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Induced resistance to cyst and root-knot nematodes in cereals by DL-β-amino-*n*-butyric acid

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Abstract

Foliar sprays and soil drenches with DL-β-amino-*n*-butyric acid (BABA) reduced the number of *Heterodera avenae* and *H. latipons* cysts on wheat and barley. Foliar sprays of wheat with 8000 mg l^{-1} BABA reduced the number of *H. avenae* cysts by 90%, whereas 2000 mg l^{-1} BABA was enough to reduce the number of *H. latipons* cysts by 79%. Multiple spray treatments with 2000 mg l^{-1} BABA at 10-day intervals reduced the number of *H. avenae* cysts on wheat and barley. A soil drench of wheat with 125 mg l^{-1} BABA reduced the number of *H. latipons* cysts by 93% and *H. avenae* cysts by 43%. Second-stage juveniles of these nematodes penetrated and formed syncytia in wheat roots soil-drenched with BABA. More adult males of *H. avenae* were produced in BABA ($\leq$250 mg l^{-1})-treated wheat roots ($\sim$76%) than in untreated roots (27%). Soil drenches with higher concentrations of BABA inhibited development of adult males and females. Several chemical elicitors of induced resistance were tested for their ability to reduce the number of *H. avenae* cysts on wheat. Only BABA was found to be an effective resistance inducer. The number of egg masses of an unidentified *Meloidogyne* sp. root-knot nematode, which infects only monocots, was also reduced by 95% by a soil drench of wheat with 500 mg l^{-1} BABA. Development of this nematode inside the BABA-treated roots was also inhibited.

Abbreviations: AABA – DL-α-amino-*n*-butyric acid; BABA – DL-β-amino-*n*-butyric acid; BTH – benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester; GABA – γ-aminobutyric acid; INA – 2,6-dichloroisonicotinic acid; J2 – second-stage juveniles; SA – salicylic acid.

Introduction

Root-knot and cyst nematodes are the most important nematodes in agriculture worldwide. Control of these nematodes has become difficult because effective nematicides, such as DBCP (dibromochloropropane) and EDB (ethylene dibromide), have been banned as hazardous to the environment and human health. Moreover, methyl bromide, which is the most widely used fumigant for the control of soilborne diseases including nematodes has already been banned in some countries, and is due to be phased out in most other countries in the near future. In addition to the use of nematicides, resistant varieties, new cultural practices and other environmentally friendly measures for nematode control need to be developed.

Induced resistance is a plant defense mechanism which can be triggered by incompatible or weak pathogens (Kuc, 1990), or chemical inducers (Kessmann et al., 1994). In contrast to genetic resistance, induced resistance protects plants from a broad spectrum of pathogens, and works systemically in many instances. Systemically induced resistance triggered by local infection with a pathogen is known as systemic acquired resistance (SAR) (Hunt and Ryals, 1996; Ryals et al., 1996). Salicylic acid (SA) is an

effective SAR-inducer in plants, and the mechanism of its action in SAR is under study (Ryals et al., 1996; Sticher et al., 1997; Dempsey et al., 1999). Pathogenesis-related (PR) proteins and plant defense genes are generally involved in SAR (Ryals et al., 1996; Sticher et al., 1997). 2,6-Dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) have also been found to be effective SAR inducers (Vernooij et al., 1995; Friedrich et al., 1996). These chemicals can induce several plant genes involved in plant defense (Vernooij et al., 1995; Görlach et al., 1996). BTH is a commercial SAR inducer, which is currently used for fungal disease control of cereals.

Induced resistance to plant-parasitic nematodes has not been as thoroughly studied as that to fungi and bacteria. SAR to the root-knot nematode *Meloidogyne hapla* was obtained in tomato and pyrethrum plants by prior inoculation with other host-incompatible species, *M. incognita* or *M. javanica* (Ogallo and McClure, 1995, 1996). In the previous study, several chemicals that induce PR proteins or plant resistance were tested for their ability to induce resistance in tomato plants against *M. javanica* (Oka et al., 1999). DL-β-Amino-*n*-butyric acid (BABA), which is known as an inducer of resistance to fungal pathogens (Cohen, 1993, 1994, 1996; Cohen and Gisi, 1994; Cohen et al., 1994, 1999; Sunwoo et al., 1996; Hwang et al., 1997), has been found to induce resistance to *M. javanica* in tomato, either by foliar spray or soil drench. This resistance was expressed as inhibition of the nematode's invasion of and development in tomato roots, probably due to smaller and more vacuous giant cells, which supply nutrients to the nematode (Oka et al., 1999).

In the present study, the ability of BABA to induce resistance in cereals to the cyst nematodes *Heterodera avenae* Woll. and *H. latipons* Franklin, and to an unidentified *Meloidogyne* sp. root-knot nematode was tested.

Materials and methods

Nematodes

Second-stage juveniles (J2) of *H. avenae* pathotype Ha21 (Mor et al., 1992a) and *H. latipons* were hatched from newly formed, dark brown cysts collected from a culture maintained on wheat (*Triticum aestivum* L. cv. Bet-Shita) (Oka et al., 1997). An unidentified *Meloidogyne* sp. isolated from a commercial turf nursery was propagated on wheat (cv. Prince), and J2 were hatched from eggs extracted from the roots with sodium hypochlorite solution (Hussey and Barker, 1973). This *Meloidogyne* sp. was found to infect some monocotyledonous plants (Y. Oka, unpubl.). Hatched J2 of the cyst and root-knot nematodes were collected daily and stored at 4 and 15 °C, respectively.

Plant material

Wheat (cv. Bet-Shita) and barley (*Hordeum vulgare* L. cv. Ingrid) plants susceptible to *H. avenae* pathotype Ha21 and *H. latipons* were used in experiments with these nematodes. Wheat (cv. Prince) plants were used in experiments with the *Meloidogyne* sp. Seeds were surface-sterilized with 0.1% $HgCl_2$ solution (Oka et al., 1997), and germinated in a petri dish. Germinating seeds (with about 1 cm roots) were planted in 250-ml plastic pots or 50-ml plastic test tubes.

Chemicals

DL-α-Amino-*n*-butyric acid (AABA) and BABA were purchased from Sigma Chemical Co. (St. Louis, MO). INA (formulated as 25% wettable powder) and BTH (formulated as 50% wettable granules) were a gift of Novartis (Basel, Switzerland).

Direct effect of BABA on nematodes

To test the direct effect of BABA on nematodes, about 60 J2 of *H. avenae*, *H. latipons* and the *Meloidogyne* sp. were incubated in 500 µl of a 500 mg l^{-1} BABA solution or in water as a control in a 24-well plate (Corning Glass Works, New York) for 72 h at 15 °C (with *Heterodera*) or 25 °C (with *Meloidogyne*). After incubation, percentages of immobilized nematodes were recorded. These experiments were performed with six-fold replication.

Effect of BABA on infection by cyst nematodes

Foliar sprays

Three-day-old barley (cv. Ingrid) and wheat (cv. Bet-Shita) seedlings grown in sandy soil (pH 7.8) in 250 ml plastic pots were inoculated with 50 *H. avenae* J2, 4, 6 and 13 days after planting. Starting 2 days before the first nematode inoculation, the seedlings were sprayed to run-off one to five times with a 2000 mg l^{-1} BABA

solution containing 0.01% (v/v) Tween-20 at 10-day intervals. Soil surfaces of the pots were covered with paper filters to prevent dripping of the BABA solution on the soil. The seedlings were kept at 15 °C and 11 h days, and fertilized every other week with a 0.1% solution of 20-20-20 (N-P-K). Control plants were sprayed with 0.01% Tween-20 in water. The plants were uprooted 3 months after planting, and fresh shoot weights and numbers of cysts were recorded. There were six replicates of each treatment combination.

In a similar experiment, 6-day-old wheat seedlings grown in sandy soil in 50-ml test tubes were inoculated with about 100 *H. avenae* or *H. latipons* J2. The seedlings were sprayed to run-off with BABA solutions at concentrations of 0, 1000, 2000, 4000 or 8000 mg l^{-1}, 6 and 13 days after inoculation. The seedlings were kept at 15 °C and 11 h days. Fresh shoot weights and numbers of cysts were recorded 3 months after inoculation. There were eight replicates of each treatment.

Soil drenches

Three-day-old wheat and barley seedlings grown in sandy soil in 50 ml tubes were soil-drenched with 10 ml of BABA solution at concentrations of 0, 125, 250, 500 or 1000 mg l^{-1}. Two days after BABA treatment, wheat and barley seedlings were each inoculated with about 100 *H. avenae* J2, and further wheat seedlings were inoculated with about 100 *H. latipons* J2. The seedlings were kept at 15 °C and 11 h days. Nematode invasion of and development in roots was observed under a stereomicroscope after staining with acid fuchsin (Bybd et al., 1983). Numbers of cysts on the roots were counted 100 days after inoculation. Each treatment had five replicates. In another experiment, 5-day-old wheat plants grown in 50 ml test tubes were inoculated with about 200 *H. avenae* J2. The plants were soil-drenched with 10 ml of 500 mg l^{-1} BABA, 1, 2, 4, 8 or 16 days after inoculation. The numbers of adult females on the roots were counted 53 days after inoculation. Each treatment had five replicates.

Effect of resistance inducers on H. avenae *infection of wheat*

Five-day-old wheat seedlings grown in 50-ml test tubes were soil-drenched with 10 ml of solutions of BABA (500 mg l^{-1}), AABA (500 mg l^{-1}), SA (690 mg l^{-1}), INA (50 mg l^{-1}) or BTH (50 mg l^{-1}). The seedlings were inoculated with about 100 *H. avenae* J2, 2 days after treatment. Numbers of cysts were counted after 100 days. Each treatment had six replicates.

Effect of BABA on H. avenae *sex differentiation on wheat*

Three-day-old wheat seedlings grown in the test tubes were soil-drenched with 12 ml of BABA solutions at concentrations of 0, 125, 250, 500 or 1000 mg l^{-1}. The seedlings were inoculated with about 200 *H. avenae* J2, 2 days after treatment. Forty-five days after inoculation, adult males in the soil and inside the roots were extracted by sieving the soil with 285- and 30 μm sieves and by root incubation, respectively; adult females on the root surface were counted under a stereomicroscope. Each treatment had five replicates.

Effect of BABA on root-knot nematode infection of wheat

Foliar sprays

Seven-day-old wheat (cv. Prince) seedlings planted in 50-ml test tubes were sprayed to run-off with BABA solutions of 0, 1000, 2000, 4000, 6000 or 8000 mg l^{-1} containing 0.01% Tween-20. Two days after treatment, the seedlings were inoculated with about 300 *Meloidogyne* sp. J2. The seedlings were kept at 25 °C and 13 h long days, and fertilized once every other week. Numbers of egg masses on the roots were counted 45 days after inoculation. Each treatment had six replicates.

Soil drenches

Seven-day-old wheat (cv. Prince) seedlings grown in test tubes were soil-drenched once or twice with 12 ml of solutions of BABA at concentrations of 0, 62.5, 125, 250 and 500 mg l^{-1}. The seedlings were inoculated with about 300 J2 of the *Meloidogyne* sp. 2 days after the first treatment. The second soil drench with BABA was performed 14 days after inoculation. Nematode development in untreated and BABA-treated roots was observed after staining. Numbers of egg masses on the roots were counted 40 days after inoculation. Each treatment had five replicates.

Data analysis

Data were subjected to analysis of variance (ANOVA). Where a significant ANOVA was observed, regression

analysis was performed to find a correlation between BABA treatment and number of cysts or egg masses. In exponential regressions, P and r^2 values were derived from linear regressions of $\log_e(y)$ on x, where y and x represent number of cysts or egg masses, and BABA treatment, respectively. Means were separated according to the Turkey test ($P = 0.05$). All calculations were performed with JMP (SAS Institute, Cary, NC).

Results

Direct effect of BABA on nematodes

Immobilization of *H. avenae*, *H. latipons* and the *Meloidogyne* sp. J2 in a 500 mg l^{-1} BABA solution did not differ from that in water (data not shown).

Effect of BABA on infection by cyst nematodes

Foliar sprays

Negative correlations were found between the numbers of sprays with 2000 mg l^{-1} of BABA and the numbers of *H. avenae* cysts on wheat and barley ($P < 0.001$) (Figure 1a). Shoot fresh weights of wheat and barley were not affected by the numbers of sprays with BABA ($P > 0.09$) (Figure 1b). The number of cysts of *H. avenae* and *H. latipons* on wheat was negatively correlated with concentrations of BABA ($P = 0.007$ and 0.016, respectively) (Figure 2a). Fresh shoot weights of wheat plants were not influenced by increasing concentrations of BABA ($P > 0.3$) (Figure 2b). No phytotoxicity symptoms were observed in these experiments.

Soil drenches

Exponential correlations were found between increasing concentrations of BABA and the numbers of

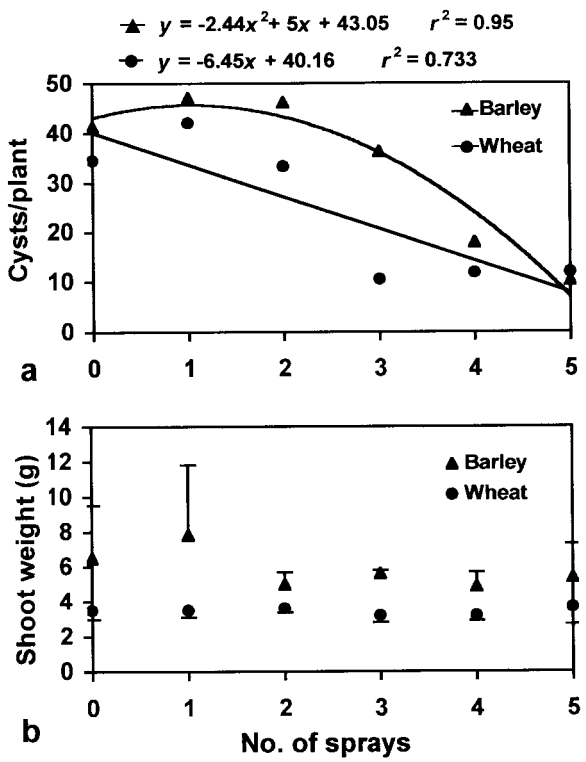

Figure 1. Effect of spraying with 2000 mg l^{-1} DL-β-amino-*n*-butyric acid (BABA) on *Heterodera avenae* infection of barley and wheat plants (a) and plant growth (b). Each point represents the mean of six replicates. Regressions are based on treatment means. Vertical lines are standard deviations.

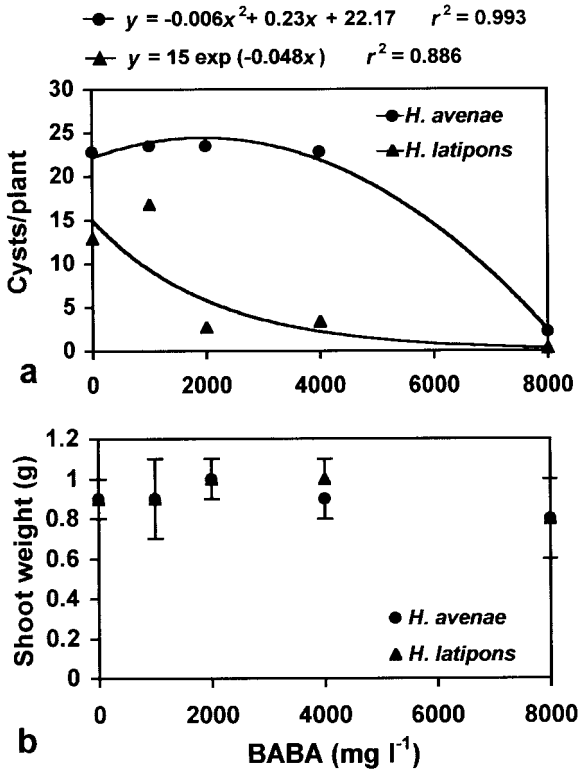

Figure 2. Effect of spraying wheat plants with DL-β-amino-*n*-butyric acid (BABA) on infection of *Heterodera avenae* and *H. latipons* (a) and plant growth (b). Each point represents the mean of eight replicates. Regressions are based on treatment means. Vertical lines are standard deviations.

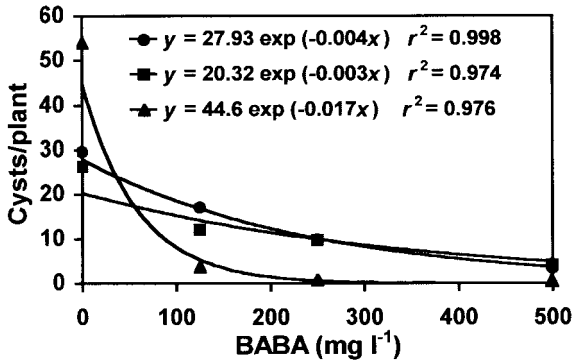

Figure 3. Effect of soil drenches with DL-β-amino-*n*-butyric acid (BABA) on cyst formation of *Heterodera avenae* and *H. latipons* on wheat and barley. (●): *H. avenae* on wheat, (■): *H. avenae* on barley, (▲): *H. latipons* on wheat. Each point represents the mean of five replicates. Regressions are based on treatment means.

H. avenae cysts on wheat and barley ($P < 0.001$), and *H. latipons* cysts on wheat ($P = 0.001$) (Figure 3). Almost no *H. latipons* cysts were found on wheat roots soil-drenched with 250 and 500 mg l^{-1} solutions of BABA. A 1000 mg l^{-1} BABA solution was needed to obtain almost total control of *H. avenae* cysts on both wheat and barley (data not shown). *H. avenae* J2 penetrated both BABA-treated and untreated wheat roots and induced typical galls at the infection sites. Syncytia were observed inside the galls, and lateral roots emerged from the galls. *H. avenae* J2 in control plant roots developed to adult males and females, whereas nematode development was inhibited in 500 mg l^{-1} BABA-treated plant roots. *H. latipons* J2 development in BABA-treated plant roots was similarly inhibited. When 500 mg l^{-1} BABA was applied after nematode inoculation, 21.6 ± 7.7 (mean ± SD) *H. avenae* adult females were counted on untreated wheat roots, whereas none were found on roots soil-drenched with BABA, 1, 2, 4 or 8 days after inoculation. The number of females on roots soil-drenched 16 days after inoculation was 3.0 ± 3.0. Nematode infection sites were observed on all roots that had undergone soil-drenching with BABA.

Effect of resistance inducers on H. avenae *infection of wheat*

Only a soil drench with BABA significantly reduced the number of *H. avenae* cysts on wheat (Figure 4). Phytotoxic symptoms (small shoots and short roots as compared to control plants) were observed on plants treated with INA and BTH (results not shown).

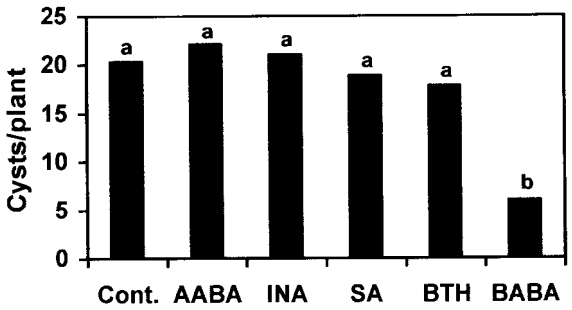

Figure 4. Effect of chemical resistance inducers (AABA, INA, SA, BTH, BAAB) on cyst formation of *Heterodera avenae* on wheat. Values with a same letter do not differ significantly, according to the Turkey test ($P = 0.05$).

Table 1. Effect of soil drench with DL-β-amino-*n*-butyric acid (BABA) on number of females and males differentiation of *Heterodera avenae* on wheat

BABA (mg l^{-1})	Female	Male	Male (%)[a]
0	25.0 ± 3.4	11.2 ± 7.0	26
125	21.3 ± 7.9	32.0 ± 5.3	61
250	8.8 ± 7.9	25.8 ± 11.5	76.6
500	1.8 ± 4.0	7.8 ± 7.1	88.2
1000	0	0.4 ± 0.9	100

Values are means and standard deviations of five replicates. [a]Calculated from replicates in which adult males or females were recovered.

Effect of BABA on H. avenae *sex differentiation on wheat*

Soil drenches with 125 mg l^{-1} BABA increased the number of adult males found on plants, whereas the number of adult females did not differ from controls, thereby resulting in an increase in the percentage of adult males (Table 1). Soil drenches with a 250 mg l^{-1} BABA solution decreased the number of adult females relative to controls, while the number of adult males was higher than in controls. Soil drenches with higher concentrations of BABA decreased the numbers of both adult males and females.

Effect of BABA on root-knot nematode infection of wheat

Foliar sprays

A linear correlation was found between the number of *Meloidogyne* egg masses and increasing concentrations of BABA ($P < 0.001$) (Figure 5a). A foliar spray

with 8000 mg l^{-1} BABA decreased the number of egg masses by 72%.

Soil drenches

The number of *Meloidogyne* sp. egg masses was decreased exponentially with increasing concentrations of BABA ($P < 0.006$). A single soil drench with BABA at a concentration of 500 mg l^{-1} reduced the number of egg masses by 94% (Figure 5b). No significant difference in the number of egg masses was found between one or two soil drenches at the same concentrations ($P > 0.13$). Nematode development in BABA-treated wheat roots was inhibited (Figure 6). No phytotoxicity was observed in plants as a result of these treatments.

Discussion

BABA induces plant resistance to several fungal pathogens, such as *Phytophthora infestans*, *P. capsici*, *Peronospora tabacina* and *Plasmopara viticola* (Cohen, 1993, 1994, 1996; Cohen and Gisi, 1994; Cohen et al., 1994, 1999; Sunwoo et al., 1996; Hwang et al., 1997). In the present study, BABA reduced the numbers of cysts and egg masses of cyst and root-knot nematodes on wheat and barley. The other chemicals used in this study, SA, INA, BTH and AABA, did not induce resistance to *H. avenae* in wheat by soil drench. Although lower concentrations of INA and BTH compared to BABA were used in the experiment, they caused phytotoxic effects without reducing the numbers of cysts. BABA at a concentration of

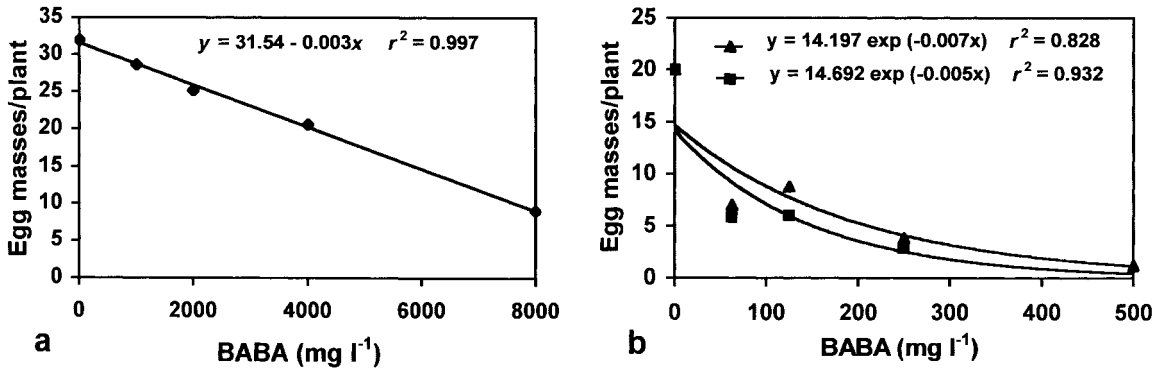

Figure 5. Egg mass production of *Meloidogyne* sp. on wheat plants sprayed (a) and soil-drenched (b) with of DL-β-amino-*n*-butyric acid (BABA). Each point represents the mean of six replicates, and the regression is based on treatment means in (a). In (b), wheat seedlings were soil-drenched one (▲) or two (■) times. Each point represents the mean of five replicates, and regressions are based on treatment means.

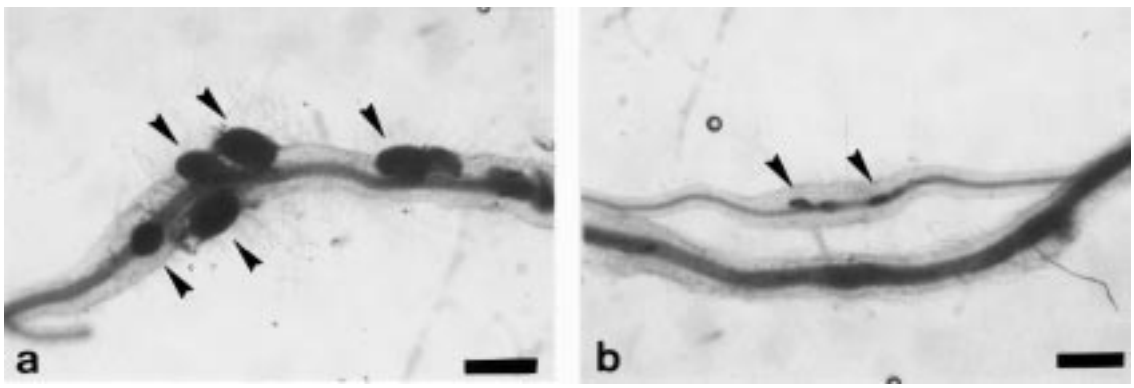

Figure 6. Infection sites and development of *Meloidogyne* sp. (arrowheads) on wheat roots 30 days after inoculation. (a) Untreated wheat. (b) Wheat soil-drenched with 500 mg^{-1} l of DL-β-amino-*n*-butyric acid. Bars = 1 mm.

500 mg l^{-1} did not kill or affect the mobility of the tested nematodes *in vitro*. Moreover, J2 of *H. avenae* and *H. latipons* penetrated wheat roots soil-drenched with 1000 mg l^{-1} BABA, and induced feeding sites. These results suggest that BABA is not itself nematicidal to the nematodes used in these experiments. In a previous study, BABA also did not affect the mobility of *M. javanica* J2 *in vitro* (Oka et al., 1999). A far higher concentration of BABA was required to induce resistance in plants by foliar spray as compared to soil drench. Only a small portion of BABA applied to leaves translocates to the root system in tomato, cucumber, wheat, barley and grapevine; nevertheless, BABA accumulated in *M. javanica* galls on cucumber and tomato whose leaves had been treated with BABA (Cohen and Gisi, 1994; Cohen et al., 1999; Oka et al., 2000). We do not know whether a signal molecule is involved in the systemic induction of resistance by BABA, or if BABA itself is translocated to the roots, and induces the resistance.

The mechanism by which SA, INA or BTH trigger induced resistance is not fully understood. Plant defense genes, including chitinases, glucanases and PR-proteins, are induced in plants by these chemicals (Vernooij et al., 1995; Friedrich et al., 1996; Dempsey et al., 1999). Chitinases and glucanases have deleterious effects on fungi but may not affect nematodes, because the substrates of the enzymes, i.e. chitin and glucan, are not present in the latter, except for a chitin layer in the eggshell. Only a few indications of a resistance mechanism to fungal pathogens in BABA-treated plants have been reported: accumulation of PR-proteins (Cohen et al., 1994; Hwang et al., 1997), elevated peroxidase activity (Bitton and Cohen, unpubl.), lignin formation at pathogen infection sites (Cohen et al., 1999) and modification of plant cell walls (Cohen and Gisi, 1994). These phenomena are also suggested to be part of the plant's resistance mechanisms to plant-parasitic nematodes (Zacheo and Bleve-Zacheo, 1995). Higher peroxidase activity, which suggests accelerated lignin synthesis, has been found in roots of BABA-treated tomato and cucumber plants relative to untreated plants (Y. Oka, unpubl.).

The most specific event in the interaction between cyst and root-knot nematodes and their hosts is the formation of feeding sites inside the roots. J2 of cyst nematodes (e.g. *Heterodera* and *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) penetrate host roots and induce sophisticated feeding sites by injecting secretions into root cells. Generally, vascular parenchyma cells are chosen for the feeding sites. Cyst nematodes form a so-called syncytium by causing the dissolution of the cell walls of neighboring cells, thus producing a large multinucleate cell (Jones, 1981). In contrast, *Meloidogyne* spp. form metabolically active giant cells via hypertrophy and mitosis without cytokinesis, again resulting in large multinucleate cells (Jones, 1981). The nematodes take up nutrients from these cells for their development, and maintain the feeding sites via continuous secretion of stimuli. In most plants resistant to cyst and root-knot nematodes, feeding-site formation is inhibited mainly by a hypersensitive reaction in the host cells, or by the early degeneration of the feeding sites. In wheat and barley roots treated with BABA, *H. avenae* syncytia and *Meloidogyne* sp. giant cells did form. This suggests that the mechanism of BABA-induced resistance is expressed at a relatively late stage of the nematode's establishment in the hosts. In fact, BABA application up to 16 days after nematode infection was also effective at reducing the number of adult females. These results indicate that feeding sites in BABA-treated roots did not supply enough nutrients to the nematodes, or that some substance(s) that inhibit(s) nematode development was (were) produced in or supplied through the feeding sites.

In wheat and oat cultivars resistant to *H. avenae*, several resistance reactions have been observed: necrosis of cells adjacent to a syncytium, early degradation of the syncytium, and hypersensitive reaction (Grymaszewska and Golinowski, 1991; Williams and Fisher, 1993; Bleve-Zacheo et al., 1995). In some resistant cultivars, juveniles develop mainly to adult males (Y. Oka, unpubl.). Larger numbers of *H. avenae* developed to adult males on wheat plants soil-drenched with 125 and 250 mg l^{-1} BABA relative to the control, but the number of adult females was fewer in wheat roots treated with 250 mg l^{-1}. At higher concentrations, BABA inhibited development of both adult males and females. These results suggest that soil-drenching with a lower concentration (125 mg l^{-1}) of BABA increased the number of males without reducing the number of adult females. We do not know the reason of this increase in the number of adult males. A medium concentration of BABA (250 mg l^{-1}) reduced the number of adult females, probably by killing indirectly the developing females. Higher concentrations (500 and 1000 mg l^{-1}) of BABA killed both developing males and females. Increases in the percentages of *Heterodera* or *Globodera* juveniles developing into

males have been explained as resulting from adverse nutritional conditions, which may be caused by high nematode densities in roots and physiological status or genetic factors in plants (Trudgill, 1967; Grundler et al., 1991). Generally, the males require fewer nutrients for their development than do females.

H. latipons appeared to be more sensitive to the BABA-induced resistance in wheat by both foliar spray and soil drench than *H. avenae*. This may be due to differences in host–parasite relationship, e.g., differences in penetration and syncytium formation sites in the root. *H. latipons* J2 penetrate fully differentiated mature roots and usually form syncytia in the cortex, whereas *H. avenae* J2 penetrate near the root tip and form syncytia in the vascular cylinder (Mor et al., 1992b).

Recently, BABA-induced resistance in tomato and grapevine to *P. infestans* and *P. viticola*, respectively, was inactivated by treatment with L-proline or γ-aminobutyric acid (GABA) (Ovadia and Cohen, 1999). The authors suggested that BABA adversely affected L-proline and GABA transport into the host and/or the pathogen. Interestingly, proline accumulated in nematode-induced galls at abnormally high concentrations (Lewis and McClure, 1975; Bird and McClure, 1976). Moreover, the increase in proline was far greater in a cotton cultivar susceptible to *M. incognita* than in a resistant one (Lewis and McClure, 1975). Considering that proline is present at a relatively high percentage in the *Meloidogyne* eggshell (Bird and McClure, 1976), large amounts of proline seems to be essential to the nematode's development and reproduction. The involvement of proline in BABA-induced resistance to nematodes is therefore worth further examination.

In conclusion, BABA is a potent nematode control agent whose mode of action differs from commercial nematicides, e.g. acetylcholine esterase inhibitors. To find or synthesize more efficient resistance inducers, the mechanism of BABA-induced resistance must be elucidated.

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