HSD test.*

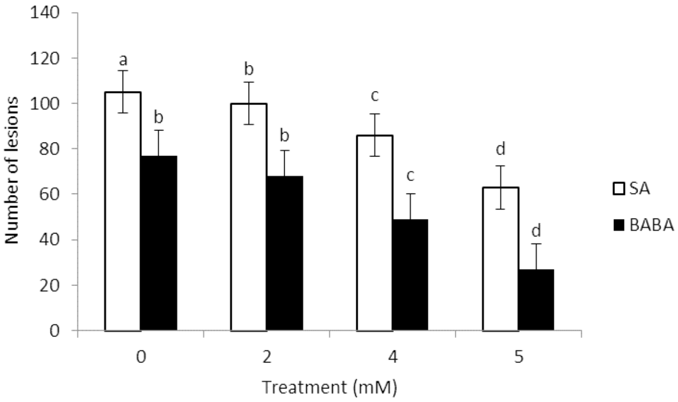

Figure 3: *Effect of SA and BABA dose on numbers of lesions against Phytophthora infestans on potato. Bars with the same letter are not significantly different, P = 0.05, Tukey's HSD test.*

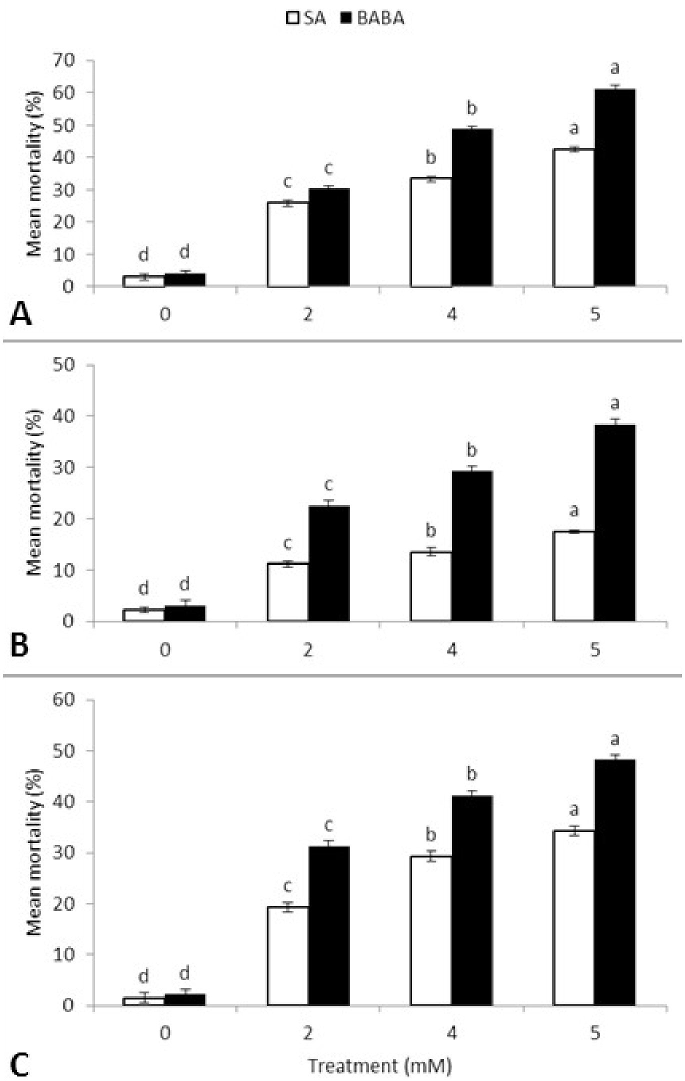

Figure 4: *Percent mean mortality of potato aphid, thrips and whitefly on potato leaves treated with aqueous solutions of SA and BABA. Bars with the same letter are not significantly different, P= 0.05, Tukey's HSD test.*

Treatment affected mortality of potato aphids, thrips and whiteflies. The resistance inducers affected mortality of potato aphid (*F*= 566.53; df= 3, 11; P<0.05) for SA and (F= 1901.91; df= 3, 11; P<0.05) for BABA. For thrips (F= 116.10; df= 3, 11; P<0.05) for SA and for BABA (F= 2345.06; df= 3, 11; P<0.05). Mortality of whitefly (F= 728.83; df= 3, 11; P<0.05) with SA and with BABA (F= 1228.71; df= 3, 11; P<0.05). Application of BABA to potato leaves increased mortality of aphids (61.2%) and whitefly (48.2%) compared with the control (Figure 4A, B). Both inducers did not modify thrips mortality compared with the control (Figure 4C).

The activity of peroxidase (POX) and polyphenol oxidase (PPO) in untreated potato leaves were lower compared to treated leaves. There was slight increase in the peroxidase activity for SA as compared with BABA treatments. The polyphenol oxidase contents increased due to application of SA and due to application of BABA. The greatest increase in polyphenol oxidase and peroxidase activities was with application of BABA. However, increase in these enzyme activities occurred in presence of BABA and SA at the highest concentration (5mM) (Figures 5 and 6).

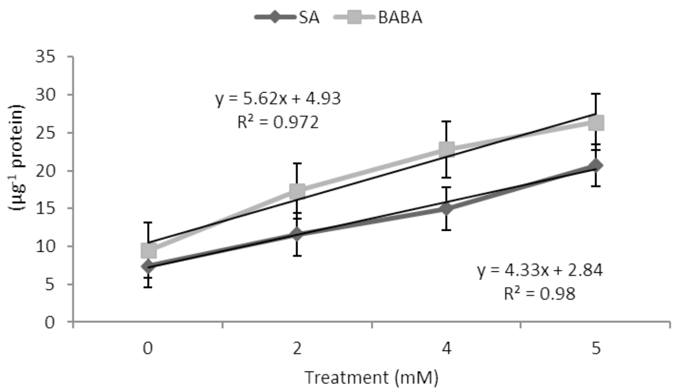

Figure 5: *Peroxidase (POX) activity in potato leaves after treatment with SA or BABA. Assays of POX activities performed 15 days post-inoculation. Bars with the same letter are not significantly different, P= 0.05, Tukey's HSD test.*

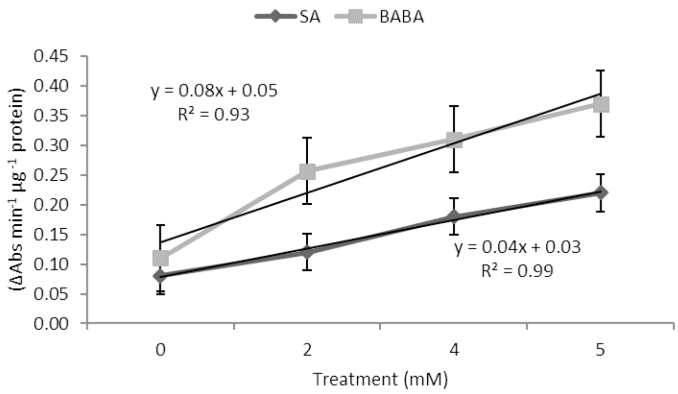

Figure 6: *Polyphenol oxidase (PPO) activity in potato leaves after treatment with SA and BABA. Assays of PPO activity performed 15 days post-inoculation. Bars with the same letter are not significantly different, P = 0.05, Tukey's HSD test.*

Application of SA and BABA reduced numbers and size of lesions produced as result of pathogen infection. Reduction in numbers and size of lesions provided protection against this disease. Sharma et al. (2010, 2012) and Tamm et al. (2011) reported reduction in *P. infestans*, blight severity from 43-100% on young leaves of tomato, a related crop, and 62% reduction in the severity of *Plasmopara viticola* by application of BABA. In the potato late blight pathosystem, BABA reduces appressoria formation (Kim and Jeun, 2007). Liljeroth et al. (2010) reported application of BABA resulted in reduction of lesion sizes compared to untreated control in different susceptible and partially resistant potato cultivars (Heller and Gessler, 1986) indicating the response is likely widespread across potato cultivars. Application of BABA *in vivo* and *in vitro* demonstrated reduction (Arıcı et al., 2007) in *P. infestans* in tomato, a related crop. Promising results from laboratory tests demonstrated antifungal activity where the BABA EC_{50} was similar to the fungicide tebuconazole effecting spore germination and hyphal growth (Sasek et al., 2012). Moushib et al. (2013) reported that BABA was effective in reducing lesion size of *P. infestans* under greenhouse conditions; Similarly, downy mildew disease severity was reduced by application of BABA compared to non-treated controls (Mersha et al., 2013) and findings agree with those reports.

The SA role in signaling disease resistance was first established by injecting tobacco leaves (*Nicotiana tabacum* L.) with SA, or aspirin, resulting in 90% reduction in lesion number (Vlot et al., 2009) indicating the response is widespread across plant types. Higher SA concentrations increased resistance against potato late blight when inserted into potato tubers (seed) before planting as stated by Quintanilla and Brishammar, 1998, but disagree with Tosun et al. (2003) as SA and HarpinEa treatments elicited systemic protection against late blight under greenhouse conditions by reducing disease severity.

Application of BABA and SA increased mortality of the three sucking insect pests. The peach-potato aphid, *M. persicae* was most susceptible, particularly to BABA; followed by whitefly; the least susceptible to both inducers were thrips as occurred in other studies (Cipolline et al., 2004; Senthil-Nathan et al., 2009; Hodge and Powell, 2011; Worrall et al., 2012; Mersha et al., 2013; Moushib et al., 2013) indicating responses in potato are similar to unrelated crops. It may be that BABA and SA induce an enhanced resistance in plants to sucking insect pests, by making them physically more difficult to penetrate or by inhibiting feeding.

Peroxidases (POX) and polyphenol oxidases (PPO) activities in potato leaves after application of BABA and SA has been reported (SI-Ammour et al., 2003; Hassan and Abo-Elyousr, 2013) as a change indicator associated with chemically induced resistance against plant pathogen, *P. infestans* and different sucking insect pests. There was an increase in activity of these enzymes by applying resistance elicitors. Diverse defense responses in form of enzymes activated upon application of elicitors result in changes in cell metabolism, activity of enzymes, particularly, peroxidase (POD), lipoxygenase (LOX), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), ß-1,3 glucanase and superoxide dismutase (SOD) (Chandra et al., 2007; Vanitha et al., 2009; Ngadze et al., 2012). Phenylpropaniod metabolism is a pathway which catalyze phenylalanine ammonia-lyase (PAL) leading to production of phenylpropaniod metabolites which are highly toxic to invade pathogens and insects (Ruuhola et al., 2008; Troncoso-Rojas et al., 2013). Phenoloxidases have been demonstrated to be important in defense against herbivores in many crops (Thaler, 2002; Kranthi et al., 2003). Oxidases play a role in defense against multiple herbivores (Constabel et al., 2000; Haruta et al., 2001; Tscharntke et al., 2001).

Conclusions and Recommendations

Defense induction with application of BABA and SA can be an alternative control strategy offering durable, broad spectrum, control of *P. infestans* and sucking insect pests of potato using the plant's own resistance. Although authentic information is available on the elicitor's role treated response of plants to certain plant pathogens and insects while little is known about how plants synchronize attacker-induced signals into specific defense response. This should be determined at the molecular level to produce more insight into events occurring during all induction processes against pathogens or insect invasion. However, pathway effects of so far un-identified regulatory factors in plant pathogens interaction and insect herbivory need further investigation.

Novelty Statement

Pre-treatment of plants with salicylic acid (SA) and β-aminobutyric acid (BABA) proved helpful induction of defense response against *Phytophthora infestans* (Mont.) de Bary and sucking insects. Potato plants treated with SA and BABA had higher peroxidase and polyphenol oxidase activity than water treated plants.

Author's Contribution

Muhammad Usman Ghazanfar conceived the idea and facilitated, guided and supervised the experiments while Waqas Wakil guided and co-supervised the experiments. Imtiaz Hussain and Misbah Iqbal Qamar helped in data collection. Waqas Raza helped in experiments, wrote and finalized the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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