

RESEARCH PAPER

L-Glutamine inhibits beta-aminobutyric acid-induced stress resistance and priming in *Arabidopsis*

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Abstract

The non-protein amino acid beta-aminobutyric acid (BABA) enhances *Arabidopsis* resistance to microbial pathogens and abiotic stresses through potentiation of the *Arabidopsis* defence responses. In this study, it is shown that BABA induces the stress-induced morphogenic response (SIMR). SIMR is observed in plants exposed to sub-lethal stress conditions. Anthocyanin, a known modulator of stress signalling, was also found to accumulate in BABA-treated *Arabidopsis*. These data and a previous microarray study indicate that BABA induces a stress response in *Arabidopsis*. High concentrations of amino acids, except for L-glutamine, cause a general amino acid stress inhibition. General amino acid inhibition is prevented by the addition of L-glutamine. L-Glutamine was found to inhibit the BABA-mediated SIMR and anthocyanin accumulation, suggesting that the non-protein amino acid BABA causes a general amino acid stress inhibition in *Arabidopsis*. L-Glutamine also blocked BABA-induced resistance to heat stress and to the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. During bacterial infection, priming of the salicylic acid-dependent defence marker *PR1* was abolished by L-glutamine treatment. These results indicate that L-glutamine removal of the BABA-mediated stress response is concomitant with L-glutamine inhibition of BABA priming and BABA-induced resistance.

Key words: Acquired thermotolerance, *Arabidopsis*, beta-aminobutyric acid, priming, *Pseudomonas syringae*, stress, stress imprinting, stress-induced morphogenic response.

Introduction

Throughout evolution, plants have developed numerous defence mechanisms manifested through altered physiology to endure environmental abiotic stress and to combat challenges arising from biotic stress. Abiotic stresses include drought, excess water, salinity, heat, cold, wounding, and exposure to chemical stress (Shao *et al.*, 2006; Wu *et al.*, 2007).

A common theme underlying responses to a range of biotic and abiotic stresses is the phenomenon of priming. Priming refers to a phenomenon where plants are sensitized to stress (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006; Van der Ent *et al.*, 2009). Typically primed plants display either faster and/or stronger, activation of the various defence responses that are induced following attack by microbial pathogens, or in response to abiotic stresses (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006; Beckers

et al., 2009). Priming is observed in plants and also in animals (Pham *et al.*, 2007; Beckers *et al.*, 2009; Jung *et al.*, 2009). Priming provides low-cost protection in relatively high disease pressure conditions (van Hulst *et al.*, 2006).

The non-protein amino acid beta amino-butyric acid (BABA) increases *Arabidopsis* resistance to different, unrelated stresses such as microbial pathogens, salt, drought, and heat shock (Zimmerli *et al.*, 2000, 2001, 2008; Ton and Mauch-Mani, 2004; Jakab *et al.*, 2005; Ton *et al.*, 2005). BABA primes *Arabidopsis* plants to respond quicker and stronger to biotic and abiotic stresses (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006). Typically, following infection by *Pseudomonas syringae* pv. *tomato* DC3000, the salicylic acid (SA)-dependent defence marker *PATHOGENESIS-RELATED* gene 1 (*PR1*) is induced earlier and stronger in

BABA-treated *Arabidopsis* (Zimmerli *et al.*, 2000; Ton *et al.*, 2005). Plant responses to different stresses are controlled at the molecular level by changes in gene expression and many genes are involved in such stress responses (Kreps *et al.*, 2002; Tardif *et al.*, 2007). A recent microarray study revealed that BABA enhances mRNA accumulation of abscisic acid (ABA) and ethylene early signalling intermediates (Zimmerli *et al.*, 2008). ABA and ethylene are two plant hormones involved in the *Arabidopsis* stress response (Xiong *et al.*, 2002; van Loon *et al.*, 2006). These observations, and the fact that many stress-responsive genes were found to be up-regulated by BABA, suggests that this chemical activates a stress response in *Arabidopsis* (Zimmerli *et al.*, 2008).

Previous plant exposure to stress can modify the plant response to a subsequent, different stress (Bruce *et al.*, 2007). Higher plants are capable of demonstrating some stress 'memory', or stress imprinting (Bruce *et al.*, 2007). Stress imprinting is usually defined as genetic or biochemical modifications induced by a first stress exposure that lead to enhanced resistance to subsequent stress. Preliminary stress exposure is indeed known to boost the stress tolerance of the plant through induction of acclimation responses (Bruce *et al.*, 2007). Tolerance can be linked to an array of morphological, physiological, and biochemical responses that decrease stress exposure damage or facilitate repair of damaged systems (Potters *et al.*, 2007).

Exposure of plants to a mild chronic stress can cause the induction of a specific, stress-induced morphogenic response (SIMR) (Potters *et al.*, 2007). These responses are characterized by a blockage of cell division in the main meristematic tissues, an inhibition of elongation, and a redirected outgrowth of lateral organs (Potters *et al.*, 2009). The SIMR is part of a general acclimation strategy, whereby plants do redirect their growth when exposed to stress. These stress responses are also characterized by the presence of antioxidants that prevent damage caused by reactive oxygen species, and the accumulation of foliar anthocyanin that acts as modulators of stress signals (Steyn *et al.*, 2002; Gould and Lister, 2006).

The objective of this work was to understand better how the priming agent BABA potentiates the *Arabidopsis* defence responses. The data presented here provide evidence that BABA induces the SIMR in *Arabidopsis*. In addition, this work demonstrates for the first time that BABA-mediated SIMR, priming and resistance to stress can be inhibited by L-glutamine. This observation suggests that the non-protein amino acid BABA primes by inducing a general amino acid inhibition stress response in *Arabidopsis*. The relationship between BABA-mediated stress imprinting and priming is discussed.

Materials and methods

Biological materials and growth conditions

Arabidopsis thaliana (L. Heyhn.) Columbia (Col-0) were grown in commercial potting soil/perlite (3:2 v/v) at 22/18 °C day/night temperatures with 9 h light per 24 h for the indicated time. The

proCYCB1;1:GUS transgenic line was kindly provided by Negi *et al.* (2008). For *in vitro* culture, seeds were first surface-sterilized with commercial bleach diluted to 1:10, washed with deionized distilled water three times, suspended in 0.15% agar (Biomax Scientific Co., Ltd., Taiwan) and stored at 4 °C in the dark. After 2–3 d of stratification, seeds were sown on sterilized half-strength MS medium [1/2 Murashige and Skoog salt (Sigma, USA), 1.5% agar (Biomax Scientific Co., Ltd, Taiwan), pH 5.7] and cultivated as indicated. Strain DC3000 of *Pst* was cultivated at 28 °C, 340 rpm in King's B medium (Biomax Scientific Co. Ltd., Taiwan) containing rifampicin (100 mg l⁻¹) for selection.

Chemical treatment

BABA (Sigma, USA) and L-glutamine (Sigma, USA) were dissolved in water and pots were soil drenched 48 h before pathogen inoculation. Pots were syringe infiltrated with a 4-fold concentrated BABA or L-glutamine solution. To get the final indicated concentration, a volume of solution of one-quarter of the final volume of the pot was used. The BABA and L-glutamine concentrations used are indicated in the figure legends.

Root growth assay

In vitro Arabidopsis seedlings were grown vertically under 16 h light conditions at 22 °C on half-strength MS medium supplemented with the indicated concentration of BABA (Sigma, USA) or L-glutamine (Sigma, USA). Root length was recorded on 12-d-old seedlings and relative root growth rates were evaluated by comparison with the water control.

Lateral root quantification

Lateral root number evaluation was performed on 15-d-old *in vitro* seedlings grown on half strength MS medium containing BABA (Sigma, USA), L-glutamine (Sigma, USA), or both chemicals at the indicated concentration. The number of lateral roots on the primary root was determined with a dissecting microscope (Negi *et al.*, 2008).

Measurement of anthocyanin content

Measurement of anthocyanin content was performed according to Mita *et al.* (1997). Approximately 100 mg of 15-d-old seedlings grown in pots were collected in Eppendorf tubes, flash-frozen in liquid N₂ and ground into powder. One ml of 1% (v/v) hydrochloric acid in methanol was added to each sample tube and the tubes were vigorously vortexed. After 1 d of incubation at 4 °C, the mixture was centrifuged at 13 000 rpm for 15 min and absorbance of the supernatant was measured at 530 nm and 657 nm. The formula $[A_{530} - (1/4) \times A_{657}]$ was used to determine anthocyanin concentration. One anthocyanin unit is equivalent to one absorbance unit $[A_{530} - (1/4 \times A_{657})]$ in 1 ml of extraction solution.

Heat-shock treatment

Heat-shock treatments were performed according to Zimmerli *et al.* (2008). One to two-hundred surface-sterilized seeds were plated in rows on sterilized half-strength MS medium and grown horizontally under continuous light conditions. *Arabidopsis*-acquired thermotolerance was evaluated by moving 12-d-old plantlets *in vitro* grown at 22 °C to 38 °C for 45 min. The chamber containing the plants was then allowed to heat up for 10 min to reach 45 °C. The plants were then kept at 45 °C for an additional 80 min. All heat-shock treatments were performed in the dark. After heat-shock treatment, the plants were returned to 22 °C in continuous light, and the evaluation of viability was assessed 4 d later. Seedlings were also photographed after 4 d. Plants were considered as survivors if no necrosis was visible on true leaves when observed at $\times 100$ magnification with a stereo microscope.

Measurement of leaf weight

Forty-eight hours after chemical treatment, rosettes from 4-week-old pot-grown *Arabidopsis* were excised for fresh weight analysis.

Pseudomonas syringae bioassays

For bacterial inoculation, cells were collected by centrifugation, resuspended in 10 mM MgSO₄·7H₂O at $A_{600}=0.2$, corresponding to a concentration of 10^8 cfu ml⁻¹. Three-week-old plants were dipped in a solution of 2×10^7 cfu ml⁻¹ bacteria containing the surfactant, Silwet L-77 (Biomax Scientific Co., Ltd., Taiwan) at a concentration of 0.01% of the volume of buffer. Plants inoculated with *Pst* DC3000 were then kept in 100% relative humidity during the first 24 h post-inoculation. For colony-forming units (cfu) determination, infected tissues were collected 48 h post-inoculation. After collection and weight evaluation, leaves were washed twice with sterile water and homogenized in 10 mM MgSO₄·7H₂O. Quantification was done by plating appropriate dilutions on King's B agar containing rifampicin (100 mg l⁻¹; Biomax Scientific Co., Ltd., Taiwan) using sterilized microbeads (Boeco, Germany). All plates were cultivated in the dark at 28 °C and colonies formed were quantified after 48 h.

qRT-PCR

For each sample, leaves from three pots containing 20–30 3-week-old *Arabidopsis* were harvested at the indicated time points, flash-frozen in liquid N₂, and kept at -80 °C. Total RNA was extracted and purified using the RNeasy Plant Mini Kit (Qiagen, Germany) with additional DNA clean-up using the RNase-Free DNase Set (Qiagen, Germany). Complementary DNA was synthesized from 2 µg of total RNA using oligo(dT) primers and the reverse transcriptase from the M-MLV kit (Invitrogen, USA). SYBR Green PCR Master Mix (Bio-Rad, USA) and the iCycler Sequence Detection System (iQ⁵ Real-Time PCR Detection System, Bio-Rad, USA) was employed for quantitative real-time PCR (qRT-PCR) analysis. The thermal cycling program was composed of an initial 3 min at 95 °C, followed by 40 cycles of 95 °C for 30 s, 54 °C for 35 s, 72 °C for 35 s. Melting curve was run from 55 °C to 95 °C with 10 s time intervals to ensure the specificity of the product. Data were analysed using Bio-Rad iQ⁵ software (version 2.0). *EF-1-ALPHA* (At5g60390), was used as the reference gene for normalization of gene expression levels in all samples. For amplification, primer sequences were AAAACTTAGCCTGGGG-TAGCGG (forward) and CCACCATTGTTACACCTCACTTTG (reverse) for *PRI* (AT2G14610), AAGCGTCTCATGATGTACC (forward) and ACTGAAAAGAGCCTGACC (reverse) for *CHS* (AT5G13930), ATGGTTAGTCAGAAAGAGACC (forward) and TAAAGTGAGTAGCGTCTTGG (reverse) for *DFR* (AT5G42800), and TGAGCACGCTCTTCTTGCTTCA (forward) and GGTGGTGGCATCCATCTTGTTACA (reverse) for *EF-1-ALPHA*.

Gus staining

For GUS expression analysis, 4-d-old seedlings were transferred to GUS staining buffer (100 mM sodium phosphate, pH 7.0, 10 mM EDTA, 0.5 mM K₄Fe(CN)₆, 0.5 mM K₃Fe(CN)₆, and 0.1% Triton X-100) containing 1 mM 5-bromo-4-chloro-3-indolyl β-D-GlcUA (X-gluc) and incubated at 37 °C for the specified time.

Results

BABA induces a SIMR in *Arabidopsis*

A recent study suggests that BABA induces a stress response in *Arabidopsis* (Zimmerli *et al.*, 2008). Plants exposed to sub-lethal stresses exhibit the SIMR (Potters

et al., 2007, 2009). Since BABA is known to inhibit root growth (Zimmerli *et al.*, 2008), the possibility that BABA induces a SIMR in *Arabidopsis* was further investigated by testing whether BABA-mediated root growth inhibition is correlated with reduced cell division in the meristematic root tissue. The mitotic activity of the root meristem was evaluated by analysing the promoter activity of the mitotic cyclin *CYCBI;1* (DiDonato *et al.*, 2004; Negi *et al.*, 2008). BABA reduced cell cycle activity of the root meristem, as monitored by the *proCYCBI;1:GUS* reporter activity (DiDonato *et al.*, 2004; Negi *et al.*, 2008) (Fig. 1A). This observation suggests that BABA reduced cell division in the root meristematic zone. BABA also increased lateral organs by increasing lateral root density (Fig. 1B). As already observed (Zimmerli *et al.*, 2008), BABA inhibited root

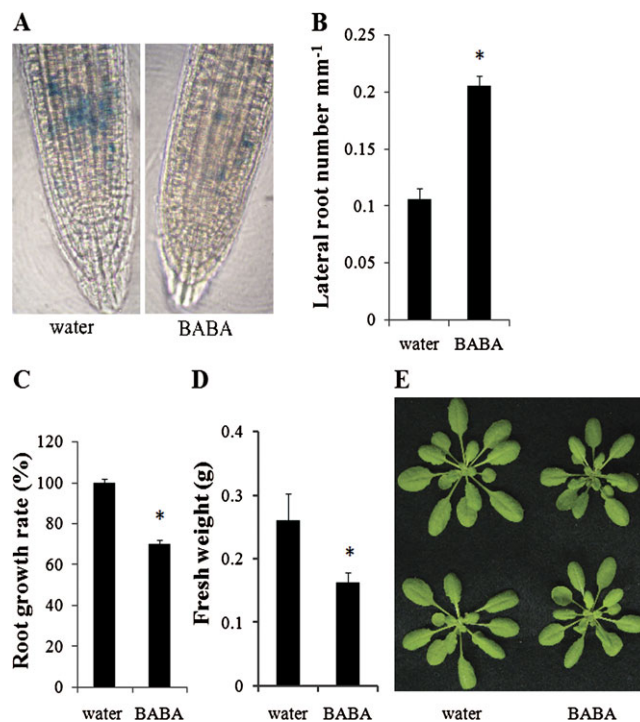


Fig. 1. BABA provokes a stress-induced morphogenic response in *Arabidopsis*. (A) BABA affects cell cycle activity of the root meristem. Cell cycle activity was evaluated by measuring primary root tip *proCYCBI;1:GUS* reporter activity (Negi *et al.*, 2008). Five-d-old seedlings were vertically grown in the presence of water or 500 µM BABA and stained for 2 h for GUS activity. (B) Effects of BABA on lateral root density. Lateral root density was evaluated on 15-d-old *in vitro* plants vertically grown on half-strength MS medium supplemented with 75 µM BABA or water (control). The data are means ± SE ($n > 30$). (C) Comparison of primary root length of 15-d-old *Arabidopsis* seedlings vertically grown on half-strength MS medium containing 75 µM BABA with the water control. (D, E) BABA inhibits *Arabidopsis* growth. Fresh weight (D) and size (E) were evaluated 3 d after water (control) or 300 µM BABA treatments. Error bars are SD. All experiments were repeated at least three times. Representative results are shown. (B, C, D) The asterisk indicates significant difference ($P < 0.01$, Student's t-Test).

growth (Fig. 1C). In addition, BABA-treated plants were found to be smaller with a reduced fresh weight as compared to the wild type (Fig. 1D, E). Together these data suggest that BABA acts as a stress agent that provokes a SIMR in *Arabidopsis*.

BABA induces accumulation of anthocyanin

Foliar anthocyanin acts as modulators of stress signals (Steyn *et al.*, 2002; Gould and Lister, 2006). BABA-treated *Arabidopsis* accumulated more anthocyanin in the petioles of 4-week-old *Arabidopsis* compared to the non-treated control (Fig. 2A). In addition, the total anthocyanin content in 5-week-old BABA-treated *Arabidopsis* was significantly higher than the water control (Fig. 2B). The anthocyanin pathway in *Arabidopsis* is catalysed by several important regulatory enzymes, including chalcone synthase (CHS) and dihydroflavonol-4-reductase (DFR) (Lillo *et al.*, 2008). qRT-PCR analyses revealed that the expression levels of these genes were elevated in BABA-treated plants (Fig. 2C). These results indicate that BABA may stimulate

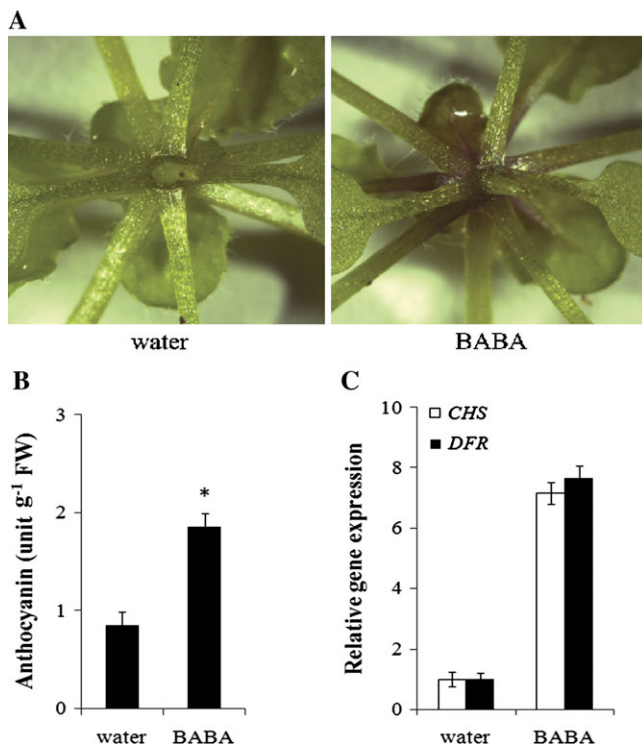


Fig. 2. BABA induces accumulation of anthocyanin. Foliar anthocyanin accumulation was observed in 4-week-old (A) or in 5-week-old (B) *Arabidopsis*. The asterisk indicates significant difference ($P < 0.01$, Student's t-Test). (C) Effect of BABA on the transcript levels of *CHS* and *DFR* genes in *Arabidopsis*. qRT-PCR relative expression levels were monitored in 3-week-old *Arabidopsis*. *EF-1-ALPHA* was used as an internal standard control. Expression levels of BABA-treated *Arabidopsis* were compared to water-treated controls (defined value of 1). Error bars are SD ($n=3$ technical replicates). For all experiments, *Arabidopsis* were treated with water (control) or 200 μ M BABA and samples were collected 48 h later. Experiments were repeated twice with similar results.

anthocyanin biosynthesis by regulating the expression of *CHS* and *DFR*.

L-Glutamine reduces the BABA-mediated SIMR

All amino acids, except for L-glutamine, cause the so-called general amino acid inhibition (Bonner *et al.*, 1996; Bonner and Jensen, 1997). The molecular basis for this phenomenon is not clear, but it is prevented by L-glutamine (Bonner *et al.*, 1996; Bonner and Jensen, 1997). Since the chemical BABA is a non-protein amino acid, it may, like natural amino acids, induce the general amino acid inhibition response. To confirm this hypothesis, inhibition of BABA-mediated SIMR by L-glutamine was evaluated. L-Glutamine was found to reduce BABA-mediated root growth inhibition and the BABA-mediated increase in lateral root density (Fig. 3A, B, C). In addition, BABA reduction of *CYCB1:1* promoter activity as demonstrated by *proCYCB1:1:GUS* staining was partially abolished by L-glutamine (Fig. 3D). L-Glutamine treatment also partially restored a normal vegetative growth pattern in *Arabidopsis* treated with BABA (Fig. 3E, F). Together these data indicate that L-glutamine can largely rescue the BABA-mediated SIMR. These results also suggest that BABA may cause a general amino acid inhibition in *Arabidopsis*.

L-Glutamine inhibits BABA-mediated accumulation of anthocyanin

Arabidopsis plants showed increased accumulation of anthocyanin after treatment with BABA (Figs 2A, B, 4A). To test whether L-glutamine can also rescue this phenotype, anthocyanin concentration was analysed in plants treated with both BABA and L-glutamine. Similar to the effect of L-glutamine on the BABA-mediated SIMR, L-glutamine reduced the BABA-mediated increase of anthocyanin accumulation (Fig. 4A, B). L-Glutamine also inhibited the accumulation of *CHS* and *DFR* transcripts of the anthocyanin biosynthetic pathway in BABA-treated *Arabidopsis* (Fig. 4C). Together these data shows that L-glutamine treatment restores a normal level of anthocyanin in BABA-treated plants.

BABA-enhanced *Arabidopsis* thermotolerance is altered by L-glutamine

L-Glutamine treatment was found to inhibit the BABA-mediated SIMR and anthocyanin accumulation in *Arabidopsis*. To test further whether L-glutamine can counteract the BABA effect, its impact on BABA-mediated *Arabidopsis* acquired thermotolerance was analysed (Zimmerli *et al.*, 2008). Ten-day-old *Arabidopsis* seedlings were heat acclimated at 38 °C for 45 min and then heat shocked at 45 °C for 90 min (Zimmerli *et al.*, 2008). As preliminary experiments observed (Zimmerli *et al.*, 2008), most of the BABA-treated *Arabidopsis* survived this heat shock treatment, while the water-treated controls did not (Fig. 5A, B). L-Glutamine did not affect the level of heat resistance, but when added with BABA, L-glutamine dramatically reduced BABA-induced heat protection (Fig. 5A, B). These results

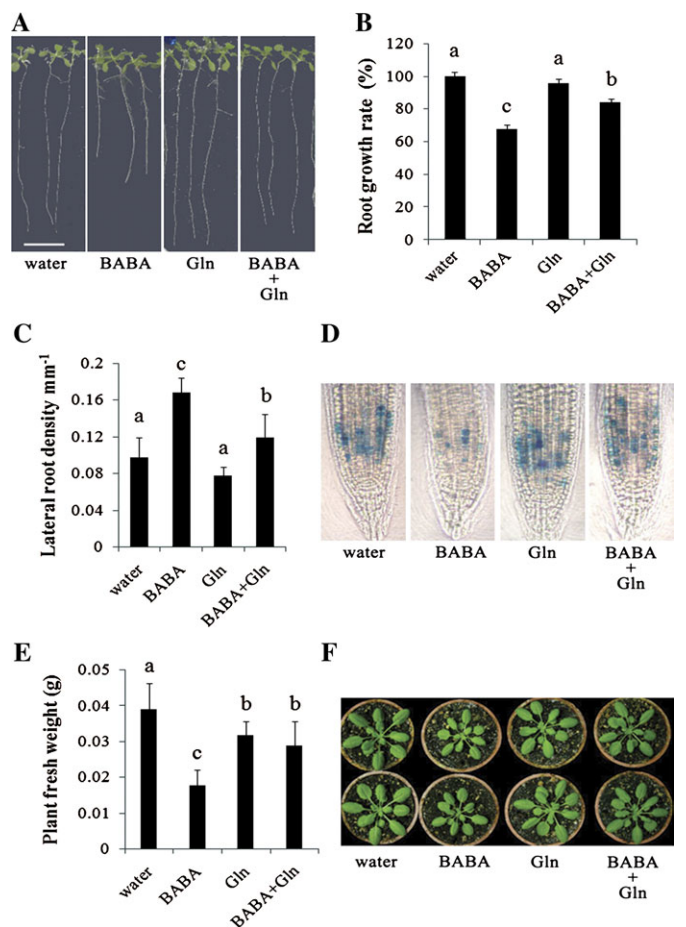


Fig. 3. L-Glutamine partially rescues the BABA-mediated stress-induced morphogenic response. (A, B) Comparison of primary root length (A) and growth rate (B) of 15-d-old *Arabidopsis* seedlings grown on half-strength MS medium containing 75 μ M BABA, 75 μ M L-glutamine (Gln) or both L-glutamine and BABA (BABA+Gln). Scale bar=1 cm. (C) Lateral root density. Treatments were performed as in (A). The data are means \pm SE ($n > 30$). (D) L-Glutamine removes BABA inhibition on cell cycle activity. Cell cycle activity was evaluated by measuring primary root tip *proCYCB1;1::GUS* reporter activity (Negi *et al.*, 2008). Five-day-old seedlings were grown in the presence of 500 μ M BABA, 500 μ M L-glutamine (Gln) or BABA and L-glutamine together (BABA+Gln) and stained for 2 h for GUS activity. (E, F) Fresh weight (E) and plant size (F) 3 d after treatment with 300 μ M BABA, 10 mM L-glutamine (Gln) or both BABA and L-glutamine (BABA+Gln). Error bars are SD ($n > 30$). Experiments were repeated three times. Representative results are shown. (B, C, D) Means with different letters are significantly different ($P < 0.05$) based on a Least Significant Different (LSD) test.

indicate that L-glutamine inhibits BABA-mediated *Arabidopsis* acquired thermotolerance and further support the idea that L-glutamine counteracts the BABA effect in *Arabidopsis*.

L-Glutamine inhibits BABA-induced resistance to *Pst* DC3000

BABA enhances *Arabidopsis* resistance to biotic stresses (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006). The

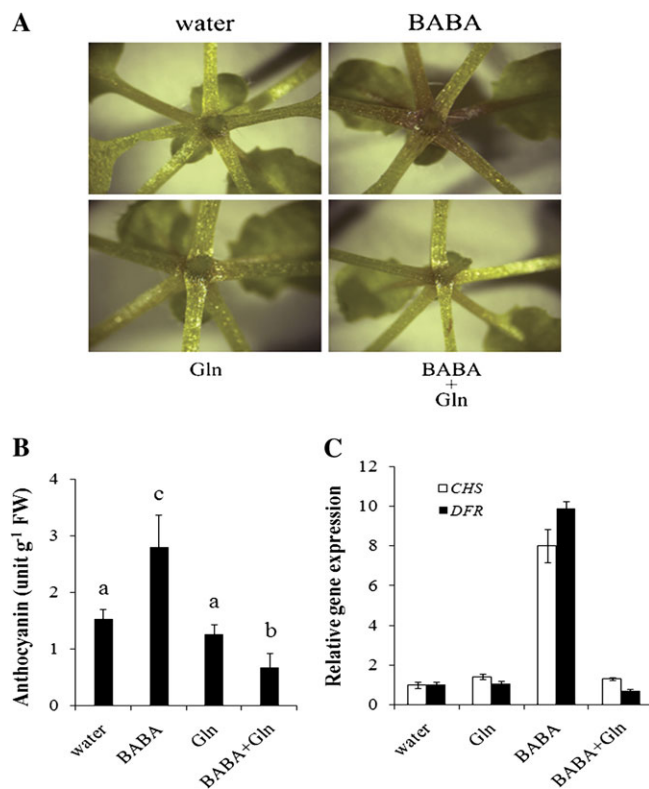


Fig. 4. L-Glutamine inhibits BABA mediated anthocyanin accumulation. (A) Foliar anthocyanin accumulation in 3-week-old *Arabidopsis*. (B) Evaluation of anthocyanin content in 5-week-old *Arabidopsis*. Means with different letters are significantly different ($P < 0.05$, LSD test). (C) BABA-mediated up-regulation of *CHS* and *DFR* mRNAs is inhibited by L-glutamine. qRT-PCR relative expression levels were monitored in 3-week-old *Arabidopsis*. *EF-1-ALPHA* was used as an internal standard control. Expression levels were compared to water-treated controls (defined value of 1). Error bars are SD ($n=3$ technical replicates). For all experiments, *Arabidopsis* samples were collected 48 h post-treatment with 250 μ M BABA, 10 mM L-glutamine (Gln), or both BABA and L-glutamine (BABA+Gln). Experiments were repeated twice with similar results.

effect of L-glutamine on BABA-induced resistance was further evaluated on the symptoms of *Arabidopsis* infected with the virulent bacterial pathogen *Pst* DC3000 3 d post-inoculation (dpi). In addition, bacterial titres were also evaluated at 2 dpi. L-Glutamine reduced BABA-induced resistance at the symptom and titre levels (Fig. 6A, B). These data indicate that L-glutamine treatment partially counteracts BABA-induced resistance against virulent bacteria.

BABA-induced priming of PR1 expression is inhibited by L-glutamine

BABA enhances *Arabidopsis* resistance to *Pst* DC3000 through priming of the SA defence signalling (Zimmerli *et al.*, 2000; Ton *et al.*, 2005). Since L-glutamine treatment inhibits BABA-induced resistance to *Pst* DC3000 (Fig. 6A, B), the possibility that L-glutamine also blocks BABA-mediated priming of the SA defence marker gene *PR1* was

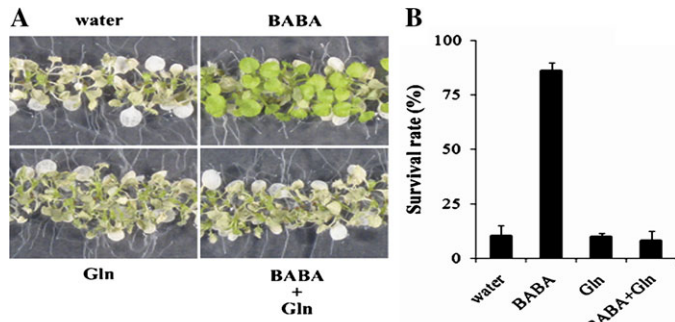


Fig. 5. BABA-enhanced *Arabidopsis* acquired thermotolerance is inhibited by L-glutamine. (A, B) Symptoms (A) and survival rate (B) of 2-week-old heat-shocked *Arabidopsis* seedlings (see details in the Materials and methods) pretreated with 500 μ M BABA, 500 μ M L-glutamine (Gln) or both BABA and L-glutamine (BABA+Gln). Heat-induced and damaged cells in (A) were evaluated 4 d after the heat shock treatment. Survival rates represent the mean percentage survivors and SD ($n > 10$ plates consisting of approximately 150 seedlings per plate). Experiments were repeated three times. Representative results are shown.

tested. The expression level of *PR1* was evaluated by qRT-PCR at 18 h post *Pst* DC3000 inoculation. As expected, BABA primed *PR1* expression (Fig. 6C). Without bacterial infection, L-glutamine treatment alone or mixed with BABA did not affect *PR1* expression levels. However, *Arabidopsis* treated with both L-glutamine and BABA did not demonstrate a primed *PR1* expression after bacterial infection (Fig. 6C). L-Glutamine thus strongly reduced BABA priming of *PR1*. This observation suggests that L-glutamine-mediated inhibition of BABA-induced resistance against *Pst* DC3000 functions through a blockage of BABA priming.

Discussion

BABA induces a SIMR in *Arabidopsis*

In *Arabidopsis*, the chemical BABA has been shown to enhance disease resistance and to increase salt, drought, and thermotolerance (Zimmerli *et al.*, 2000, 2001, 2008; Ton and Mauch-Mani, 2004; Jakab *et al.*, 2005; Ton *et al.*, 2005). BABA does not activate the defence response directly but rather sensitizes plants to respond more quickly and strongly to biotic and abiotic stresses. This process is referred to as priming (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006). The mechanisms underlying the BABA mode of action and, particularly, the priming phenomenon are still poorly understood. In addition, the metabolic pathways through which BABA mediates both abiotic and biotic stress resistance are still being elucidated. In the present study, it was shown that BABA induces a phenotypical response similar to the recently described SIMR (Potter *et al.*, 2007, 2009). BABA-treated seedlings clearly demonstrated an inhibition of cell division in the meristematic root tissue (Fig. 1A) and a concomitant reduction in root growth

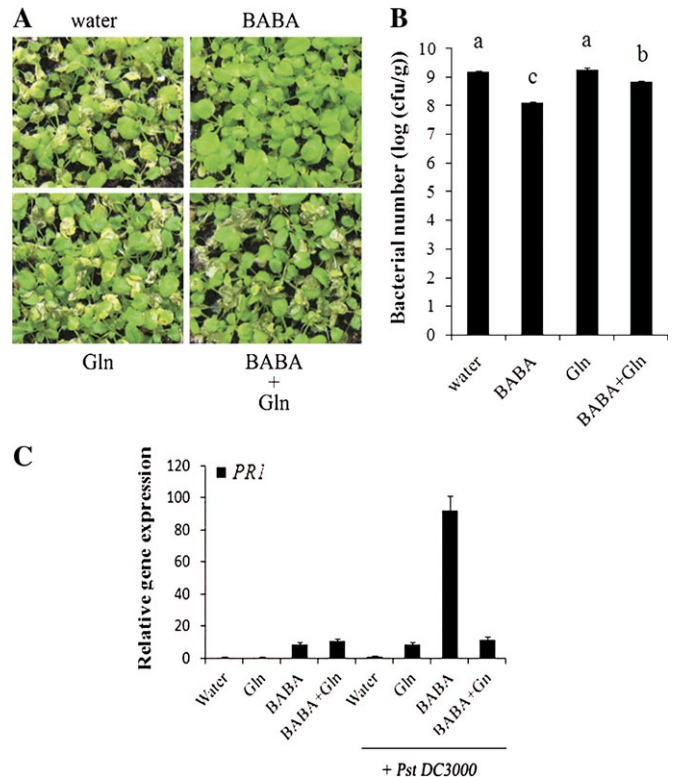


Fig. 6. L-Glutamine inhibits BABA-induced resistance to bacteria and priming. (A) Disease symptoms were evaluated 3 dpi with the virulent bacterial pathogen *Pst* DC3000. (B) Bacterial growths were evaluated 48 h post-bacterial dip inoculation. Means with different letters are significantly different ($P < 0.05$, LSD test). (C) BABA priming of *PR1* expression is inhibited by L-glutamine. qRT-PCR relative expression levels at 18 h post-bacterial inoculation. *EF-1-ALPHA* was used as an internal standard control. Expression levels were compared to water-treated controls (no bacterial inoculation, defined value of 1). For all experiments, three-week-old *Arabidopsis* were treated 48 h before *Pst* DC3000 inoculation with 150 μ M BABA, 10 mM L-glutamine (Gln) or both BABA and L-glutamine (BABA+Gln). Error bars are SD ($n = 3$ technical replicates). Experiments were repeated three times. Representative results are shown.

is observed (Fig. 1C; Zimmerli *et al.*, 2008). Secondly, BABA was found to increase lateral organs by increasing lateral root density (Fig. 1B). Furthermore BABA-treated *Arabidopsis* seedlings demonstrated a reduced vegetative growth and were found to be slightly stunted (Fig. 1D, E). All of these phenotypes are characteristic of the SIMR (Potter *et al.*, 2007, 2009). Consequently, BABA may act as a stressing agent in *Arabidopsis*. This hypothesis has been further confirmed as BABA-treated *Arabidopsis* activate ABA and ethylene stress signalling concomitantly with an accumulation of stress-induced transcripts (Zimmerli *et al.*, 2008). In addition, known modulators of stress signalling such as anthocyanin (Steyn *et al.*, 2002; Gould and Lister, 2006) accumulated upon BABA treatment (Fig 2A, B). Taken together, it suggests that BABA provokes a mild chronic stress in *Arabidopsis* that would explain the observed SIMR.

What kind of mild chronic stress may BABA induce?

Since BABA is a non-protein amino acid and is not metabolized *in planta* (Zimmerli *et al.*, 2000), it was speculated that this chemical provokes a general amino acid inhibition, as do amino acids when supplied to the plant at high concentration (Bonner *et al.*, 1996).

The molecular basis for this phenomenon is still not clear, it is, however, known that exogenous application of amino acids to suspension culture of *Nicotiana glauca* cells is toxic and inhibits cell growth (Bonner *et al.*, 1996; Bonner and Jensen, 1997). It has been shown that this 'general amino-acid stress' is prevented by the addition of L-glutamine (Bonner *et al.*, 1996; Bonner and Jensen, 1997). The removal of BABA-induced stress effects in *Arabidopsis* by L-glutamine was thus tested. BABA-induced SIMR was found to be largely reduced by L-glutamine treatment and, concomitantly, BABA-induced resistance to heat shock and to virulent bacteria such as *Pst* DC3000 was also reduced by L-glutamine. These observations suggest that BABA induces a general amino acid stress inhibition. It is, however, still possible that L-glutamine and BABA may share a common transporter and L-glutamine compete for BABA transport. L-Glutamine may thus inhibit BABA translocation into the cell in the presence of excess of L-glutamine. Future work is needed to elucidate this point. Importantly, it is clear that L-glutamine attenuates the BABA effect in *Arabidopsis*. It is worthwhile realizing that both the BABA-mediated SIMR and the protective effects are affected by L-glutamine treatment.

Does BABA prime the Arabidopsis defence response by stress imprinting?

Priming behaviour is critical for adaptation to complex, ever-changing environmental conditions (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006; Beckers and Conrath, 2007; Bruce *et al.*, 2007; Frost *et al.*, 2008). Priming is usually defined as a sensitization to stress responsiveness. As a result, priming boosts the plant's defence response and primed plants are more resistance to biotic and abiotic stress (Conrath *et al.*, 2002; Prime-A-Plant Group, 2006; Pham *et al.*, 2007; Beckers *et al.*, 2009; Jung *et al.*, 2009). It was shown here that BABA acts as a chemical stress and therefore induces the SIMR in *Arabidopsis*. The removal of the SIMR by the addition of L-glutamine was found to be correlated with a loss of BABA-mediated priming and protection. This observation suggests that BABA primes the *Arabidopsis* defence response by stress imprinting. Preliminary stress exposure or stress imprinting is indeed known to induce priming and resistance in plants (Bruce *et al.*, 2007; Galis *et al.*, 2009). Typically, repeated exposure to stressful concentrations of the phytohormone ABA impaired the *Arabidopsis* stomatal response to light (Goh *et al.*, 2003). Similarly, treatment with sub-lethal concentrations of paraquat correlates with greater oxidative resistance in plants (Ye and Gressel, 2000). *Arabidopsis* first exposed to osmotic stress demonstrate an altered Ca²⁺

response that leads to the acquisition of stress-tolerance (Knight *et al.*, 1998). Although all these examples suggest that stress imprinting is caused by previous exposure to mild stress conditions, most of them relate to priming and protection after a second exposure to the same or to a very similar stress. Soil drench treatment with the chemical BABA primes appropriate defence mechanisms and provides long-term protection against biotrophic bacteria (Zimmerli *et al.*, 2000; Ton *et al.*, 2005; Goellner and Conrath, 2008), necrotrophic fungi (Zimmerli *et al.*, 2001; Ton and Mauch-Mani, 2004; Flors *et al.*, 2008), and abiotic stresses (Jakab *et al.*, 2005). L-Glutamine was found to inhibit BABA-induced resistance to both heat shock and the bacterial pathogen *Pst* DC3000, two different type of stresses (i.e. abiotic and biotic). This suggests that this observed multifaceted BABA-mediated resistance is induced by a mild chronic stress imprinting. Together, these observations indicate that BABA-mediated stress imprinting may act at a convergent node that induces resistance to different, unrelated stresses. Determining the underlying mechanisms involved in BABA-mediated priming should thus uncover general, global components of the stress imprinting phenomenon.

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