BIOLOGIA PLANTARUM 51 (2): 377-380, 2007

BRIEF COMMUNICATION

Influence of cytokinins and novel cytokinin antagonists on the senescence of detached leaves of *Arabidopsis thaliana*

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Abstract

Cytokinins N^6-benzyladenine (BA) and 1-(2-chloropyridin-4-yl)-3-phenylurea (4PU-30) delayed the senescence of detached leaves (3^{rd} to 7^{th} leaf node) of wild and ethylene insensitive *eti5* mutant of *Arabidopsis thaliana*. The novel anticytokinins, structural analogues of purine and phenylurea cytokinins also affected the senescence of detached rosette leaves of *A. thaliana*. They diminished to a significant extent the cytokinin-induced delay of chlorophyll destruction, but without a considerable difference in their action against both types of cytokinins. These results correlated with changes observed in ribonuclease (RNase) activity.

Additional key words: anticytokinins, chlorophyll, ethylene insensitive mutant, leaf node, RNase.

Senescence (natural or induced by various artificial factors) is accompanied by destruction of photosynthetic pigments and leaf yellowing is one of the first visible symptoms of this process (Thomas and Stoddart 1980, Zacarias and Reid 1990, Sergiev *et al*. 2003, Todorov *et al*. 2003a,b, Alexieva *et al*. 2004). The leaf isolation is a key event leading to commencement of the senescence mechanisms. The growth promoting as well as senescence-delaying properties of cytokinins and their action as anti-senescence agents are wide studied (Van Staden *et al*. 1988, Zacarias and Reid 1990, Stoynova-Bakalova *et al.* 2001, Wilhelmová *et al.* 2004). On the other side, the application of cytokinin antagonists eliminates the cytokinin-induced hindrance of senescence in excised leaves and other model systems (Karanov *et al*. 1993, Alexieva *et al*. 1994, Sergiev 1999). The destruction of RNA and induction of RNase activity are characteristic for the senescence in higher plants (Dangl *et al*. 2000). To our knowledge, the effects of cytokinin antagonists in relation to senescence-induced changes in RNase activity are not studied.

Arabidopsis mutants have been used increasingly in physiological and biochemical studies (Kieber 1997). We used plants of *Arabidopsis thaliana* (L.) Heynh. wild type (WT), and the ethylene insensitive mutant *eti5*. This mutant (Harpham *et al*. 1991) possesses characteristics of delayed senescence accompanied with higher amount of leaf pigments and soluble proteins (Sergiev *et al*. 2003, Todorov *et al*. 2003a,b). In the present work we investigated the effect of synthetic purine and phenylurea cytokinins and their novel structural analogues with anticytokinin properties on chlorophyll (Chl) content and RNase activity in detached rosette leaves.

The plants were grown in plastic pots, filled with soil/*Perlite* mixture (3:1) in a growth chamber (16-h photoperiod, 70 μmol m^{-2} s^{-1} photon flux density, 24/20 ºC day/night temperature, 60 % air humidity). The plants were daily irrigated. In order to characterize the senescence of detached rosette leaves in presence of purine and phenylurea cytokinins, their antagonists

Received 21 August 2005, *accepted* 17 April 2006.
Abbreviations: BA - N^6-benzyladenine; 2PU-3 - 1-(4-chlorophenyl)-3-(pyridin-2ylmethyl)urea; 4PU-30 - 1-(2-chloropyridin-4-yl)-3-phenylurea; RNase - ribonuclease; TP-5 - 3-benzyl-7-(4-methylpiperazin-1-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine.
Acknowledgements: This work was supported by the COPERNICUS Programme ERBIC15CT960914.
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and combinations of them we used excised leaves from 30-d-old plants. The leaves (from 3rd to 7th leaf node) were cut, weighed, and put in Petri dishes (d = 10 mm) for incubation on two layers of filter paper wetted with 5 cm^3 of distilled water (control) or water solutions of 1-(4-chlorophenyl)-3-(pyridin-2ylmethyl)urea (2PU-3), 3-benzyl-7-(4-methylpiperazin-1-yl)-3*H*-[1,2,3]triazolo [4,5-*d*]pyrimidine (TP-5), N^6-benzyladenine (BA), and 1-(2-chloropyridin-4-yl)-3-phenylurea (4PU-30). All compounds were applied in concentration 0.01 mM. The samples were incubated in darkness (25 ºC) for 72 h and Chl content was measured spectrophotometrically (Arnon 1949). RNase activity was measured using the method of Merlo *et al*. (1988). The results presented are from three experiments, in three replicates each. The data presented are mean values ± standard deviation (SD).

As expected the excised *Arabidopsis* leaves from both genotypes incubated in darkness demonstrated typical symptoms of senescence, assessed by considerable loss of Chl (Fig. 1), which is in accordance with other findings in

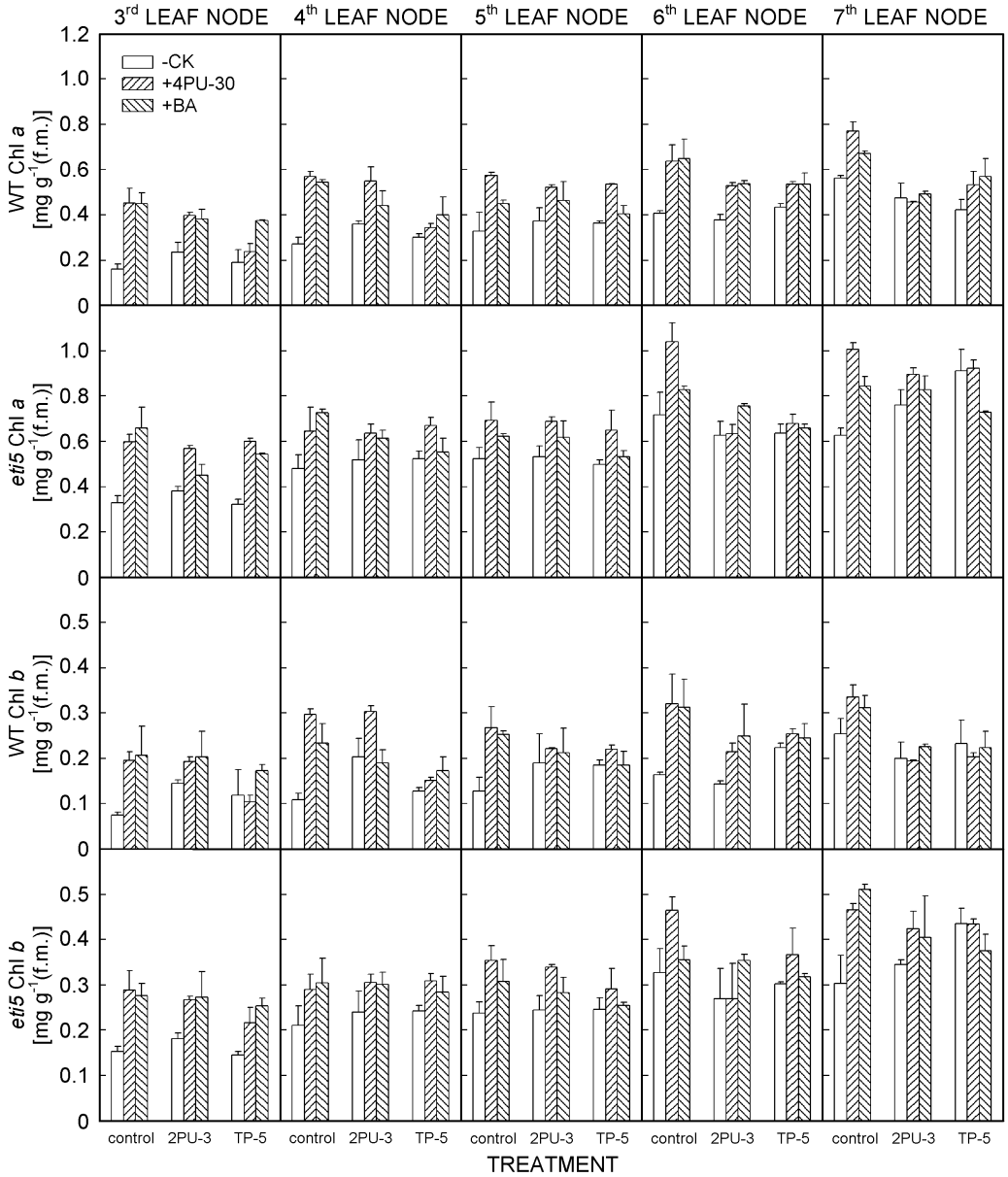

Fig. 1. Chlorophyll (Chl) content in excised rosette leaves (leaf nodes 3rd to 7th) of wild type (WT) and *eti5* mutant of *Arabidopsis* incubated on test solutions of 0.01 mM BA, 4PU-30, 2PU-3 and TP-5 for 72 h in darkness. Initial state WT Chl *a*: 3rd - 0.860, 4th - 0.790, 5th - 0.812, 6th - 1.057, 7th - 0.974; *eti5* Chl *a*: 3rd - 0.961, 4th - 1.002, 5th - 1.251, 6th - 1.196, 7th - 1.313; WT Chl *b*: 3rd - 0.363, 4th - 0.429, 5th - 0.441, 6th - 0.460, 7th - 0.590; *eti5* Chl *b*: 3rd - 0.452, 4th - 0.606, 5th - 0.590, 6th - 0.595, 7th - 0.603 mg g^{-1}(f.m.).

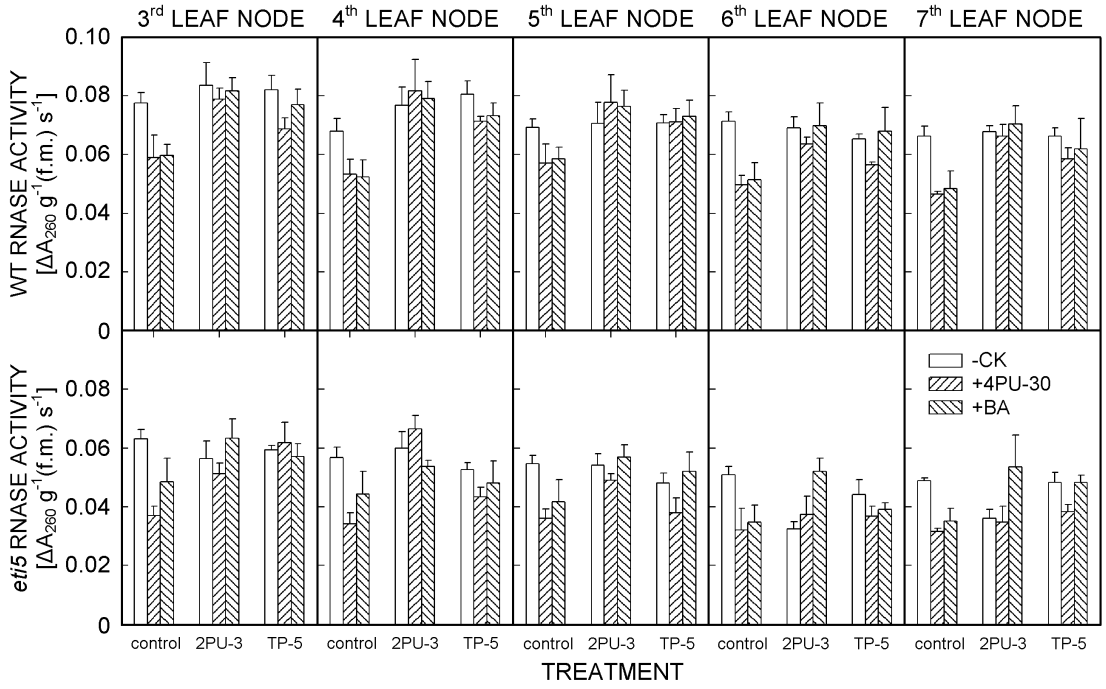

Fig. 2. RNase activity in excised rosette leaves (leaf nodes 3rd to 7th) of wild type (WT) and *eti5* mutant *Arabidopsis* incubated on test solutions of 0.01mM BA, 4PU-30, 2PU-3 and TP-5 for 72h in darkness. Initial state WT: 3rd - 0.0329, 4th - 0.0255, 5th - 0.0232, 6th - 0.0223, 7th - 0.0158; *eti5*: 3rd - 0.0234, 4th - 0.0224, 5th - 0.0210, 6th - 0.0177, 7th - 0.0132; U = ΔA_{260} g^{-1}(f.m.) s^{-1}.

leaf explants from *Arabidopsis* and other plant species (Zacarias and Reid 1990, Sergiev 1999). The Chl destruction was lesser in the mutant as compared to the WT.

Both purine and phenylurea cytokinins (BA and 4PU-30) retarded leaf senescence (Fig. 1). Their structural analogues, TP-5 and 2PU-3 described in details in another model systems (Sergiev 1999), slightly affected the Chl loss when applied alone. However, in combination with cytokinins, they decreased the cytokinin-promoted Chl retention. The effects were expressed in equal degree against both cytokinins in contrary to other test systems, senescence of barley leaf segments and growth of radish cotyledons (Sergiev 1999), where the compounds counteracted selectively the corresponding cytokinin.

Our results correlated with the changes in RNase activity (Fig. 2). RNase activity in *eti5* mutant was less than in the WT. Reduced RNase activity in mutant plant as compared to the WT *Arabidopsis* is not unexpected fact since its retarded senescence. Treatments with both cytokinins decreased the RNase activity in both genotypes. The cytokinin antagonists applied alone did not influence considerably the RNase activity with exception of 2PU-3 in the younger (6th and 7th) leaf nodes of *eti5*, where an inhibition of the enzyme was observed in relation to the respective control. Both cytokinin antagonists TP-5 and 2PU-3 eliminated the effects of BA and 4PU-30 on RNase activity when applied in combination with the cytokinins.

Our findings in excised leaves of *Arabidopsis* of both genotypes are in accordance with the reported difference in the senecsence processes of intact *Arabidopsis* plants (Todorov *et al*. 2003a,b), *i.e.* delayed Chl destruction in the rosette leaves of the mutant plants in comparison to the WT.

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