β -Aminobutyric Acid–Induced Resistance Against Root-Knot Nematodes in Rice Is Based on Increased Basal Defense

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The nonprotein amino acid β -aminobutyric acid (BABA) is known to protect plants against various pathogens. The mode of action is relatively diverse and specific in different plant-pathogen systems. To extend the analysis of the mode of action of BABA to plant-parasitic nematodes in monocot plants, we evaluated the effect of BABA against the root-knot nematode (RKN) *Meloidogyne graminicola* in rice. BABA treatment of rice plants inhibited nematode penetration and resulted in delayed nematode and giant cell development. BABA-induced resistance (BABA-IR) was still functional in mutants or transgenics defective in salicylic acid biosynthesis and response or abscisic acid (ABA) response. Pharmacological inhibition of jasmonic acid (JA) and ethylene (ET) biosynthesis indicated that BABA-IR against rice RKN likely occurs independent of JA and ET. However, histochemical and biochemical quantification in combination with quantitative real-time reverse transcription-polymerase chain reaction data suggest that BABA protects rice against RKN through the activation of basal defense mechanisms of the plant, such as reactive oxygen species accumulation, lignin formation, and callose deposition.

The nonprotein amino acid β -aminobutyric acid (BABA) is a well-known activator of plant defense responses and is active against a wide range of biotic and abiotic stress factors (Cohen 2002; Jakab et al. 2001, 2005; Pastor et al. 2013; Zimmerli et al. 2008). The mechanisms of BABA-induced resistance (BABA-IR) are diverse and appear to be pathogen- and plant-specific (Cohen 2002; Jakab et al. 2001; Piękna-Grochala and Kępczyńska 2013).

Whereas the jasmonic acid (JA) and ethylene (ET) pathways do not seem to play an important role in BABA-IR against *Pseudomonas syringae* pv. *tomato* DC3000 and *Botrytis cinerea* in *Arabidopsis*, this phenomenon was shown to be largely dependent on an intact salicylic acid (SA) signaling pathway (Zimmerli et al. 2000, 2001). In accordance with this finding, a lack of BABA-IR against *Tobacco mosaic virus* (TMV) was

observed in SA-deficient *NahG* tobacco (Siegrist et al. 2000). Nevertheless, the same *NahG* tobacco plants, when challenged with downy mildew, were protected by BABA similarly to wild-type plants (Cohen 2001), demonstrating that the mode of action of BABA-IR differs depending on the pathogen and might not be exclusively SA-dependent. Confirming this view, BABA-IR against the oomycete *Hyaloperonospora parasitica* as well as the necrotrophic fungi *Alternaria brassicicola* and *Plectosphaerella cucumerina* was unaffected in *Arabidopsis* genotypes impaired in the SA pathway (Ton and Mauch-Mani 2004; Zimmerli et al. 2000) but was correlated with an augmentation of callose-rich papillae surrounding attempted sites of penetration (Ton and Mauch-Mani 2004; Zimmerli et al. 2000). In the case of the *Arabidopsis* interaction with necrotrophic fungi, BABA-induced callose formation was shown to be controlled by the abscisic acid (ABA)-pathway, as ABA mutants did not reveal callose accumulation and were no longer protected by BABA-treatment (Ton and Mauch-Mani 2004). Also, in lettuce, BABA-IR against the oomycete *Bremia lactucae* acts through a rapid callose-rich encasement of the primary invading structures (Cohen et al. 2010). Next to callose deposition, Cohen et al. (2010) observed the accumulation of hydrogen peroxide (H_2O_2) in BABA-treated lettuce plants and this observation was corroborated in tomato and grapevine, in which a strong accumulation of reactive oxygen species (ROS) was observed in BABA-treated plants (Baysal et al. 2007; Dubreuil-Maurizi et al. 2010).

Although less-studied than for bacterial and fungal pathogens, some studies have also demonstrated the potential of BABA-IR against plant-parasitic nematodes. While generally unnoticed due to their microscopic size, nonspecific above-ground symptoms, and below-ground symptoms merely under low infection pressure, plant-parasitic nematodes are among the most damaging pathogens on plant roots. Nearly half of the total economic loss caused by nematodes derives from just two crops, rice and maize (Sasser and Freckman 1987), and one of the most damaging types of nematodes attacking rice roots is the sedentary root-knot nematode (RKN) *Meloidogyne graminicola* (Kyndt et al. 2014). The infective second stage juvenile (J2) of this RKN invades the plant root in the elongation zone and moves toward the root apex to make a U-turn into the vascular bundle, where it selects four to eight cells to induce a feeding site (Kyndt et al. 2013). Repeated rounds of nuclear division and cell growth in the absence of cytokinesis lead to the formation of multinucleate, hypertrophied giant cells up to 100-times the size of normal root vascular parenchyma cells, and hyperplasia of surrounding cells causes the formation of macroscopically visible

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root-knots or galls. Besides to this gall formation, heavy infection of rice plants causes yellowing, stunting, reduction of tillering, delayed maturation, root proliferation, and 17 to 32% grain yield losses (Kyndt et al. 2014).

Application of BABA has been shown to reduce gall formation and reproduction of *M. javanica* in tomato (Oka et al. 1999; Sahebani and Hadavi 2009), pineapple (Chinnasri et al. 2006), mung bean plants (Ahmed et al. 2009), and cucumber (Sahebani et al. 2011). Also in wheat, foliar sprays and soil drenches with BABA led to resistance against the sedentary cyst nematodes *Heterodera avenae* and *Heterodera latipons* as well as RKN *Meloidogyne* spp. (Oka and Cohen 2001). Although the mode of action of BABA-IR against nematodes has not been studied in detail, an induced H_2O_2 production was observed in BABA-treated tomato and cucumber plants when infected by *M. javanica* (Sahebani and Hadavi 2009; Sahebani et al. 2011). In the present study, we investigate i) the potential of BABA-IR against RKN infection in rice and ii) the physiological mechanisms involved. First, we show that soil drenching of rice plants with BABA is actively inducing resistance against RKN *M. graminicola*. Studies with mutants or inhibitors point out that SA, JA, and ET are not required for BABA-IR against RKN. Instead, histochemical and cytological investigation of the defense reactions demonstrate that the basal defense of rice is enhanced, involving boosted H_2O_2 , lignin, and callose accumulation in the BABA-treated plants. Our data favor a model in which BABA-IR against *M. graminicola* acts through augmenting multiple facets of the plant basal defense and is largely independent of the SA, JA, and ET pathways.

RESULTS

BABA induces resistance against RKN in rice by inhibiting nematode penetration and delaying nematode and giant cell development.

To determine whether BABA can be used as an elicitor to induce rice resistance against RKN, the effect of BABA on *M. graminicola* attraction was first examined. Second stage juveniles (J2) in the soil are attracted to the roots near the tips (Curtis et al. 2009). We examined whether there was a difference in attraction of J2 in BABA-treated versus water-treated (control, Nipponbare) roots. At 9 h postinoculation (hpi), the number of

nematodes surrounding the root elongation zone was counted. The result in Figure 1A shows that there is no significant difference ($P > 0.05$) in the number of J2 close to root tips of BABA-treated and control roots. From this data we can conclude that BABA does not influence nematode attraction to the roots.

To further evaluate the effect of BABA on *M. graminicola* penetration, the J2 inside roots were counted at 50 hpi. BABA-treated plants contain a significantly lower number of J2 ($P < 0.05$) than water-treated plants (Fig. 1B). This result illustrates that BABA-IR starts to be functional at the penetration stage. The observed effect cannot be caused by direct effects of BABA on nematodes, since nematode mobility was not impaired when J2 were incubated in a 3.5-mM BABA solution for 72 h (data not shown), and nematodes soaked in a 3.5-mM BABA solution for 1 day were equally infective when compared with the water-soaked nematodes (Supplementary Fig. S1).

The effect of BABA-IR on nematode development was evaluated at 16 days postinoculation (dpi) (Fig. 1C). Compared with control plants (water-treated), pretreatment with BABA resulted in a significantly lower number of adult females per plant (65% reduction) at 16 dpi. This indicates that BABA is an excellent inducer of rice defense against *M. graminicola*.

In order to obtain a more detailed understanding of BABA-IR against *M. graminicola*, the developmental stages of *M. graminicola* in BABA-treated plants were also recorded at 16 dpi and were compared with control plants at this time point (Fig. 2). To be able to investigate the effect of BABA on nematode development once the nematodes had already penetrated the roots, this experiment was executed with two experimental set-ups: pre- and post-treatment. In case of pretreatment (Fig. 2A), plants were inoculated with RKN 1 day after soil drenching with BABA, the substrate was washed away from the roots at 2 dpi, and the plants were transferred to Hoagland solution for another 14 days. In case of post-treatment (Fig. 2B), plants were first inoculated with RKN, the substrate was washed away from the roots 2 days later, and the plants were transferred to a 3.5-mM BABA solution for 2 days, after which the plants were transferred to Hoagland solution for another 12 days. In both cases, control plants were treated in exactly the same way, except that they received water instead of BABA. At 16 dpi, significantly fewer and smaller adult females ($P < 0.05$) were recorded in BABA-treated roots in both experimental setups. The majority of nematodes (87 to

Fig. 1. β -Aminobutyric acid (BABA) induces resistance to the rice root-knot nematode *Meloidogyne graminicola*. Rice plants were soil drenched with 3.5 mM BABA or water 1 day before *M. graminicola* inoculation. **A**, Analysis of BABA-induced resistance against *M. graminicola* on the stage of attraction. The number of J2 surrounding the root elongation zone were recorded 9 h postinoculation (hpi). **B**, Effects of BABA on invasion of *M. graminicola* into rice plants. Number of nematodes inside roots was recorded at 50 hpi. **C**, The number of adult females per plant were recorded at 16 days postinoculation (dpi). Bars represent means $\pm$ standard error of nematodes from eight plants. Different letters indicate statistically significant differences (Student *t* test with $P \leq 0.05$). Data represent one of two (A) or three (B and C) independent experiments with similar results.

94%) in control plants had developed into adult females at 16 dpi; however, much lower percentages of adult females were observed in BABA-treated plants in both pre- and post-treatment experiments, with only about 52 and 9% developed nematodes, respectively (Fig. 2A and B). Besides fewer adult females in BABA-treated roots, the total number of nematodes in pretreated roots was significantly lower, which again confirmed that fewer nematodes had penetrated the BABA-treated roots. The post-treatment even had a stronger effect on BABA-IR against RKN development, as a large portion (91%) of nematodes remained in the juvenile stage. Although the roots were washed at 2 dpi in post-treatment, and one would expect that the nematodes had already entered the root system before BABA was applied (at 2 dpi), there was still a slightly lower number of nematodes observed in BABA post-treated plants compared with control. Figure 2 shows representative galls at 16 dpi in the control and BABA pretreated plants

(Fig. 2C and D, respectively). A delay of nematode development was clearly seen in BABA-treated plants; the majority of the nematodes in the control plants were producing egg masses, while nematodes in BABA-treated plants were still in juvenile stage.

Figure 3 shows the difference between 7-dpi giant cells formed in control plants (Fig. 3A) versus BABA-treated plants (Fig. 3B). Although the nematode feeding sites were still composed of enlarged cells with dense cytoplasm, multiple nuclei, and thickened cell walls, they appeared deformed in BABA-treated roots when compared with control roots. In line with this observation (Fig. 3C), nematode development at 7 dpi was severely hampered in the BABA pretreated roots (Fig. 3D).

These data demonstrate that BABA treatment of plants not only impedes rice RKN penetration but also inhibits their development, indicating that BABA is a strong inducer of plant defense against rice RKN.

Fig. 2. Effects of β -aminobutyric acid (BABA) on the development of *Meloidogyne graminicola* in rice plants. **A**, The different developmental stages of *M. graminicola* in the roots with BABA pretreatment at 16 days postinoculation (dpi). **B**, The different developmental stages of *M. graminicola* in the roots with BABA post-treatment at 16 dpi. Different letters indicate statistically significant differences (Student *t* test with $P \leq 0.05$). a or b indicates the significant differences in the J2 stage; A or B indicates the significant differences in the J3+J4 stages; α or β indicates the significant differences in adult females; and θ or ϵ indicates the significant differences in the total number of nematodes. Data represent one of two independent experiments with similar results, each experiment contained eight plants. Developing *M. graminicola* inside water-treated galls (C) and in a 3.5 mM BABA-treated gall (D) at 16 dpi. GC, giant cells; N, nematodes; and E, egg masses.

The expression of hormone-related genes in BABA-induced resistance against the rice RKN.

To determine whether plant defense hormones are involved in BABA-IR against *M. graminicola*, the expression of several hormone biosynthesis and response genes was investigated using real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) at different time points after BABA treatment and different time points after inoculation with nematodes (Fig. 4). The scheme of different treatments and sampling times is given in Figure 4B.

To check the importance of the SA pathway in BABA-IR against *M. graminicola*, the expression levels of genes involved in SA biosynthesis (*OsICS1*), signaling (*OsWRKY45*), and SA response (*OsPR1b*) were analyzed. *OsICS1* is a rice isochorismate synthase, the first catalytic enzyme in the production of SA from chorismate during plant defense (Wildermuth et al. 2001). *OsICS1* was significantly repressed in roots infected with *M. graminicola* at 1 dpi (time 2) and 3 dpi (time 3), in comparison with control roots (water-treated and noninoculated). This gene was repressed to similar levels at both time points when the roots were pretreated with BABA before inoculation. In contrast, the transcript levels of *OsICS1* did not significantly change in BABA-treated and noninoculated roots (time 1) compared with control roots (water-treated and noninoculated). *OsWRKY45* is known to be a key regulator in the SA signaling pathway and to be involved in benzothiadiazole (an SA analog)-induced resistance to *Magnaporthe*

grisea and *Xanthomonas oryzae* pv. *oryzae* (Shimono et al. 2007, 2012). The expression level of *OsWRKY45* was repressed in *M. graminicola*-infected roots, but its expression level was significantly up-regulated in BABA-treated roots at all the tested time points and both with or without nematode infection, when compared with corresponding control roots (water-treated and noninoculated).

OsPR1b is generally seen as a systemic acquired resistance (SAR) marker gene and it has been shown to be pathogen-inducible in rice after blast fungus and bacterial blight infection (Mitsuhashi et al. 2008). The transcription of *OsPR1b* significantly increased after *M. graminicola* infection at 1 and 3 dpi (times 2 and 3, respectively), but it did not respond to BABA treatment, when compared with corresponding water-treated and noninoculated control roots.

The JA biosynthesis gene *OsAOS2* (Mei et al. 2006), which has been shown to be pathogen-inducible, and the JA-inducible MYB transcription factor gene *OsJAMYB* (homolog of *Atmyb19*, NP2000039.1 [Lee et al. 2001]) showed a similar trend at the tested time points. Both genes were significantly down-regulated in *M. graminicola*-infected roots at 1 dpi (time 2) but were up-regulated at time 3 in all BABA-treated plants in comparison with water-treated noninoculated control roots.

The ET biosynthesis gene *OsACO7* and responsive gene *OsERF1* were used to evaluate the ET pathway. The qRT-PCR data indicated that *OsACO7* showed a significant downregulation in 1-day BABA-treated and noninoculated roots (time 1) and in

Fig. 3. A, Morphology of giant cells induced by *Meloidogyne graminicola* at 7 days postinoculation (dpi) in control and B, β -aminobutyric acid (BABA)-treated rice roots. C, Developing *M. graminicola* inside 7-dpi control galls and D, 7-dpi BABA-treated galls. The nematodes were visualized with acid fuchsin staining. The giant cells were stained with toluidine blue. GC, giant cells; N, nematode.

Fig. 4. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of the expression levels of defense hormone-related genes in rice roots after soil application of β -aminobutyric acid (BABA) alone or with *Meloidogyne graminicola* infection at different times. **A**, Data are shown as relative expression levels of hormone-related genes in water-treated and *M. graminicola* inoculated (Mg), BABA-treated (BABA), and BABA-treated plus inoculated (BABA+Mg) tissue in comparison with the control tissue (water-treated and noninoculated root tissue). Bars represent mean expression levels $\pm$ standard error from two biological replicates and three technical replicates, each containing a pool of eight plants. Asterisks indicate significant differential expression ($P \leq 0.05$). Data are shown as normalized relative expression levels in comparison with the control roots (expression level set at 1). Gene expression levels were normalized using three internal reference genes, *OsEXP*, *OsEXPnarsai*, and *OsUBQ5*. **B**, The scheme of different treatments and sampling times used in qRT-PCR analyses in this study. Light gray dots indicate time points for treatments and black dots, time points for sampling. Time 1, two samples: i) roots treated with 3.5 mM BABA for 1 day (BABA), ii) control roots treated with water for 1 day. Time 2, four samples: i) roots treated with 3.5 mM BABA for two days (BABA), ii) roots treated with 3.5 mM BABA for 2 days and inoculated with *M. graminicola* for 1 day (BABA+Mg), iii) roots treated with water for 2 days and inoculated with *M. graminicola* for 1 day (Mg), and iv) control roots treated with water for 2 days. Time 3, four samples: i) roots treated with 3.5 mM BABA for 4 days (BABA), ii) roots treated with 3.5 mM BABA for 4 days and inoculated with *M. graminicola* for 3 days (BABA+Mg), iii) roots treated with water for 4 days and inoculated with *M. graminicola* for 3 days (Mg), and iv) control roots treated with water for four days.

BABA-treated and inoculated roots (time 2), when compared with corresponding control roots (water-treated and noninoculated). *OsERF1* did not significantly respond to BABA treatment or to *M. graminicola* infection.

OsNCED3 is a key enzyme in the ABA biosynthesis pathway, and *OsLIP9* is an ABA-responsive gene. These genes did not strongly respond to inoculation at 1 and 3 dpi (times 2 and 3, respectively). However, 2 days after treatment, BABA induced a strong upregulation of *OsNCED3*, both in noninoculated and inoculated roots (time 2) in comparison with water-treated and noninoculated control roots. The ABA-responsive gene *OsLIP9* showed a similar expression trend as *OsNCED3*, although it was a little less induced by BABA.

Plants impaired or less efficient in hormone-related pathways are still protected by BABA treatment.

The qRT-PCR data described above showed that some genes involved in the SA and ABA pathways were strongly induced by BABA treatment at different time points (Fig. 4). In order to obtain a more detailed understanding of the role of these defense signaling pathways in BABA-IR against rice RKN, different rice transgenic plants were used to evaluate the efficiency of BABA-IR against *M. graminicola*.

NahG plants express a bacterial salicylate hydroxylase that degrades SA to catechol (Gaffney et al. 1993), and this decreases in planta concentrations of SA. *OsWRKY45* and *OsNPR1* both function in the signaling pathways downstream of SA but are independent of each other (Shimono et al. 2007). The ABA-inducible mitogen-activated protein kinase *OsMPK5* has been shown to play important roles in various biotic and abiotic stresses in rice (Xiong and Yang 2003). In this study, *NahG*, *WRKY45* RNAi, *NPR1* RNAi, and *MPK5* RNAi transgenic plants were used for infection tests to check the importance of the SA pathway and ABA response in BABA-IR. The data (Fig. 5) showed that BABA-IR is equally as effective in all transgenic plants as in their corresponding control lines (Nipponbare or Taipei 309).

Analysis of the mode of action of BABA using inhibitors of secondary metabolites.

To further test whether the resistance induced by BABA depends on the accumulation of secondary metabolites, BABA

was applied together with different chemical inhibitors of metabolite biosynthesis, 1 day prior to nematode inoculation. The mean number of adult females in each treatment divided by the mean number of adult females in control plants ($n = 8$) at 16 dpi is illustrated in Figure 6. Application of BABA onto JA biosynthesis-inhibited plants (Nipp+DIE+BABA) resulted in a similar effect of BABA-IR against RKN as in the control, with about a 53% reduction rate compared with about 59% in the control. Inhibiting ET biosynthesis by aminooxyacetic acid (AOA) also did not influence the effect of BABA-IR against RKN. Phenylalanine ammonia-lyase (PAL) is the first enzyme in the phenylpropanoid pathway and is involved in the biosynthesis of the polyphenols (Hahlbrock and Grisebach 1979). Blocking PAL activity by L-2-aminoxy-3-phenylpropionic acid (AOPP) significantly decreased BABA-IR against *M. graminicola*, with only about a 40% reduction compared with about 59% reduction in the control. Similarly, blocking ABA biosynthesis by fluridone resulted in only 38% nematode reduction by BABA treatment (Fig. 6). In conclusion, inhibiting the biosynthesis of JA or ET resulted in a similar BABA-IR as in control plants, whereas BABA-IR is less effective in PAL and ABA inhibitor-treated plants. These results indicate that PAL activity and ABA biosynthesis are both partially involved in BABA-IR against RKN, while JA and ET biosynthesis are not involved.

BABA-treated plants have increased lignin accumulation and expression of lignin-related genes in roots.

The observation in the inhibitor experiments (Fig. 6) indicated that PAL may be involved in BABA-IR against RKN; therefore, we further investigated the role of the corresponding pathway. PAL is an important enzyme in the phenylpropanoid pathway that catalyzes the deamination of phenylalanine to transcinnamic acid, a precursor of the lignin and flavonoid biosynthetic pathways (Dixon and Paiva 1995). Lignin confers mechanical strength to plant secondary cell walls, which contributes to basal defense against pathogens (Lewis and Yamamoto 1990), including plant-parasitic nematodes (Fogain and Gowen 1995; Wuyts et al. 2006, 2007).

Using biochemical quantification, significantly higher lignin content was found in rice roots 1 day after BABA treatment, in comparison with water-treated control roots (Fig. 7A). In line

Fig. 5. Effects of β -aminobutyric acid (BABA) against *Meloidogyne graminicola* in lines impaired in salicylic acid and abscisic acid signaling and corresponding control lines. BABA (3.5 mM) was soil-drenched 1 day before *M. graminicola* inoculation. The number of adult females per plant was counted at 16 days postinoculation (dpi). Bars represent means $\pm$ standard error of adult females from eight plants. Different letters indicate statistically significant differences (Duncan multiple range test with $\alpha = 0.05$). Data represent one of two independent experiments with similar results.

with these results, qRT-PCR on the expression of three important genes involved in lignin biosynthesis showed them to be significantly induced by BABA treatment at different time points (Fig. 7B). In contrast, in water-treated and nematode-inoculated roots, a downregulation of cinnamate 4-hydroxylase *OsC4H* (at 1 dpi, time 2) and *OsPAL* (at 3 dpi, time 3) was observed, confirming the previously observed downregulation of the phenylpropanoid pathway in giant cells and galls (Ji et al. 2013; Kyndt et al. 2012a). However, the expression of cinnamyl alcohol dehydrogenase *OsCAD6* did not respond to nematode infection.

To examine the role of lignin in BABA-IR against RKN, lignin was visualized in gall sections at different time points by Wiesner staining. A strong staining (red) was observed around feeding sites at all time points in BABA-treated plants (Fig. 7C, left), while it was not or was less-detected in feeding sites formed in control roots (Fig. 7C, right).

ROS generation in BABA-treated plants.

The production of ROS during the oxidative burst is characteristic of the defense response in plants (Levine et al. 1994). The hydrogen peroxide (H_2O_2) level was measured in rice roots upon BABA treatment. Increased levels of H_2O_2 were found in BABA-treated plants at 1 and 3 dpi, whether inoculated with *M. graminicola* or not (Fig. 8A). However, at 5 dpi, H_2O_2 levels were all similar between the different treatments.

The NADPH oxidases, which catalyze the reduction of oxygen to generate the superoxide anion, in plants also termed respiratory burst oxidase homolog (Rboh), were shown to be required for ROS accumulation in plant defense (Simon-Plas et al. 2002; Torres et al. 2006; Yoshioka et al. 2003). Here, we investigated the expression of one rice NADPH oxidase gene

(*OsRbohB*) by qRT-PCR (Fig. 8B). *OsRbohB* was strongly induced 2 days after BABA treatment (time 2) in both non-inoculated and nematode-inoculated plants.

Callose deposition is enhanced in BABA-treated galls.

Many researchers have reported that BABA induces callose deposition upon pathogen attack (Cohen et al. 2010, 2011; Hamiduzzaman et al. 2005; Ton and Mauch-Mani 2004; Walz and Simon 2009; Zimmerli et al. 2001). To further explore the mechanisms of BABA-IR against *M. graminicola*, callose was visualized in galls by aniline blue staining (Adam and Somerville 1996). Two days after *M. graminicola* infection, rice roots were washed and transferred to a 3.5-mM BABA solution or water for another 2 days, and then, root galls were used for callose staining (Fig. 9A). The results showed that callose deposits were strongly induced by BABA in galls. The formed callose spots were denser and bigger in BABA-treated galls than in control galls. The big bright dots seen in the BABA-treated roots were probably the aggregation of many callose speckles, as reported by Ham et al. (2007), upon *Pseudomonas syringae* pv. *phaseolicola* NPS3121 infection in *Arabidopsis*.

Callose deposition is a dynamic process coordinated through the activities of callose synthase and the callose hydrolyzing enzyme β -1,3-glucanase (Hao et al. 2008). The expression patterns of three callose synthase-encoding genes (*OsGSL1*, *OsGSL3*, and *OsGSL5*) and one callose hydrolyzing gene (*OsGNS5*) were investigated by qRT-PCR (Fig. 9B and C). Upon BABA-treatment, *OsGSL1* and *OsGSL5* expression levels were increased in roots, while *OsGNS5* showed a significant downregulation (Fig. 9B).

When looking at infected tissue, *OsGSL3* was significantly up-regulated in BABA-treated galls compared with water-

Fig. 6. Effects of β -aminobutyric acid (BABA) in plants treated with inhibitors of secondary metabolites. Either 3.5 mM BABA, the inhibitors, or both were soil-drenched 1 day before *Meloidogyne graminicola* inoculation. The number of adult females per plant was recorded at 16 days postinoculation. The data are represented as the ratio of the mean number of adult females in each treatment divided by the mean number of adult females in control plants ($n = 8$). Different letters indicate statistically significant differences (Duncan multiple range test with $\alpha = 0.05$). Data represent one of two independent experiments with similar results. Nipp, Nipponbare; DIE, diethyldithiocarbamic acid (DIECA); AOA, aminooxyacetic acid; AOPP, L-2-aminooxy-3-phenylpropionic acid; and Fluri, fluridone.

treated and noninoculated root tips as well as with water-treated control galls (Fig. 9C). Although the other two callose synthase genes showed a slightly higher expression level in BABA-treated galls, these differences were not significant. Interestingly, *OsGNS5* was clearly up-regulated in control galls, while, in contrast, the expression level of this gene remained at the basal expression level in BABA-treated galls. The results of qRT-PCR confirmed our microscopic observations that BABA induced the accumulation of callose, possibly by upregulating callose biosynthesis and repressing its degradation. *OsGNS5* was strongly induced in water-treated galls, exemplifying once again that *M. graminicola* strongly suppresses the plant innate immunity system.

DISCUSSION

BABA has been reported for years to be a potent inducer of resistance in plants against a broad-spectrum of stress factors (Cohen 2002; Jakab et al. 2001, 2005; Pastor et al. 2013; Zimmerli et al. 2008). However, the molecular mechanisms underlying BABA-IR remain largely unknown, especially in the interaction of plants with plant-parasitic nematodes. The research described here seeks to provide a characterization of the role of BABA-IR against the rice RKN *M. graminicola* and the related defense mechanisms.

The data presented in this report show that BABA is a potent inducer of root defense against RKN attack in rice. Although this chemical did not influence nematode attraction toward the roots, treatment with BABA made rice roots more difficult for RKN to invade, as indicated by a lower number of nematodes in roots at 50 hpi. Furthermore, the development of juveniles into adult females was heavily inhibited, and giant cells in BABA-treated plants were less developed than those in control plants. Studies performed on many plant pathogens and nematodes (Cohen 2002; Jakab et al. 2001; Oka and Cohen 2001) showed that there was no direct toxic effect to the pathogens by BABA treatment, and in vivo toxicity of BABA metabolites can also be ruled out, as experiments using C^{14} -labeled BABA clearly demonstrated that the substance was not metabolized by the plant (Cohen and Gisi 1994). However, BABA-IR against *Sitobion avenae* in wheat seedlings is possibly due to direct toxicity of high BABA contents in the plant phloem (Cao et al. 2014). The action of BABA against RKN in our study is probably not based on a direct toxic effect since 3.5 mM BABA incubation did not exhibit any influence on nematode mobility and infectivity.

Although most nematodes had already entered the roots at the moment of BABA application, we did observe a slightly lower total number of nematodes inside the roots in the post-treatment experiment. A possible explanation for this is that nematodes that

failed to establish feeding sites due to induced resistance either died or left the hostile roots, which has been observed in several RKN-resistant cultivars, such as soybean (Herman et al. 1991) and alfalfa (Griffin and Elgin 1977; Reynolds et al. 1970).

To elucidate possible mechanisms contributing to BABA-IR against RKN, the involvement of hormone-dependent defense pathways was examined. It has been reported that BABA-IR against TMV in tobacco and *Pseudomonas syringae* and *Botrytis cinerea* in *Arabidopsis* is based on an intact SA signaling pathway (Siegrist et al. 2000; Zimmerli et al. 2000). In this study, our data showed that i) the transcripts of SAR marker *PR1b* and SA biosynthesis gene *ICS1* did not accumulate upon BABA treatment and ii) BABA-IR was fully functional in the *NahG* line and the *NPR1* RNAi lines. Although the expression of *OsWRKY45* was significantly potentiated in BABA-treated roots, BABA successfully triggered defense against RKN in the *WRKY45* RNAi line, indicating that activation of *OsWRKY45* by BABA was probably not a prerequisite for defense against RKN. However, we could not exclude that residual *WRKY45* expression in the RNAi lines triggered defense responses. Hence, *OsWRKY45* knockout lines will be essential to further study the role of *OsWRKY45* in BABA-IR. From these observations, we conclude that the SA-dependent defense pathway does not significantly contribute to BABA-IR against RKN. In addition, we showed that inhibition of JA and ET biosynthesis does not significantly influence BABA-IR in rice against *M. graminicola*. As a consequence, defense mechanisms other than the classical SA, JA, and ET pathways must be responsible for the protective effect of BABA-treatment against RKN in rice.

Hydrogen peroxide accumulation has been shown to perform several important functions in early defense responses of plants against pathogens (Apel and Hirt 2004; Lamb and Dixon 1997). It may cause direct pathogen destruction (Bestwick et al. 1998), trigger hypersensitive cell death and activate defense-related genes (Apel and Hirt 2004; Levine et al. 1994), or serve as a secondary messenger in the systemic signaling network of plant cells (Shetty et al. 2003). The accumulation of H_2O_2 in BABA-treated plants has been repeatedly described. For instance, in lettuce, BABA-treated plants induced accumulation of H_2O_2 in the mycelia of *Bremia lactucae* and adjacent mesophyll cells and altered the color of mycelia to red (Cohen et al. 2011). In tomato, BABA induced an early oxidative burst and antioxidative defense mechanism that led to a suppressive effect on *Pseudomonas syringae* growth (Baysal et al. 2007). In grapevine, a strong H_2O_2 production was observed in BABA-treated leaves after downy mildew *Plasmopara viticola* infection, and this process was correlated with an increased resistance (Dubreuil-Maurizi et al. 2010). Furthermore, BABA also induced H_2O_2 production in tomato (Sahebani and Hadavi

Fig. 7. β -Aminobutyric acid (BABA) treatment increased lignin accumulation. **A**, Lignin content in the roots of rice seedlings. Bars represent the mean $\pm$ standard error (SE) of lignin content from 20 plants. Different letters indicate statistically significant differences (Student *t* test with $P \leq 0.05$). CWR, cell wall residues. **B**, Analysis of the expression levels of three genes involved in lignin biosynthesis in rice roots after soil application of BABA alone (BABA), only with *Meloidogyne graminicola* (Mg) infection, and BABA treatment plus *M. graminicola* infection (BABA+Mg) at different times compared with control (water-treated and noninoculated). *OsPAL*, *OsC4H*, and *OsCAD*, *O. sativa* phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, and cinnamyl alcohol dehydrogenase genes, respectively. Bars represent mean expression levels $\pm$ SE from two biological replicates and three technical replicates, each containing a pool of eight plants. Asterisks indicate significant differential expression ($P \leq 0.05$). Data are shown as relative expression levels in comparison with the control roots (expression level set at 1). Gene expression levels were normalized using two internal reference genes, *OsEXPnarsai* and *OsUBQ5*. Time 1, two samples: i) roots treated with 3.5 mM BABA for 1 day (BABA), ii) control roots treated with water for 1 day. Time 2, four samples: i) roots treated with 3.5 mM BABA for two days (BABA), ii) roots treated with 3.5 mM BABA for 2 days and inoculated with *M. graminicola* for 1 day (BABA+Mg), iii) roots treated with water for 2 days and inoculated with *M. graminicola* for 1 day (Mg), and iv) control roots treated with water for 2 days. Time 3, four samples: i) roots treated with 3.5 mM BABA for 4 days (BABA), ii) roots treated with 3.5 mM BABA for 4 days and inoculated with *M. graminicola* for 3 days (BABA+Mg), iii) roots treated with water for 4 days and inoculated with *M. graminicola* for 3 days (Mg), and iv) control roots treated with water for four days. **C**, Histochemical staining for lignin (pink-red color) in BABA-treated and control galls induced by *M. graminicola* at different time points. Sections (100 μ m) were stained with the Wiesner reagent to visualize lignin. Scale bar = 100 μ m. Yellow stars indicate giant cells.

2009) and cucumber (Sahebani et al. 2011), when infected by *M. javanica*. In our experiments, treatment with BABA resulted in a strong H₂O₂ accumulation in the rice roots both with and without nematode inoculation, and these high levels of H₂O₂ lasted until 3 days post-nematode inoculation. Moreover, the transcript level of *OsRbohB* was strongly induced 2 days after BABA treatment in both noninoculated and inoculated plants.

Hydrogen peroxide could, for instance, also contribute to lignin formation. Lignin, a major component of the secondary cell wall of vascular plants, is considered to be the first line of defense against the penetration of invading pathogens (Lewis and Yamamoto 1990; Nicholson and Hammerschmidt 1992; Vance et al. 1980). Lignin can enhance mechanical strength of plant cell walls and make them less accessible to cell wall-degrading enzymes, which are secreted by plant-parasitic nematodes during

penetration and migration in plant roots (Gheysen and Jones 2006). Therefore, differences in lignin content between BABA-treated and water-treated plants were analyzed. The lignin content was found to be significantly enhanced in BABA-treated roots. Also, BABA-treated galls were characterized by a more pronounced lignin deposition than control galls, and genes involved in lignin biosynthesis were more strongly expressed in BABA-treated plants. RNAi-mediated transient gene silencing of lignin biosynthesis genes in wheat led to a higher penetration efficiency of *Blumeria graminis* f. sp. *tritici* than control (Bhuiyan et al. 2009). Banana (*Musa* spp.) resistant to the burrowing nematode *Radopholus similis* had constitutively higher levels of lignin in the

vascular bundle than susceptible cultivars (Fogain and Gowen 1995; Wuyts et al. 2007). Transgenic *Arabidopsis* containing elevated levels of syringyl lignin significantly reduced the fecundity and development of *M. incognita* compared with the wild-type control (Wuyts et al. 2006). Accordingly, tobacco plants with reduced syringyl lignin content resulted in a faster life cycle of *M. incognita*. Hence, the high level of lignification in BABA-treated roots probably restricted *M. graminicola* invasion and, therefore, resulted in less nematode penetration in our experiments. Lignin components are produced by the phenylpropanoid pathway. Genes involved in this pathway, such as *PAL*, *OsC4H*, caffeate *O*-methyltransferase (*COMT*), and

Fig. 8. β -Aminobutyric acid (BABA) treatment increased reactive oxygen species generation. **A**, The H_2O_2 content per gram of fresh root tissue in water-treated (control), water-treated and inoculated with *Meloidogyne graminicola* (Mg), BABA-treated (BABA), and BABA-treated plus inoculated (BABA+Mg) roots. BABA (3.5 mM) or water was soil-drenched 1 day prior to nematode inoculation. Bars represent the mean $\pm$ standard error (SE) of H_2O_2 content from six replicates, each containing a pool of four roots. Data represent one of two independent experiments with similar results. Different letters indicate statistically significant differences (Duncan multiple range test with $\alpha = 0.05$). dpi: day postinoculation. **B**, Analysis of *OsRbohB* expression in BABA-treated (BABA), BABA-treated plus inoculated (BABA+Mg), and water-treated and inoculated (Mg) roots compared with water-treated and noninoculated control at different time points. Bars represent mean expression levels $\pm$ SE from two biological replicates and three technical replicates, each containing a pool of eight plants. Asterisks indicate significant differential expression ($P \leq 0.05$). Data are shown as relative expression levels in comparison with the control roots (expression level set at 1). Gene expression levels were normalized using two internal reference genes, *OsEXPnarsai* and *OsUBQ5*. Time 1, two samples: i) roots treated with 3.5 mM BABA for 1 day (BABA), ii) control roots treated with water for 1 day. Time 2, four samples: i) roots treated with 3.5 mM BABA for two days (BABA), ii) roots treated with 3.5 mM BABA for 2 days and inoculated with *M. graminicola* for 1 day (BABA+Mg), iii) roots treated with water for 2 days and inoculated with *M. graminicola* for 1 day (Mg), and iv) control roots treated with water for 2 days. Time 3, four samples: i) roots treated with 3.5 mM BABA for 4 days (BABA), ii) roots treated with 3.5 mM BABA for 4 days and inoculated with *M. graminicola* for 3 days (BABA+Mg), iii) roots treated with water for 4 days and inoculated with *M. graminicola* for 3 days (Mg), and iv) control roots treated with water for four days.

OsCAD, were strongly repressed in giant cells induced by *M. javanica* in tomato and *Arabidopsis* plants (Portillo et al. 2013) as well as galls and giant cells formed by RKN in rice plants (Ji et al. 2013; Kyndt et al. 2012a). These observations were confirmed by our experiments, exemplifying the strong defense-repressing capacity of this nematode. Based on the fact that BABA-IR was partially compromised in PAL inhibitor-treated plants, we speculate that greater *PAL* expression upon BABA treatment leads to increased levels of lignin, which contributes to either less-efficient penetration, delay of RKN development, or both. However, the role of other phenylpropanoid-derived compounds, such as flavonoids and some phytoalexins in BABA-IR, still needs to be investigated. For instance, it has been found that many other phenylpropanoids, such as sinapic acid and sinapoyl malate, accumulate in *Arabidopsis* after BABA treatment (Pastor et al. 2014).

Callose plays important roles during plant development (Chen et al. 2007) and is induced as a plant resistance response after exposure to a range of biotic and abiotic stresses (Chen and Kim 2009; Jacobs et al. 2003; Luna et al. 2011; Ueki and Citovsky 2005). Callose deposition is one of the earliest plant responses against invading nematodes (Grundler et al. 1998; Hussey et al. 1992). It is generally deposited between the plasma membrane and the cell wall, is synthesized by callose synthase, and is degraded by callose-degrading enzymes (Chen and Kim 2009). Overexpression of *RAP2.6*, an ethylene-response transcription-factor gene in *Arabidopsis* plants, enhanced callose deposition in syncytia and increased resistance against the beet cyst nematode *Heterodera schachtii* (Ali et al. 2013). Similarly, callose deposition on the sieve plates of resistant rice is an important mechanism for reducing phloem sap to the brown planthopper pest (Hao et al. 2008). In our experiment, abundant, compact callose deposits were observed in galls formed in BABA-treated

Fig. 9. Callose deposition is enhanced in β -aminobutyric acid (BABA)-treated galls. **A**, Callose detection in 4 days postinoculation (dpi) water-treated (control) and BABA-treated (BABA) galls. Scale bar = 1 mm. **B**, Analysis of the relative expression levels of three callose-synthesis genes (*OsGSL1*, *OsGSL3*, *OsGSL5*) and one callose-degrading gene (*OsGNS5*) in BABA-treated and water-treated roots. **C**, Analysis of the relative expression levels of three callose-synthesis genes (*OsGSL1*, *OsGSL3*, *OsGSL5*) and one degrading gene (*OsGNS5*) in water-treated but noninoculated root tips, BABA-treated galls, and water-treated galls. Bars in quantitative reverse transcription-polymerase chain reaction data represent mean expression levels $\pm$ standard error from two independent biological replicates and three technical replicates, each containing a pool of eight plants. Asterisks indicate significant differential expression ($P \leq 0.05$). Gene expression levels were normalized using two internal reference genes, *OsEXPnarsai* and *OsUBQ5*.

roots, whereas the callose signals were much fainter in water-treated plants. Corroborating this observation, the callose synthase gene *OsGSL3* was significantly up-regulated in BABA-treated galls, compared with uninfected root tips or water-treated galls. Expression of the callose degrading enzyme *OsGNS5*, on the other hand, was strongly down-regulated in BABA-treated roots, a phenomenon which may allow callose to be maintained in plant roots. However, RKN feeding significantly induced the expression of *OsGNS5* in galls, which is probably one of the mechanisms of RKN to overcome the physical barriers of the basal defense in susceptible rice plants. Similarly, the brown planthopper could unplug sieve tube occlusions by activating callose-degrading enzymes in susceptible rice plants (Hao et al. 2008). Taken together, we conclude that BABA activates genes for callose synthesis and suppresses callose degradation, leading to the accumulation of callose in BABA-treated roots. This phenomenon may inhibit RKN penetration, giant cell expansion, and ingestion of cytoplasm from giant cells. Several studies have reported that BABA-induced callose deposition is based on the ABA-signaling pathway (Flors et al. 2005; Ton and Mauch-Mani 2004; Ton et al. 2005). There is an indication in our experiments that ABA is required for BABA-IR against RKN, as BABA-IR was slightly impaired when the plants were cotreated with the ABA-inhibitor fluridone. However, since fluridone inhibits ABA biosynthesis via the carotenoid pathway from which only a small fraction may be required for ABA biosynthesis (Taylor et al. 2000), fluridone probably provoked other perturbations (e.g., decrease of astaxanthin and lutein) in addition to the inhibition of ABA biosynthesis (Achu 2005). Therefore, whether fluridone can specifically inhibit ABA production in our experimental conditions and if callose deposition is dependent on the ABA-pathway needs to be further explored.

Our understanding of the mechanisms of BABA-IR against the rice RKN is encapsulated in a model shown in Figure 10. BABA induces a multifaceted plant basal defense response in the rice roots, which inhibits nematode penetration and development. However, the major defense hormone-dependent signal pathways play no pertinent roles in this induced resistance mechanism.

Fig. 10. Model illustrating the mechanisms of β -aminobutyric acid (BABA)-induced resistance against *Meloidogyne graminicola* in rice. Lines ending with arrows show activation. Lines ending with perpendicular short lines indicate suppression.

MATERIALS AND METHODS

Plant materials and growth conditions.

Rice (*Oryza sativa* L.) lines used in this work included *O. sativa* cvs. Nipponbare and Taipei 309. The seeds of cv. Nipponbare were provided by the United States Department of Agriculture (GSOR-100) and the corresponding SA-deficient *NahG* line (Yang et al. 2004) and RNAi *OsMPK5* transgenic plants (Xiong and Yang 2003) were provided by Y. Yang (Pennsylvania State University); the *OsWRKY45* RNAi transgenic line was a gift from H. Takatsuji (Plant Disease Resistance Research Unit, National Institute of Agrobiological Sciences, Ibaraki, Japan); the *NPR1* RNAi transgenic line and its corresponding wild-type Taipei 309 were provided by Z. He (National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China). Seeds were germinated on wet filter paper for 4 days at 30°C, were then transferred to a sand and absorbent polymer (SAP) substrate (Reversat et al. 1999) in polyvinyl-chloride tubes, and were further grown at 26°C under a 16-h light and 8-h dark regime at a relative humidity of 70 to 75%.

Infection experiments.

The *M. graminicola* culture was provided by D. De Waele (Catholic University Leuven, Belgium) and was originally isolated in The Philippines. *M. graminicola* was maintained on *O. sativa* cv. Nipponbare in potting soil under the same temperature and light conditions as described above, and the nematodes were extracted using a modified Baermann funnel.

In general, pretreatment with BABA was performed as follows: 14-day-old rice plants were soil drenched with 3.5 mM BABA (or water as control), and 1 day later, they were inoculated with about 200 second-stage juveniles (J2) of *M. graminicola* per plant. In a developmental assay, the SAP substrate was washed away from the roots 2 days after inoculation, and the plants were transferred to a hydroponic system, with Hoagland solution, for another 14 days.

To test the direct effect of BABA on nematode infectivity and development, J2 of *M. graminicola* were soaked in 3.5 mM BABA (Mg+BABA) or water (as control) for 1 day at 26°C. Then, all the soaked nematodes were washed by tap water and were inoculated on rice plants (200 J2 per plant). The number of J2 and adult females inside the roots was recorded at 50 hpi and 15 dpi, respectively. Ten plants were included in each treatment.

To study the effect of BABA on penetration of *M. graminicola* into rice roots, about 200 J2 were inoculated 1 day after soil was drenched with 3.5 mM BABA or water. The number of nematodes inside roots was recorded at 50 hpi. To evaluate the effect of BABA once the nematodes had already established in the roots, the following post-treatment setup was used: 2 days after *M. graminicola* infection, roots were washed and transferred to 3.5 mM BABA solution (or water as control) for 2 days. Subsequently, plants were transferred to pure Hoagland solution for another 12 days.

To visualize the nematodes inside the roots, the infected tissue was boiled for 3 min in 0.8% acetic acid and 0.013% acid fuchsin. Roots were then washed with running tap water and were destained in acidified glycerol (5:100 HCL/glycerol, vol/vol). Eight plants were included in each experiment and all the experiments were independently repeated once or twice, with similar results.

Attraction assay.

O. sativa cv. Nipponbare seedlings (14 days old) were soil drenched with 3.5 mM BABA or water. One day after treatment,

root tips were excised (about 5 mm long) and each tip was put into a 1.5-cm-diameter well in a 24-well cell-culture microplate. Each well contained about 150 J2 suspended in 500 µl of 0.8% water-agar. The microplate was incubated in a growth chamber (26°C) for 9 h, and then, the number of nematodes at the elongation zone of the root tip was counted. Eight replicates were included in the experiment and the experiment was done twice, with similar results.

Inhibitor experiments.

Chemical treatment was done with AOA, diethyldithiocarbamic acid (DIECA), fluridone, and L-2-aminooxy-3-phenylpropionic acid (AOPP), inhibiting ET, JA, ABA biosynthesis, and PAL activity, respectively. All chemicals were purchased from Sigma-Aldrich and were dissolved in a few drops of ethanol before diluting in distilled water. The chemicals and concentrations used are as follows: AOA (50 mM), DIECA (100 µM), fluridone (0.1 µM), and AOPP (100 µM). Intact 14-day-old seedlings were soil drenched with i) an inhibitor or ii) an inhibitor plus 3.5 mM BABA. This treatment was done 1 day before nematode inoculation. Eight plants were included in each experiment and the experiment was independently repeated, with similar results.

RNA extraction, cDNA synthesis, and qRT-PCR.

RNA extraction and cDNA synthesis were performed on total roots, galls, or root tips and were analyzed as described by Kyndt et al. (2012b). The primer sequences used for qRT-PCR are listed in Supplementary Table S1. All qRT-PCR reactions were done in triplicate, on cDNA from two independent biological replicates. A biological replicate contained a pool of the respective plant tissue harvested from at least eight plants. BABA application was done on day 0, and nematodes were inoculated on day 1. Samples were collected at three time points, as visualized in Figure 4B. At each sample point, control samples were taken from plants that were grown in the same way except that they received only water treatments. Two samples were taken at time 1: roots treated with 3.5 mM BABA for 1 day (BABA), control roots treated with water for 1 day. Four samples were taken at time 2: roots treated with 3.5 mM BABA for 2 days (BABA), roots treated with 3.5 mM BABA for 2 days and inoculated with *M. graminicola* for 1 day (BABA+Mg), roots treated with water for 2 days and inoculated with *M. graminicola* for 1 day (Mg), and control roots treated with water for two days. Four samples were taken at time 3: roots treated with 3.5 mM BABA for 4 days (BABA), roots treated with 3.5 mM BABA for 4 days and inoculated with *M. graminicola* for 3 days (BABA+Mg), roots treated with water for 4 days and inoculated with *M. graminicola* for 3 days (Mg), and control roots treated with water for 4 days.

Tissue processing for light microscopy.

Galls on the roots were excised from water-treated and BABA-treated plants 7 days after inoculation. Fixation of galls was done in 2% glutaraldehyde in 1× piperazine-*N,N'*-bis(2-ethanesulfonic acid) buffer overnight and dehydration was then done in a series of ethanol dilutions. Embedding of galls was conducted using Technovit 7100 (Kulzer, Germany). Sections (10 µm thick) were cut with a Leica RM2265 motorized rotary microtome (Leica Microsystems, Nussloch, Germany) and were stained with 0.05% toluidine blue O. Digital images were acquired with an Olympus BX51 microscope equipped with an Olympus ColorView III camera. Images were processed with the Olympus analysis cell^F software (Olympus Soft Imaging Solutions, Munster, Germany).

Lignin analyses.

Root tissues were collected 1 day after soil drench with 3.5 mM BABA or water. Dry roots (about 6 mg) in 2-ml vials

were subjected to sequential extractions, each time for 30 min, at near-boiling temperatures in water (98°C), ethanol (76°C), chloroform (59°C), and acetone (54°C). The remaining cell-wall residue was dried under vacuum and was weighed again. Lignin quantification was performed according to Vanholme et al. (2013) and was optimized for small amounts of plant tissue. Absorbance was measured at 280 nm using a Nano-Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, U.S.A.). Twenty samples were measured for each treatment. The lignin concentrations were calculated by means of the Bouguer-Lambert-Beer law: $A = \epsilon \times l \times c$, with $\epsilon = 17.75 \text{ l g}^{-1} \text{ cm}^{-1}$ (Vega-Sánchez et al. 2012) and $l = 0.1 \text{ cm}$.

Lignin histochemistry was examined using Wiesner reagent (Pomar et al. 2004). Galls harvested at different time points from BABA or water treatments were embedded in 7% agarose and were cut into 100 µm sections with a vibroslicer. Sections were incubated for 5 min in Wiesner reagent (1% phloroglucinol in 95% ethanol+HCL), which was subsequently replaced by water. Sections were directly observed with a binocular microscope (S8APO, Leica).

Hydrogen peroxide content assay.

Hydrogen peroxide levels were determined according to Velikova et al. (2000). Root tissues (about 100 mg) were homogenized in an ice bath with 0.3 ml 0.1% (wt/vol) trichloroacetic acid. The homogenate was centrifuged at 14,000 rpm for 15 min and 0.1 ml of the supernatant was added to 0.1 ml of 10mM potassium phosphate buffer (pH 7.0) and 0.2 ml of 1M KI. The absorbance of supernatant was read at $\lambda_{\text{max}} = 390 \text{ nm}$. The hydrogen peroxide content was calculated based on a standard curve made by measuring known hydrogen peroxide concentrations in the same assay. Six replicates were included, with each containing a pool of four roots. The experiment was independently repeated, with similar results.

Callose staining.

The method of detection of callose deposition was adapted from Millet et al. (2010). Two days after nematode or water inoculation, plants were transferred to 3.5 mM BABA or water for another 2 days. Whole roots were immediately fixed in a 3:1 ethanol/acetic acid solution for 1 day. The fixative was changed three times to ensure thorough fixing and clearing of the tissues. Seedlings were rehydrated in 70% ethanol for 3 h, 50% ethanol for 3 h, 30% ethanol for an additional 2 h, and water overnight. After three water washes, roots were treated with 10% NaOH and were placed at 37°C for 2 h to make the tissues transparent. After three water washes, seedlings were incubated in 0.01% aniline blue (Sigma-Aldrich) in 150 mM K_2HPO_4 solution (pH 9.5) for 25 min. The roots, including galls, were mounted on slides, and callose was observed immediately under UV (excitation, 390 nm; emission, 460 nm).

Statistical analysis.

Data obtained from qRT-PCR was analyzed using the software Rest 2009 ($P \leq 0.05$). All other statistical analyses were performed using the software SPSS (version 21) or Student *t* test ($P \leq 0.05$). The normality of data were checked by the Kolmogorov-Smirnov test of composite normality ($\alpha = 0.05$). Homogeneity of variance was checked by the Levene test ($P \leq 0.05$). Analysis of variance (one-way ANOVA) and multiple comparisons of differences between treatments was then performed by Duncan's multiple range test ($\alpha = 0.05$).

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LITERATURE CITED

- Achu, E. A. 2005. Influence of salicylic and abscisic acid on the resistance of tomato to biotrophic and necrotrophic pathogens. PhD. thesis, Ghent University, Belgium.
- Adam, L., and Somerville, S. C. 1996. Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant J.* 9:341-356.
- Ahmed, N., Abbasi, M., Shaukat, S., and Zaki, M. 2009. Induced systemic resistance in mung bean plant against root-knot nematode *Meloidogyne javanica* by DL- β -amino butyric acid. *Nematologia Mediterranea* 37: 67-72.
- Ali, M. A., Abbas, A., Kreil, D. P., and Bohlmann, H. 2013. Overexpression of the transcription factor RAP2.6 leads to enhanced callose deposition in syncytia and enhanced resistance against the beet cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. *BMC Plant Biol.* 13:47.
- Apel, K., and Hirt, H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373-399.
- Baysal, Ö., Gürsoy, Y. Z., Örnek, H., Çetinel, B., and Teixeira da Silva, J. A. 2007. Enhanced systemic resistance to bacterial speck disease caused by *Pseudomonas syringae* pv. tomato by DL- β -aminobutyric acid under salt stress. *Physiol. Plant.* 129:493-506.
- Bestwick, C. S., Brown, I. R., and Mansfield, J. W. 1998. Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. *Plant Physiol.* 118:1067-1078.
- Bhuiyan, N. H., Selvaraj, G., Wei, Y., and King, J. 2009. Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J. Exp. Bot.* 60:509-521.
- Cao, H. H., Zhang, M., Zhao, H., Zhang, Y., Wang, X. X., Guo, S. S., Zhang, Z. F., and Liu, T. X. 2014. Deciphering the mechanism of β -aminobutyric acid-induced resistance in wheat to the grain aphid, *Sitobion avenae*. *PLoS ONE* 9:e91768. Published online.
- Chen, X. Y., and Kim, J. Y. 2009. Callose synthesis in higher plants. *Plant Signal. Behav.* 4:489-492.
- Chen, R., Zhao, X., Shao, Z., Wei, Z., Wang, Y., Zhu, L., Zhao, J., Sun, M., He, R., and He, G. 2007. Rice UDP-glucose pyrophosphorylase1 is essential for pollen callose deposition and its cosuppression results in a new type of thermosensitive genic male sterility. *Plant Cell* 19:847-861.
- Chinnasri, B., Sipes, B. S., and Schmitt, D. P. 2006. Effects of inducers of systemic acquired resistance on reproduction of *Meloidogyne javanica* and *Rotylenchulus reniformis* in pineapple. *J. Nematol.* 38:319-325.
- Cohen, Y. 2001. The BABA story of induced resistance. *Phytoparasitica* 29: 375-378.
- Cohen, Y. R. 2002. β -aminobutyric acid-induced resistance against plant pathogens. *Plant Dis.* 86:448-457.
- Cohen, Y., and Gisi, U. 1994. Systemic translocation of ^{14}C -DL-3-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans*. *Physiol. Mol. Plant Pathol.* 45:441-456.
- Cohen, Y., Rubin, A. E., and Kilfin, G. 2010. Mechanisms of induced resistance in lettuce against *Bremia lactucae* by DL- β -amino-butyric acid (BABA). *Eur. J. Plant Pathol.* 126:553-573.
- Cohen, Y., Rubin, A. E., and Vaknin, M. 2011. Post infection application of DL-3-amino-butyric acid (BABA) induces multiple forms of resistance against *Bremia lactucae* in lettuce. *Eur. J. Plant Pathol.* 130:13-27.
- Curtis, R. H., Robinson, A. F., and Perry, R. N. 2009. Hatch and host location. Page 139-161 in: *Root-Knot Nematodes*. R. N. Perry, M. Mones, and J. L. Starr, eds. CABI, Wallingford, U. K.
- Dixon, R. A., and Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* 7:1085-1097.
- Dubreuil-Maurizi, C., Trouvelot, S., Frettinger, P., Pugin, A., Wendehehenne, D., and Poinssot, B. 2010. β -aminobutyric acid primes an NADPH oxidase-dependent reactive oxygen species production during grapevine-triggered immunity. *Mol. Plant-Microbe Interact.* 23:1012-1021.
- Flors, V., Ton, J., Jakab, G., and Mauch-Mani, B. 2005. Absciscic acid and callose: Team players in defence against pathogens? *J. Phytopathol.* 153: 377-383.
- Fogain, R., and Gowen, S. 1995. Investigations on possible mechanisms of resistance to nematodes in *Musa*. *Euphytica* 92:375-381.
- Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261: 754-756.
- Gheysen, G., and Jones, J. 2006. Molecular aspects of plant-nematode interactions. Pages 234-254 in: *Plant nematology*. R. N. Perry, and M. Mones, eds. CABI, Wallingford, U. K.
- Griffin, G. D., and Elgin, J. H., Jr. 1977. Penetration and development of *Meloidogyne hapla* in resistant and susceptible alfalfa under differing temperatures. *J. Nematol.* 9:51-56.
- Grundler, F. M., Sobczak, M., and Golinowski, W. 1998. Formation of wall openings in root cells of *Arabidopsis thaliana* following infection by the plant-parasitic nematode *Heterodera schachtii*. *Eur. J. Plant Pathol.* 104: 545-551.
- Hahlbrock, K., and Grisebach, H. 1979. Enzymic controls in the biosynthesis of lignin and flavonoids. *Annu. Rev. Plant Physiol.* 30:105-130.
- Ham, J. H., Kim, M. G., Lee, S. Y., and Mackey, D. 2007. Layered basal defenses underlie non-host resistance of *Arabidopsis* to *Pseudomonas syringae* pv. *phaseolicola*. *Plant J.* 51:604-616.
- Hamiduzzaman, M. M., Jakab, G., Barnavon, L., Neuhaus, J. M., and Mauch-Mani, B. 2005. β -Aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Mol. Plant-Microbe Interact.* 18:819-829.
- Hao, P., Liu, C., Wang, Y., Chen, R., Tang, M., Du, B., Zhu, L., and He, G. 2008. Herbivore-induced callose deposition on the sieve plates of rice: An important mechanism for host resistance. *Plant Physiol.* 146: 1810-1820.
- Herman, M., Hussey, R. S., and Boerma, H. R. 1991. Penetration and development of *Meloidogyne incognita* on roots of resistant soybean genotypes. *J. Nematol.* 23:155-161.
- Hussey, R., Mims, C., and Westcott, S., III. 1992. Immunocytochemical localization of callose in root cortical cells parasitized by the ring nematode *Criconebella xenoplax*. *Protoplasma* 171:1-6.
- Jacobs, A. K., Lipka, V., Burton, R. A., Panstruga, R., Strizhov, N., Schulze-Lefert, P., and Fincher, G. B. 2003. An *Arabidopsis* callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell* 15:2503-2513.
- Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Métraux, J. P., and Mauch-Mani, B. 2001. β -Aminobutyric acid-induced resistance in plants. *Eur. J. Plant Pathol.* 107:29-37.
- Jakab, G., Ton, J., Flors, V., Zimmerli, L., Métraux, J.-P., and Mauch-Mani, B. 2005. Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiol.* 139: 267-274.
- Ji, H., Gheysen, G., Denil, S., Lindsey, K., Topping, J. F., Nahar, K., Haegeman, A., De Vos, W. H., Trooskens, G., Van Criekeing, W., De Meyer, T., and Kyndt, T. 2013. Transcriptional analysis through RNA sequencing of giant cells induced by *Meloidogyne graminicola* in rice roots. *J. Exp. Bot.* 64:3885-3898.
- Kyndt, T., Denil, S., Haegeman, A., Trooskens, G., Bauters, L., Van Criekeing, W., De Meyer, T., and Gheysen, G. 2012a. Transcriptional reprogramming by root knot and migratory nematode infection in rice. *New Phytol.* 196:887-900.
- Kyndt, T., Nahar, K., Haegeman, A., De Vleeschauwer, D., Höfte, M., and Gheysen, G. 2012b. Comparing systemic defence-related gene expression changes upon migratory and sedentary nematode attack in rice. *Plant Biol Stuttg* 14 (Suppl 1):73-82.
- Kyndt, T., Vieira, P., Gheysen, G., and de Almeida-Engler, J. 2013. Nematode feeding sites: Unique organs in plant roots. *Planta* 238: 807-818.
- Kyndt, T., Fernandez, D., and Gheysen, G. 2014. Plant-parasitic nematode infections in rice: Molecular and cellular insights. *Annu. Rev. Phytopathol.* 52:135-153.
- Lamb, C., and Dixon, R. A. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:251-275.
- Lee, M. W., Qi, M., and Yang, Y. 2001. A novel jasmonic acid-inducible rice myb gene associates with fungal infection and host cell death. *Mol. Plant-Microbe Interact.* 14:527-535.
- Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79:583-593.
- Lewis, N. G., and Yamamoto, E. 1990. Lignin: Occurrence, biogenesis and biodegradation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:455-496.
- Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., and Ton, J. 2011. Callose deposition: A multifaceted plant defense response. *Mol. Plant-Microbe Interact.* 24:183-193.
- Mei, C., Qi, M., Sheng, G., and Yang, Y. 2006. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid

- level, PR gene expression, and host resistance to fungal infection. *Mol. Plant-Microbe Interact.* 19:1127-1137.
- Millet, Y. A., Danna, C. H., Clay, N. K., Songnuan, W., Simon, M. D., Werck-Reichhart, D., and Ausubel, F. M. 2010. Innate immune responses activated in *Arabidopsis* roots by microbe-associated molecular patterns. *Plant Cell* 22:973-990.
- Mitsuhashi, I., Iwai, T., Seo, S., Yanagawa, Y., Kawahigasi, H., Hirose, S., Ohkawa, Y., and Ohashi, Y. 2008. Characteristic expression of twelve rice PR1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). *Mol. Genet. Genomics* 279:415-427.
- Nicholson, R. L., and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annu. Rev. Phytopathol.* 30:369-389.
- Oka, Y., and Cohen, Y. 2001. Induced resistance to cyst and root-knot nematodes in cereals by DL- β -amino-n-butyric acid. *Eur. J. Plant Pathol.* 107:219-227.
- Oka, Y., Cohen, Y., and Spiegel, Y. 1999. Local and systemic induced resistance to the root-knot nematode in tomato by DL- β -amino-n-butyric acid. *Phytopathology* 89:1138-1143.
- Pastor, V., Luna, E., Ton, J., Cerezo, M., García-Agustín, P., and Flors, V. 2013. Fine tuning of reactive oxygen species homeostasis regulates primed immune responses in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 26:1334-1344.
- Pastor, V., Balmer, A., Gamir, J., Flors, V., and Mauch-Mani, B. 2014. Preparing to fight back: Generation and storage of priming compounds. *Front. Plant Sci.* 5:295.
- Piękna-Grochala, J., and Kępczyńska, E. 2013. Induction of resistance against pathogens by β -aminobutyric acid. *Acta Physiol. Plant.* 35:1735-1748.
- Pomar, F., Novo, M., Bernal, M. A., Merino, F., and Barceló, A. R. 2004. Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt in *Capsicum annuum*. *New Phytol.* 163: 111-123.
- Portillo, M., Cabrera, J., Lindsey, K., Topping, J., Andrés, M. F., Emiliozzi, M., Oliveros, J. C., García-Casado, G., Solano, R., Koltai, H., Resnick, N., Fenoll, C., and Escobar, C. 2013. Distinct and conserved transcriptomic changes during nematode-induced giant cell development in tomato compared with *Arabidopsis*: A functional role for gene repression. *New Phytol.* 197:1276-1290.
- Reversat, G., Boyer, J., Sannier, C., and Pando-Bahuon, A. 1999. Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory. *Nematology* 1:209-212.
- Reynolds, H. W., Carter, W. W., and O'Bannon, J. H. 1970. Symptomless resistance of alfalfa to *Meloidogyne incognita acrita*. *J. Nematol.* 2:131-134.
- Sahebani, N., and Hadavi, N. 2009. Induction of H₂O₂ and related enzymes in tomato roots infected with root knot nematode (*M. javanica*) by several chemical and microbial elicitors. *Biocontrol Sci. Techn.* 19:301-313.
- Sahebani, N., Hadavi, N. S., and Zade, F. O. 2011. The effects of β -amino-butyric acid on resistance of cucumber against root-knot nematode, *Meloidogyne javanica*. *Acta Physiol. Plant.* 33:443-450.
- Sasser, J. N., and Freckman, D. W. 1987. A world perspective on nematology: The role of the society. Pages 7-14 in: *Vistas on Nematology*, J. A. Veech, and D. W. Dickson, eds. Society of Nematologists, Inc., Hyattsville, MD, U.S.A.
- Shetty, N., Kristensen, B., Newman, M.-A., Møller, K., Gregersen, P. L., and Jørgensen, H. L. 2003. Association of hydrogen peroxide with restriction of *Septoria tritici* in resistant wheat. *Physiol. Mol. Plant Pathol.* 62:333-346.
- Shimono, M., Sugano, S., Nakayama, A., Jiang, C.-J., Ono, K., Toki, S., and Takatsuji, H. 2007. Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* 19:2064-2076.
- Shimono, M., Koga, H., Akagi, A., Hayashi, N., Goto, S., Sawada, M., Kurihara, T., Matsushita, A., Sugano, S., Jiang, C. J., Kaku, H., Inoue, H., and Takatsuji, H. 2012. Rice WRKY45 plays important roles in fungal and bacterial disease resistance. *Mol. Plant Pathol.* 13:83-94.
- Siegrist, J., Orober, M., and Buchenauer, H. 2000. β -Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiol. Mol. Plant Pathol.* 56:95-106.
- Simon-Plas, F., Elmayan, T., and Blein, J. P. 2002. The plasma membrane oxidase NtrbohD is responsible for AOS production in elicited tobacco cells. *Plant J.* 31:137-147.
- Taylor, I. B., Burbidge, A., and Thompson, A. J. 2000. Control of abscisic acid synthesis. *J. Exp. Bot.* 51:1563-1574.
- Ton, J., and Mauch-Mani, B. 2004. β -amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* 38:119-130.
- Ton, J., Jakab, G., Toquin, V., Flors, V., Iavicoli, A., Maeder, M. N., Métraux, J. P., and Mauch-Mani, B. 2005. Dissecting the β -aminobutyric acid-induced priming phenomenon in *Arabidopsis*. *Plant Cell* 17:987-999.
- Torres, M. A., Jones, J. D., and Dangel, J. L. 2006. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 141:373-378.
- Ueki, S., and Citovsky, V. 2005. Identification of an interactor of cadmium ion-induced glycine-rich protein involved in regulation of callose levels in plant vasculature. *Proc. Natl. Acad. Sci. U.S.A.* 102:12089-12094.
- Vance, C., Kirk, T., and Sherwood, R. 1980. Lignification as a mechanism of disease resistance. *Annu. Rev. Phytopathol.* 18:259-288.
- Vanholme, B., Cesarino, I., Goeminne, G., Kim, H., Marroni, F., Van Acker, R., Vanholme, R., Morreel, K., Ivens, B., Pinosio, S., Morgante, M., Ralph, J., Bastien, C., and Boerjan, W. 2013. Breeding with rare defective alleles (BRDA): A natural *Populus nigra* HCT mutant with modified lignin as a case study. *New Phytol.* 198:765-776.
- Vega-Sánchez, M. E., Verhertbruggen, Y., Christensen, U., Chen, X., Sharma, V., Varanasi, P., Jobling, S. A., Talbot, M., White, R. G., Joo, M., Singh, S., Auer, M., Scheller, H. V., and Ronald, P. C. 2012. Loss of Cellulose synthase-like F6 function affects mixed-linkage glucan deposition, cell wall mechanical properties, and defense responses in vegetative tissues of rice. *Plant Physiol.* 159:56-69.
- Velikova, V., Yordanov, I., and Edreva, A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* 151:59-66.
- Walz, A., and Simon, O. 2009. β -Aminobutyric acid-induced resistance in cucumber against biotrophic and necrotrophic pathogens. *J. Phytopathol.* 157:356-361.
- Wildermuth, M. C., Dewdney, J., Wu, G., and Ausubel, F. M. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414:562-565.
- Wuyts, N., Lognay, G., Swennen, R., and De Waele, D. 2006. Nematode infection and reproduction in transgenic and mutant *Arabidopsis* and tobacco with an altered phenylpropanoid metabolism. *J. Exp. Bot.* 57: 2825-2835.
- Wuyts, N., Lognay, G., Verscheure, M., Marlier, M., De Waele, D., and Swennen, R. 2007. Potential physical and chemical barriers to infection by the burrowing nematode *Radopholus similis* in roots of susceptible and resistant banana (*Musa* spp.). *Plant Pathol.* 56:878-890.
- Xiong, L., and Yang, Y. 2003. Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 15:745-759.
- Yang, Y., Qi, M., and Mei, C. 2004. Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. *Plant J.* 40:909-919.
- Yoshioka, H., Numata, N., Nakajima, K., Katou, S., Kawakita, K., Rowland, O., Jones, J. D., and Doke, N. 2003. *Nicotiana benthamiana* gp91phox homologs NbrbohA and NbrbohB participate in H₂O₂ accumulation and resistance to *Phytophthora infestans*. *Plant Cell* 15: 706-718.
- Zimmerli, L., Jakab, G., Métraux, J.-P., and Mauch-Mani, B. 2000. Potentiation of pathogen-specific defense mechanisms in *Arabidopsis* by β -aminobutyric acid. *Proc. Natl. Acad. Sci. U.S.A.* 97:12920-12925.
- Zimmerli, L., Métraux, J.-P., and Mauch-Mani, B. 2001. β -Aminobutyric acid-induced protection of *Arabidopsis* against the necrotrophic fungus *Botrytis cinerea*. *Plant Physiol.* 126:517-523.
- Zimmerli, L., Hou, B. H., Tsai, C. H., Jakab, G., Mauch-Mani, B., and Somerville, S. 2008. The xenobiotic β -aminobutyric acid enhances *Arabidopsis* thermotolerance. *Plant J.* 53:144-156.