Control of *Bremia lactucae* in Field-Grown Lettuce by DL-3-Amino-n-Butanoic Acid (BABA)

Y. Cohen[1], A. Baider[1], D. Gotlieb[1], E. Rubin[1]

Key words: lettuce, induced resistance, downy mildew, elicitors, *Bremia lactucae*

Abstract

DL-3-amino-n-butanoic acid (BABA) was effective in controlling downy mildew incited by Bremia lactucae Regel in lettuce plants. The two isomers of BABA, DL-2-amino-n-butanoic acid and 4-amino-butanoic acid and its s-enantiomer were ineffective compares to BABA, while the r-enantiomer was more effective. The SAR compound NaSA and its functional analogue BTH (Bion) were also ineffective compared to BABA. In growth chambers, BABA was effective when applied as a foliar spray or as a soil drench. Effective control of the disease was apparent when BABA was applied up to 5 days before inoculation or 3 days after inoculation. A foliar spray of 125 mg/L reduced disease by 50% and full control of the disease was achieved with 500 mg/L. A soil drench with 1.25 mg /pot was required for >90% control the disease. In the field, 2-4 sprays with 1g/L BABA reduced disease severity by 90% as compared to control untreated plants. BABA had no adverse effect on sporangial germination of Bremia lactucae in vitro, germination on plant leaf surface or, fungal penetration into the host. However, it prevented the colonization of the host with the pathogen.)

Introduction

Downy mildew caused by Bremia lactucae is the most serious fungal disease of lettuce. The disease can be controlled by chemicals including phenylamide (e.g. metalaxyl)-based compounds but mutant isolates insensitive to these compounds have been recorded (Crute et al., 1985; Crute et al., 1994; LeRoux et al., 1988). Disease resistance (R)-genes are also used to control the disease but recombinant isolates evading recognition conferred by the R-genes can occur.

An alternative procedure to protect plants against disease is to activate their own defense mechanisms by specific biotic or abiotic elicitors (reviewed by (Walters et al., 2005)). The classical type of induced resistance is often referred to as systemic acquired resistance (SAR). Sodium salicylate (NaSA), 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole-S-methyl ester (BTH), are well known elicitors of SAR in various plants against disease (Sticher et al., 1997).

The non-protein amino acid DL-3-amino-*n*-butanoic acid (BABA) also activates an induced resistance response. It is capable of inducing systemic resistance against numerous pathogens (Cohen 2002). One objective of this study was to evaluate the efficacy of BABA in controlling downy mildew in lettuce, especially under field conditions. Field studies with BABA in lettuce were not reported before. Another objective was to study the mechanism of action of BABA against *Bremia lactucae* in lettuce.

[1] The Mina & Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel <ycohen@mail.biu.ac.il>

Materials and methods

The cultivar Noga (cup type, Hazera Genetics, Mivhor, Israel) of lettuce (*Lactuca sativa* L) was used. Isolate Isr-60 of *Bremia lactucae* Regel carrying 13 virulence factors (0, 1, 2, 3, 4, 5/8, 6, 7, 10, 11, 13, 15, 16, and 17) was used for inoculations. For growth chamber studies, plants were grown from seeds in 100 ml pots containing 40g peat/vermiculite mixture (1/1, v/v), 20 plants per pot. Plants were grown in the greenhouse (18-32°C) and used one week after seeding, when have developed two cotyledon leaves. DL-3-amino-*n*-butanoic acid (BABA), DL-2- amino-*n*-butanoic acid (AABA), 4- amino- butanoic acid (GABA), sodium salicylate (NaSA) and calcofluor were purchased from Sigma, Israel. Benzothiadiazole-S-methyl ester (BTH, Bion) was a gift from Syngenta, Switzerland. All compounds were dissolved in water before use.

Four field experiments were conducted during 2005-2006 to evaluate the efficacy of BABA in controlling *Bremia lactucae* in lettuce plants. Plants were raised from seeds in Speedling trays in the greenhouse. When plants had 4 true leaves, they were transplanted into polystyrene containers (1.2×0.6×0.2 m) filled with peat + vermiculite (1/1, v/v), 8 plants/container. Containers were located in shade houses in the field at Bar-Ilan University Farm. Shade houses were covered with 50 mesh white plastic nets to avoid aphid and viral infections. At about four weeks after planting, when they reached the 10-12 leaf stage, the plants were treated with BABA and inoculated with sporangial suspension of *B. lactucae* (1×10^3/ml) on the same evening. After inoculation plants were covered with plastic sheets for the night to assure infection. BABA, of various concentrations, was sprayed with aid of a backpack manual sprayer at a rate of about 20-30 ml/plant. Experiments were done in a full randomized block design with 3-6 replicates per dose treatment. Each replicate consisted of 3 containers with 8 plants in a container. Disease records were taken by counting the number of downy mildew lesions per plant or by visual assessment of the infected leaf area in each plant.

Results

Resistance induced by aminobutanoic acids and SAR compounds. The efficacy of a foliar spray with DL-AABA, DL-BABA and GABA in protecting plants at their cotyledon stage against downy mildew is shown in Fig. 1A. Of the three isomers tested, only DL-BABA was effective. Efficacy of 70% was exhibited at a concentration of 125 mg/L and complete inhibition of the disease was achieved with 500 mg/L. Similar results were obtained in plants treated via the root system (Fig.1B): a soil drench with 2.5 mg BABA per pot (100 ml, containing 40 g of potting mixture) totally inhibited the disease. Figures 1C and 1D show that NaSA and its functional analogue BTH (Bion) applied to the foliage or the root system were both ineffective in protecting against the disease, suggesting that the protection by BABA is probably mediated by a SA-independent pathway(s).

Field experiments. Results from four field experiments are presented in Figure 2A-D. Mean percent infected leaf area at the end of the season in control-untreated plants was 31, 29 and 45% in experiments A, C and D, respectively, whereas a mean of 63 downy mildew lesions/plant was counted in control-untreated plants in experiment B. In all experiments, a significant (P=0.05) reduction in disease level was achieved with 125 mg/L BABA or above. The higher the concentration of BABA was, the stronger and more significant was the suppression of the disease. In experiment C, BABA of 250 mg/L or above was more effective than mancozeb of 2000 mg/L (Fig. 2C). Percent disease control by each concentration of BABA in each experiment is given in

Figure 4E. Mean % disease control for all 4 experiments is shown in Figure 4F. A correlation of R2=0.95 was found between Ln concentration of BABA and mean % control of the disease (Fig 2F). The calculated dose of BABA required to achieve 50% and 90% control of the disease was 201 and 1039 mg/L, respectively (Fig. 4F).

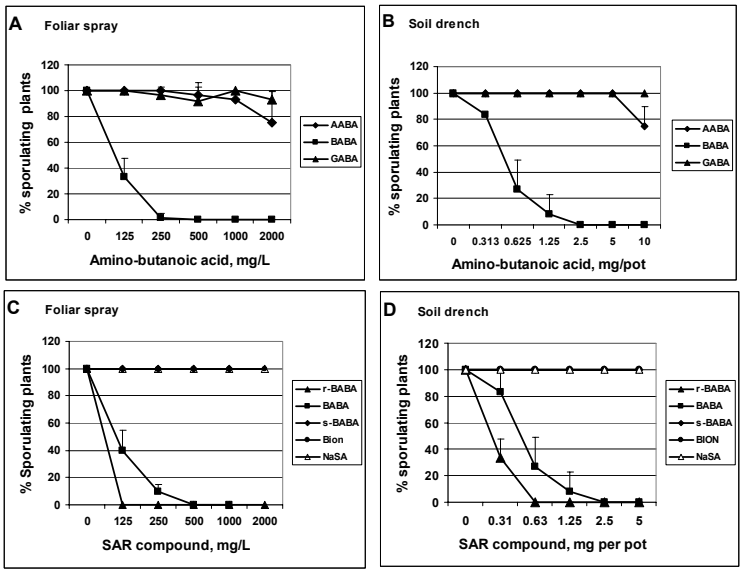

Figure 1: Control of *Bremia lactucae* in lettuce by isomers or enantiomers of aminobutanoic acid or SAR inducing compounds. Plants at their cotyledon stage (20 plants/pot, n=4) were sprayed **(A,C)** or treated by soil drench **(B,D)** with various concentrations of AABA (DL-2-amino-*n*-butanoic acid), BABA (DL-3-amino-*n*-butanoic acid) or GABA (4-aminobutanoic acid), r-BANA, s-BABA, Bion (BTH) or NaSA and inoculated with sporangia of the pathogen one day after treatment. At 5 dpi plants were transferred to 100% RH at 20°C (12h light/day) to induce sporulation of the pathogen. Two days later (7 dpi) the number of sporulating plants was recorded. In (B) and (D), 5 ml of solution (suspension in Bion) was applied to the soil surface of each pot (40 g potting mixture/pot

Discussion

DL-3-amino-*n*-butanoic acid (DL-β-aminobutyric acid, BABA) is a non-protein amino acid shown to induce resistance against about 50 plant pathogens in a large number of annual and perennial agricultural crops (Cohen, 2002). Here we show that BABA was effective in controlling downy mildew in lettuce caused by the oomycete *Bremia lactucae*. In potted plants, a foliar spray with 250 mg/L, or a soil drench with 1.25 mg/pot soil, was sufficient to suppress the disease by ≥90%. The two isomers AABA and GABA were ineffective. The s-enantiomer of BABA was ineffective whereas the r-enantiomer of BABA was more effective compared to BABA. The SAR inducing compound NaSA and its functional analogue BHT (Bion) were ineffective compared to BABA. A major finding of the present study was that BABA was efficient in controlling downy mildew in lettuce under field conditions. Foliar sprays with 201 and 1039 mg/L

resulted with 50 and 90% control of the disease, respectively. This may encourage the introduction of BABA to agriculture as a SAR compound against lettuce downy mildew. Due to the fact that BABA occurs naturally in tomato plants (unpublished data) it might also be considered for application in organic farming. Only a limited number of studies were conducted with BABA in the field. Our own studies showed efficacy against downy mildew in grapevines (Reuveni et al., 2001), late blight in potato and tomato (Cohen, 2002), rust in sunflower (Amzalek and Cohen, 2007) and moldy core in apple (Reuveni et al., 2003).

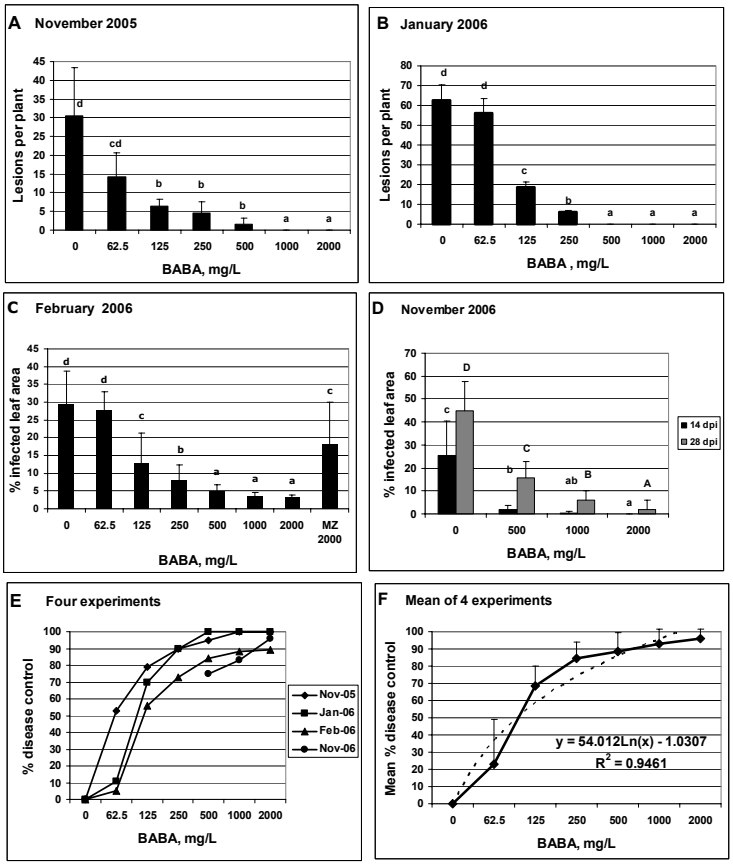

Figure 2. Control of downy mildew in lettuce by BABA under field conditions. A, B, C and D: The effect BABA on disease development in four experiments conducted during November 2005-November 2006. **E**: Percent disease control in experiments **A-D. F:** Mean % disease control for experiment **A-D**. Plants were grown in 1.2×0.6×0.2m polystyrene containers filled with peat+vermiculite (1/1, v/v) in four net houses (50×6 m each) located at BIU Farm. Plants were sprayed with BABA (six concentrations, 62.5-2000 mg/L, in experiments **A-C** and three concentrations, 500-2000 mg/L, in experiment **D**) and spray inoculated with sporangia of *Bremia lactucae* a few hours later. To assure infection, plants were covered with plastic sheets for one night following inoculation. A total of 2, 4, 4, and 3 sprays were applied in experiments **A, B, C,**

and **D**, respectively. Sprays were applied at 7-8 days intervals. In experiment **C**, the protectant fungicide mancozeb was included for comparative purposes. Experiments were conducted in fully randomized design with 3-6 replicates per dose treatment. Each replicate consisted of 24-48 plants, with 8 plants /container. Disease records (visual assessment of the percent leaf area covered with lesions of downy mildew in each plant, or the number of lesions/plant) were taken 17, 22, 25 dpi in experiments **A-C** and at 14 and 28 dpi in experiment **D**. Data were subjected to Anova analysis and separated according to Fisher's least significant difference test at $P<0.05$. Different letters on columns indicate a significant difference in disease levels. Percent disease control in **E** was calculated as 100(x/y-1), when x = mean % diseased leaf area in BABA-treated plants and y = mean % diseased leaf area in control-untreated plants.

Conclusions

BABA is shown here to effectively control downy mildew development in lettuce in growth chambers and the field. It is effective when applied to the foliage or the roots. It exhibits post infection (curative) efficacy and provides durable resistance against the disease. BABA stops disease development by suppressing fungal colonization.

Acknowledgments

The authors gratefully acknowledge funding from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project QUALITYLOWINPUTFOOD, FP6-FOOD-CT-2003- 506358.

References

Amzalek, E. and Cohen, Y. (2007): Comparative efficacy of SAR compounds against rust infection in sunflower plants. Phytopathology 97: In Press.

Cohen, Y. (2002): β-aminobutyric acid-induced resistance against plant pathogens. Plant Dis. 86:448-457.

Crute, I.R., Gordon, P.I., and Moss, N.A. (1994): Variation for responses to phenylamides in UK populations of *Bremia lactucae* (lettuce downy mildew) and *Peronospora parasitica* (Brassica downy mildew). In: Heaney, S., Slawson, D., Hollomon, D.W., Smith, M., Russel, P.E., Parry, D.W. (eds.): British Crop Protection Council Monograph No 60, pp. 155-162.

Crute, I.R., Norwood, J.M., and Gordon, D.L. (1985): Resistance to phenylamide fungicides lettuce and Brassica downy mildew. Proceedings of the Mixture Centenary Meeting, Bordeaux, British Crop Protection Council Monograph No 31:311-314.

LeRoux, P., Maisonneuve, B., and Bellec, Y. (1988): Detection en France de souches de Bremia lactucae, agent du mildiou de la laitue, resistantes au metalaxyl et a l'oxadixyl. PHM-Revue Horticole 292:37-40.

Reuveni, M., Sheglov, D., and Cohen, Y. (2003): Control of moldy-core decay in apple fruits by β-aminobutyric acids and potassium phosphites. Plant Dis. 87:933-936.

Reuveni, M., Zahavi, T., and Cohen, Y. (2001): Controlling downy mildew (*Plasmopara viticola*) in field-grown grapevine with β-aminobutyric acid (BABA). Phytoparasitica 29:125-133.

Sticher, L., Mauch-Mani, B., and Metraux, J.P. (1997): Systemic acquired resistance. Annu. Rev. Phytopathol. 35:235-270.

Walters, D., Walsh, D., Newton, A., and Lyon, G. (2005): Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology 95:1368-1373.