

Effect of beta aminobutyric acid (BABA), ABA and ethylene synthesis inhibitor (CoCl₂) on seed germination and seedling growth of *Brassica napus* L.

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ABSTRACT

Interactions among hormones effects on developmental process. There is much evidence indicating interactions between ABA and ethylene signaling cascades. BABA is a non-protein amino acid. It has been shown that BABA induces stress-induced morphogenic response. BABA activates some ABA signaling intermediate and also alters the expression level of some genes implicated in signaling by ethylene. The aim of this study was to examine whether BABA alters seed germination and some growth parameters via involvement of ABA and/or ethylene signaling or not. In this study, it is shown that BABA had no significant effect on seed germination of canola but in ABA and CoCl₂ treated seeds, germination was reduced compare to the control. ABA inhibited root growth more than BABA. Combined use of ABA and BABA treatment, had an additional inhibitory effect on shoot and root growth. Application of Cobalt chloride as an inhibitor of ethylene synthesis, increased root length but decreased shoot length. Use CoCl₂ with BABA improved significantly reduction caused by BABA. The results demonstrated that the effects of BABA on seed germination and growth parameters involves an ABA /ethylene-independent function.

Key words: BABA, ABA, ethylene synthesis inhibitor, *Brassica napus* L.

INTRODUCTION

Interactions among hormones effects on developmental process. There is much evidence indicating interactions between ABA and ethylene signaling cascades in *Arabidopsis* [1]. Ethylene influences plant growth, it acts as a stress hormone during biotic and abiotic stress conditions [2]. The ethylene pathway has a negative effect on seed dormancy. Ethylene inhibits ABA signaling, while these two pathways synergistically inhibit root growth [7]. It has been proved that endogenous ABA limits synthesis of ACC (aminocyclo propane 1 carboxylic acid) or Conversion of ACC to ethylene [8]. ABA and ethylene modulate both plant stresses and developmental responses [10]. BABA is a non-protein amino acid which naturally cannot be found in the plants. This chemical enhances resistance of plants to microbial pathogens and abiotic stresses, such as drought, salinity, low potassium and heavy metal toxicity [9, 3, 4]. In biotic stresses BABA applied its function via priming of SA-dependent defense mechanisms and subsequently occurs systemic acquired resistance (SAR). In other cases, BABA protects plants against abiotic

stresses by strengthening of ABA-dependent defense mechanisms [12]. It has been shown that BABA induces stress-induced morphogenic response such as reduction in root growth, increasing lateral root density and reduces vegetative growth [11]. It has been reported that BABA treatment activates some ABA signaling intermediate and also alters the expression level of some genes implicated in signaling by ethylene [12]. In this study we examined whether BABA alters seed germination and some growth parameters via involvement of ABA and/or ethylene signaling or not.

MATERIALS AND METHODS

Seeds of *Brassica napus* L. (c.v. Madonna) were used in this study. Seeds were sterilized in sodium hypochlorite (0.1%) solution for 10 min, rinsed in distill water several times and plated onto Petri plates containing agar and nutrients. The medium included 1.2% agar, 0.5% sucrose, 4 mM KNO₃, 1 mM Ca(NO₃)₂, 0.3 mM MgSO₄, 2 mM KH₂PO₄, 89 μM Miron citrate, 10 μM H₃BO₃, 2 μM MnCl₂, 0.77 μM ZnSO₄, 0.31 μM CuSO₄, 0.13 μM MoO₃, and 0.1 μM NiCl₂ [6]. Seeds were placed on media containing 100 μM BABA or/and 3 μM ABA, 3mM CoCl₂ or/and 100 μM BABA dissolved in water. CoCl₂ was added to the medium two days before adding BABA.

Germination percentage

The plates were placed in germinator at 22°C with 16 h photoperiod. All plants were grown at a light intensity of about 100 μmol m⁻² s⁻¹. The germination percentage was recorded after 72h of incubation. Experiments were repeated 3 times.

Growth parameters

The length of the shoot and root and fresh weight of seedlings were measured 10 days after germination. The seedlings were placed in an oven with a Temperature of 70 ° C for 48 h to determine the dry weight of the sample.

Statistical analysis

Data for each parameter were subjected to ANOVA and significant differences between treatment means were determined by LSD test using the SPSS software. Data are shown as means with three replicates and significance was determined at the 95% confidence (p ≤ 0.05) limits.

RESULTS AND DISCUSSION

Recent study showed that BABA had no significant effect on seed germination of canola but in ABA and CoCl₂ treated seeds, germination was reduced compare to the control. The ethylene regulates seed dormancy negatively by inhibiting ABA signaling. Combined treatment with ABA and BABA decreased germination percentage of canola seeds. Both BABA and ABA adversely affected root growth, shoot and root length. The relative reduction in root growth was 6% for BABA and 15% for ABA. This result showed that, ABA inhibited root growth more than BABA. Combined use of ABA and BABA treatment, had an additional inhibitory effect on shoot and root growth (Fig 1; table 1). This result suggests that BABA act independently of ABA, in root and shoot growth reduction.

Table 1. The effects of ABA (0 and 3 μM) and BABA (0 and 100 μM) on the seed germination, growth parameters in the *Brassica napus* L

ABA	BABA	Germination (%)	Root length (cm)	Shoot length (Cm)	Fresh weight (g)
0	0	85±3.5	7.96±0.32	4.8±0.2	0.03±0.001
0	300	82±2.1	7.4±0.29	4±0.05	0.025±0.002
3	0	66±3.5	6.8±0.14	3.6±0.3	0.019±0.005
3	300	68±1.5	5.4±0.3	2.7±0.14	0.014±0.006

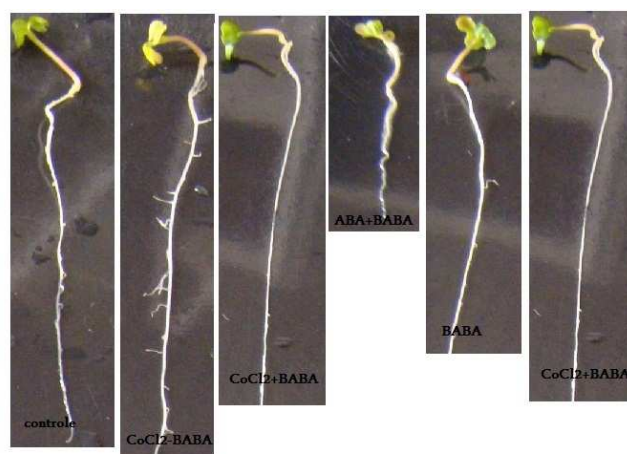


Fig 1. Effect of BABA, ABA and CoCl_2 on root length of canola seedlings

Co^{2+} ions inhibit ACC oxidase thus blocking ethylene synthesis. Application of Cobalt chloride as an inhibitor of ethylene synthesis, increased root length but decreased shoot length. Use CoCl_2 with BABA improved significantly reduction caused by BABA (table 2).

Table 2. The effects of BABA (0 and 100 μM) and CoCl_2 (0 and 3mM) on the seed germination, growth parameters, in the *Brassica napus* L

CoCl_2	BABA	Germination (%)	Root length (cm)	Shoot length (Cm)	Fresh weight (g)
0	0	85 \pm 3.5	7.96 \pm 0.32	4.8 \pm 0.2	0.03 \pm 0.001
0	300	82 \pm 2.1	7.4 \pm 0.29	4 \pm 0.05	0.025 \pm 0.002
3	0	60 \pm 2.3	10.2 \pm 0.65	3.4 \pm 0.2	0.02 \pm 0.004
3	300	58 \pm 2.6	8.7 \pm 0.1	2.5 \pm 0.1	0.016 \pm 0.005

The results demonstrated that the effects of BABA on seed germination and growth parameters involves an ABA /ethylene-independent function. Although ABA and CoCl_2 inhibits seed germination but BABA had no similar effect on germination. This observation is consistent with Wu *et al.*, 2009 in *Arabidopsis*. They showed that BABA inhibited root growth. BABA reduced cell division of the root meristem. In addition, fresh weight of BABA-treated plants reduced as compared to the control. Root elongation was also inhibited by ABA. Zimmerli *et al.* (2008) showed there is no substantial overlap with the genes upregulated by BABA and ABA expression profiles. They also noted that probably BABA and ABA upregulate different sets of genes encoding proteins with similar functions. Jakab *et al.*, (2005) showed that the plants pretreated with BABA accumulated higher levels of ABA in comparison to control plants [5]. BABA activates the accumulation of transcripts involved in developmental responses. The BABA-mediated induction of ABA- and ethylene-signaling genes. In conclusion, the chemical BABA has been shown to act as stressing factor and activate ABA and ethylene stress signaling but it appears to effect vegetative growth independent of ABA or ethylene.

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