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ARTICLE

Analysis of BABA (β-aminobutyric acid)–induced female sterility in Arabidopsis flowers

Marianna Kocsis*, Gábor Jakab

Department of Plant Physiology, University of Pécs, Pécs, Hungary

ABSTRACT The beta-aminobutyric acid (BABA) induces resistance in plants against a wide range of pathogen, affecting callose production and deposition in *Arabidopsis*. Repeated treatment with BABA, however effects the pollination of the ovules resulting female sterility. The main reasons of this phenomenon out of the structural changes in the treated *Arabidopsis* plants can be the interrupted pollen tubes and the callose deposition at the ovule micropyle in flowers and buds. **Acta Biol Szeged 52(1):247-249 (2008)**

KEY WORDS

Arabidopsis
BABA (ß-aminobutyric acid)
female sterility
fluorescence microscopy

The non-protein amino acid ß-aminobutyric acid (BABA) protects the plants against diseases attacking foliage, roots and fruits (Cohen 2002). As reported in 1976 by Aist, BABA-treated plants reacted with increased papilla formation after challenge with *Hylaperonospora parasitica* (formely Peronospora parasitica) led to the hypothesis that it might somehow affect callose production in *Arabidopsis*, since callose is one of the main components of papillae. Callose is also known to be involved in pollen – ovule interactions (de Martinis and Mariani 1999) and in some cases there was a correlation between the degree of self-sterility and the amount of callose deposition (Kerhoas et al. 1983). Based on the observation that repeated treatment with BABA triggers female sterility, Jakab et al. (2001) have isolated mutants with an impaired BABA-induced sterility (ibs) response. This chemical inducer in plants triggered a faster and stronger accumulation of callose, a higher level of cell wall lignification, or resulted in a faster activation of defense-related gene expression (Ton et al. 2005).

The present study concentrates on the induced female sterility based on the treatment of a simple chemical compound, BABA. It has known biological effects in animals and plants, but its mode of action remains unclear in plants (Zimmerli et al. 2000). The goals of our research are to describe the morphological changes led to female sterility and to identify the molecular mechanisms regulating these morphological alterations using mutant plants, which do not become sterile due to BABA-treatment. Our further aim is to know the genes and mechanisms involved in BABA-mediated protection. Knowledge about molecular mechanisms of how plants perceive and transmit stress signals, how plants regulate stress gene expression will allow help secure agriculture and environment under changing global conditions.

*Corresponding author. E-mail: mkocsis@gamma.ttk.pte.hu

Materials and Methods

Arabidopsis (Col0) plants starting bolting were treated with 30 ppm (final concentracion in the soil) of BABA ones pro week. BABA - which can be taken up by the roots and translocated through the plantlets (Cohen and Gisi 1994) - has been applied as a soil drench to the root system. We bred four series of 3-5 plants and investigated 1-3 fruits, flowers and buds pro plants. In all 40 fruits, 80 flowers (different age flowers), 36 buds of the control plants and 43 fruits, 82 flowers, 37 buds of the treated plants were investigated. After 5-6 weeks we stopped the BABA treatment.

Structure of the tissues was studied at different times, after each BABA-treatment with aniline blue staining. The staining method is used to reveal callose structures in plant tissue. Fruits, flowers and buds were placed in microtiter plates and fixed in 200 µl acetic acid and ethanol at a ratio of 3:1 for 1.5 hours or more. To soften the tissues they were submerged in 1 N NaOH for 15 min in 60ºC thermostat, then washed 3-times with destilled water and stained with 200 µl aniline blue for 5-10 minutes. The stain used was 0.1% aniline blue in 0.1 N potassium phosphate (http://preuss.bsd.uchicago.edu/protocols/aniline.html - with some modifications to shorten the procedures).

The structure and the callose deposition of the tissues were determined by fluorescence microscopy (Nicon Eclipse 80i microscope with UV light – adapted system, with illumination from an Osram HBO 100 W/2 mercury lamp), the photos were taken by the Spot Basic 4.0 software.

Results and discussion

Our study showed several differences in the structures of the flowers, buds and fruits between control and BABA-treated plants.

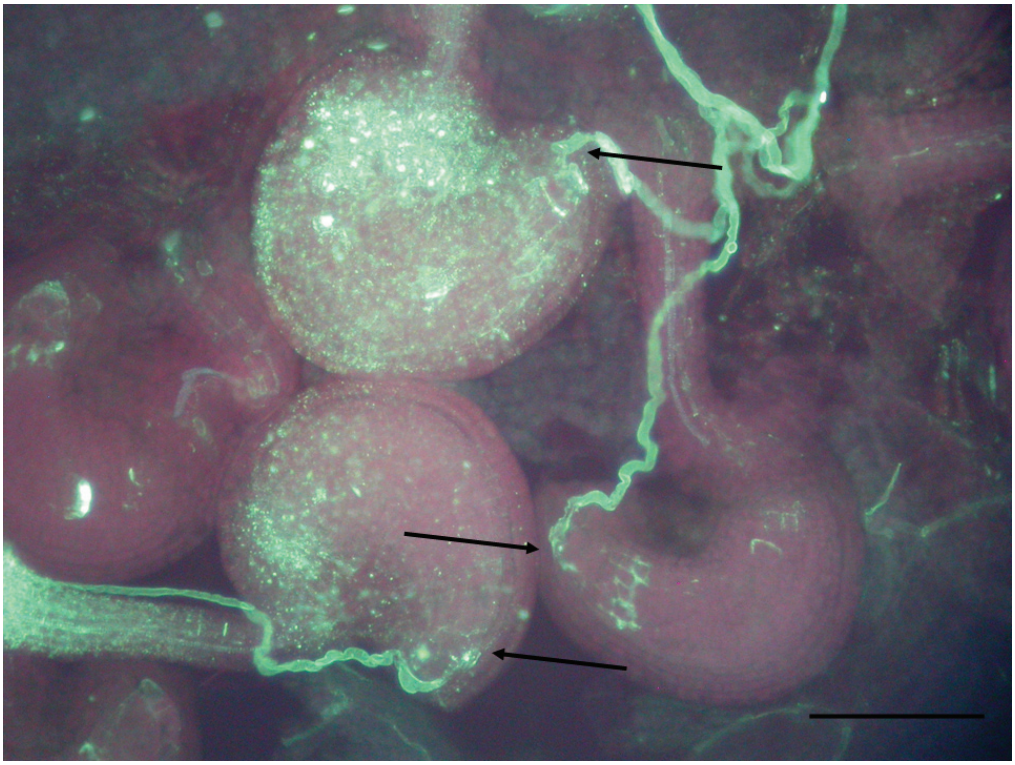

Figure 1. Part of the pistil in the flower of a control plant. The arrows indicate pollen tube entry into the micropyle. Bar= 50 µm.

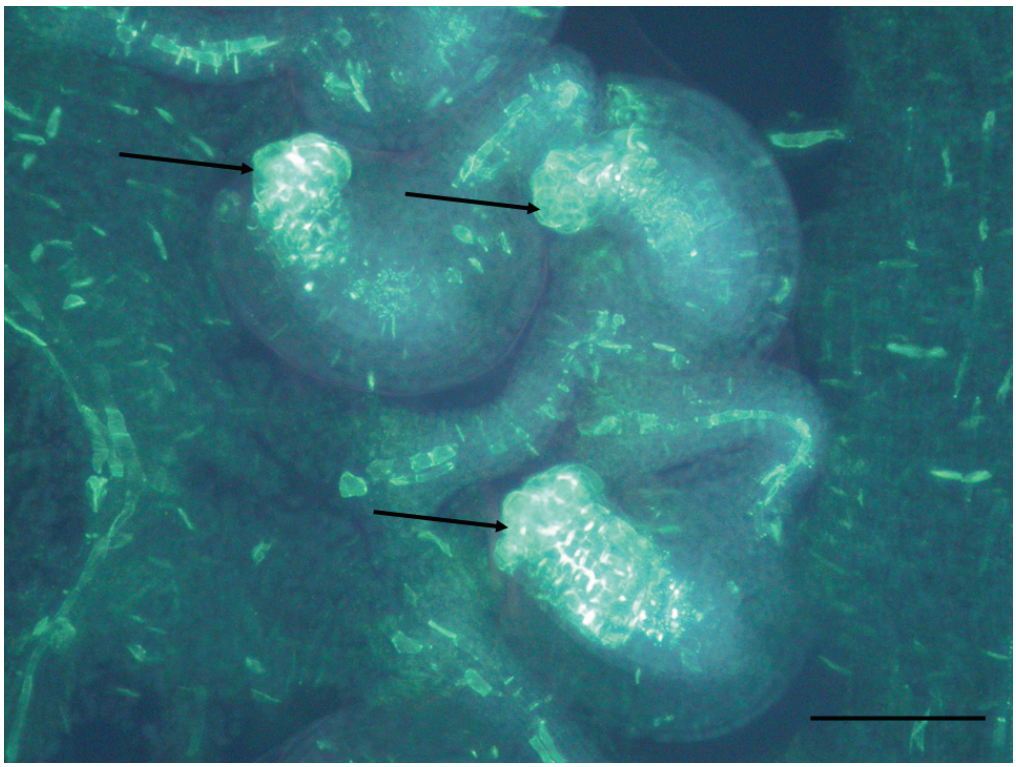

Figure 2. Part of the pistil in the flower of a BABA-treated plant. The arrows indicate the callose deposit at the ovule micropyle. Bar= 50 µm.

In normal case the gynoecium elongated and extended beyond the top of the stamens after pollination. The sepals, petals and stamens withered and fell from the fruit. These organs in BABA-treated plants turned yellow and fell earlier, - already in flowering stadium - than in the control plants. Callose deposition in sepals and petals of the treated plants could be found as lightened dots. The BABA-treated and untreated plants could be well distinguished through their fruits. The normal plants had about 2 cm long ripened fruits, the treated ones‘ maximal size were 2-3 mm. In the treated siliques none of the ovules developed into seed, they were shrunken and dried.

In untreated flowers the stamens extended above the top of the stigma at the beginning of the flowering stadium. The flowers opened and self pollinated. The stigma was comprised of a single layer of elongated papillar cells specialized for the germination of pollen (Roeder et al. 2006). In case of BABA treated plants, microscopic observations sometimes showed longer papillae covering the stigma, but the pollen germination were not affected. The style guided the pollen tubes from the stigma to the ovary. The callose plugs of the pollen tubes were similar in size and arrangement in the untreated control and treated plants. The main difference was – after the 2nd or 3rd BABA-treatment - that pollen tubes in the treated plants did not reach the ovary sacs, but already were interrupted in the style or in the upper part of the ovary, which forms the majority of the *Arabidopsis* fruit. The majority (90%) of the investigated flowers of BABA-treated plants showed this phenomenon. In contrary, the pollen tube tips of control flowers were guided into the ovule micropyle (Fig 1.) and the fertilization occurred resulting in seed set. After the 3rd or 4th treatment BABA effected vigorous callose deposition and „bubble-like“ formation at the micropylar region (Fig. 2). At the same time the ovule micropyle in the buds showed the above mentioned changes.

One explanation of the observed changes can be the perturbation of micropylar pollen tube guidance. Loss of a synergid-expressed MYB98 gene abolished the ovule’s ability to attract pollen tubes (Kasahara et al. 2005). Long distance guided pollen tube is controlled by the seven-celled female gametophyte, the embryo sac, although it is not clear whether the synergids are the source of the attracting signal or just the physical interactions interface (Chen et al. 2007). Our hypothesis is, that BABA-induced callose deposition in the micropyle may abolishe micropylar pollen tube guidance.

The BABA is rarely occurs naturally in plants, which accumulate this chemical – based on our observation - only for a while. The aborted BABA-treatment resulted normal fruits after 2 weeks. BABA, however, can be a useful and efficient tool to study the molecular mechanisms regulating both pollination and resistance against pathogens (Jakab et al. 2001; Ton et al. 2005).

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