Induction of resistance to *Meloidogyne incognita* by DL-Beta amino butyric acid under salt stress condition

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Abstract

Root-knot nematodes cause severe yield losses in crops. Though chemicals have been widely used to manage them, these chemicals have been restricted due to their hazardous environmental and health effects. In recent years, the use of bio-control agents and the induction of resistance are prominent control methods. In the present study, DL-β-Amino Butyric Acid (BABA) was tested to control *Meloidogyne incognita* under salt stress conditions. The results indicate that 100 mM NaCl in combination with BABA (125 µg/ml) significantly reduced gall index by 45% compared to the control. Interestingly, the lower dose of BABA combined with abiotic stress resulted in a similar efficacy to the higher dose of BABA (250 µg/ml).

Keywords BABA · Induced resistance · Root-knot nematode

Root-knot nematodes are polyphagous pests that cause severe damage to crops. Over a 100 species of root-knot nematodes have been described. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the most common and important species in tropical and subtropical regions (Karssen and Moens 2006). These species are widely present in protected vegetable production areas in Turkey (Devran and Söğüt 2009, 2010; Devran and Özalp 2017).

Chemicals were once used to control root-knot nematodes (Devran and Söğüt 2010), but increasing environmental and health concerns have restricted their use (Nyczepir and Thomas 2009; Wesemael et al. 2011). Recently, alternative control measures are being considered, such as the usage of resistant plants and induction of plant resistance using different plant activators. The induction of resistance in plants can be done either through pre-inoculation with incompatible strains of pathogens or by using chemicals with no directly inhibitory effect on plant pathogenic organisms despite their remarkable inductive effects *in planta* (Salazar et al. 2007; Borges and Sandalio 2015). These effects may have physiological and molecular parameters.

DL-β-amino butyric acid is a chemical plant defence activator that induces plant resistance to pathogens (Cohen 2002). However, its mode of action is uncertain because the induced reactions are dependent not only on the pathosystem, but also on the mode of application (Jakab et al. 2001). The translocation and accumulation of BABA in young leaves has resulted in enhanced resistance against fungal and bacterial pathogens (Cohen 2002; Baysal et al. 2005). Previous studies have reported a novel alternative that simultaneously uses BABA and low, tolerable levels of salt stress to control bacterial spot disease caused by *Pseudomonas syringae* pv. *tomato* (Baysal et al. 2007).

The aim of this study was to induce plant resistance to *Meloidogyne incognita* through BABA treatment and investigate its synergistic behaviour under abiotic stress.

The isolate of *M. incognita* used in this study was identified in a previous study (Devran and Söğüt 2009). Commercial tomato variety, Tueza F1, (Multi Tohum, Antalya, Turkey) with four true leaves was used for experiments. Plants were grown in pots containing sandy soil in a growth chamber at 25 °C with 65% humidity during a 16:8 h photoperiod. The plants were watered and fertilized as required.

DL-β-amino butyric acid was prepared as an aqueous solution with a final concentration of 125, 250 and 500 µg/ml. Twenty-four h prior to inoculation, the adjusted BABA solutions were applied as drenching through roots (ca. 50 ml per plant). Control plants were treated with water. The plants were immersed in a 100 mM NaCl solution, and exposed to salinity stress for 10 min. Uninoculated control plants were treated with sterile distilled water for the same period of time as the

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treated plants. BABA application (125 µg/ml) was also done at 24 h after treatment as indicated above. *Meloidogyne incognita* was reared on susceptible tomato cultivar Tuez F1 (Multi Tohum, Antalya, Turkey). Plants were inoculated at the four true leaf stage with 1000 s stage juveniles of *M. incognita*. Plants were arranged in randomized block design with 5 replicates. All experiments were conducted in growth chamber at 25 °C. Experiments ended at 8 weeks after inoculation and each plant was harvested and root systems were carefully washed under tap water.

Egg masses in each root system were counted under the stereo binocular microscope and root galling index was determined on each root using a gall index scale of 0–10 (Barker 1985). Number of egg masses on the roots and root galling indices in each pot were analyzed with analysis of variance procedures to determine the effects of BABA and its combination with abiotic stress on *M. incognita*. The significance of differences among experiments were tested using Tukey test at $P < 0.05$ significance level with SPSS statistical program (SPSS, 16.0).

All treatments showed remarkable differences compared to the control according to the number of egg masses and galling index on roots (Table 1). The results demonstrated a synergistic effect of low dose BABA combined with abiotic stress (salt stress) on *M. incognita*. These findings indicated that combination of salt stress (100 mM NaCl) with four-fold less BABA (125 µg/ml) had inhibitory effect as achieved with application of 500 µg/ml BABA alone. This could significantly reduce the galling index in tomato roots. The application of NaCl (100 mM) alone resulted in a more significant effect than BABA (500 µg/ml) alone against *M. incognita* (Table 1).

In previous studies, the inducer effect of BABA has been shown for fungal and bacterial pathogens on tomatoes (Lee et al. 2000; Cohen 2002; Baysal et al. 2005). BABA has also been used as a plant inducer against root-knot nematodes in tomatoes (Oka et al. 1999). Sahebani et al. (2011) investigated the effects of BABA on cucumbers infected with *M. javanica* and the accumulation of total phenolic compounds, hydrogen peroxide and the activity of some enzymes related to plant defence mechanisms. They showed that treating the cucumber seedlings with BABA significantly reduced the nematode galls, number of egg masses per plant and number of eggs per individual egg mass compared to the control. In the

present study, BABA was tested against *M. incognita* on tomatoes. Our findings were in agreement with previous studies (Sahebani et al. 2011; Oka et al. 1999).

In conclusion, these results indicate that the induction of plant resistance occurred through the simultaneous application of BABA and salt stress. Salt stress alone and its combination with low doses of BABA treatments showed a synergistic effect on tomato plants against *M. incognita*. Salt stress has an increasingly synergistic effect on BABA's efficacy.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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Table 1 Effects of treatments on *Meloidogyne incognita*

Applications	The Number of Egg Masses	Galling Index
BABA (500 µg/ml)	94.5 ± 13.2 ^{ab}	4.5 ± 0.3 ^{ab}
BABA (250 µg/ml)	46.5 ± 6.8 ^a	3.3 ± 0.3 ^a
NaCl (100 mM)	73.5 ± 6.5 ^{ab}	3.8 ± 0.3 ^a
BABA (125 µg/ml) + NaCl (100 mM)	53.8 ± 12.8 ^a	3.3 ± 0.3 ^a
Control	233.0 ± 79.5 ^b	6.0 ± 0.6 ^b

Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey test

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