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ARTICLE

Atnoa1 mutation may induce temperature acclimation mechanisms in *Arabidopsis thaliana*

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ABSTRACT The present work was focused on evaluating the effect of the Atnoa1 mutation on temperature adaptation processes and on changes in the content of stress-related compounds, polyamines and salicylic acid. Freezing tests indicated that cold acclimation substantially increased the freezing tolerance of Atnoa1 mutant plants, similarly to the wild type, suggesting that the negative changes caused by the mutation in ATNOA1 do not substantially affect hardening processes. Atnoa1 mutation does not affect significantly the antioxidant enzymes Under control conditions in Arabidopsis plants; however, the cold induced increase in the activity of glutathione reductase was more pronounced in Atnoa1 than in the wild-type. Results suggest that Atnoa1 mutant Arabidopsis plants try to compensate for the negative effects of this mutation. These adaptation processes include the stimulation of photoprotection and alterations in the salicylic acid and polyamine compositions. **Acta Biol Szeged 55(1):113-115 (2011)**

KEY WORDS

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Atnoa1 mutant plants were characterised by impaired NO production and organ growth, and by impaired abscisic acid-induced stomatal movements compared with wild-type plants (Guo et al. 2003). However, later results indicated that this protein did not have NOS-like activities, and it was renamed AtNOA1 (NO-associated 1), which seems to function as a plant cGTPase protein (Moreau et al. 2008).

Salicylic acid (SA) has long been known as a signal molecule in the induction of defence responses in plants (Horváth et al. 2007). Recent results show that not only does exogenous SA application diminish stress effects, but abiotic stress factors also alter the endogenous SA levels in plant cells. Although a relatively large number of studies have described the role of SA in stress adaptation processes (using mainly exogenous SA), information on the cross-talk between SA and other signalling pathways is very limited, as this field has only attracted attention very recently.

Polyamines are essential components of living organisms playing role in several stress-related processes. Recent results showed that certain polyamines, for example spermidine, may also play an important regulatory role in stress signalling pathways (Kasukabe et al. 2004).

Little is known concerning the function of cGTPases in plants, which could explain the behaviour of the Atnoa1 mutants. The aim of the present work was to characterise the effects of the Atnoa1 mutation on temperature adaptation processes in Arabidopsis, with special regard to the SA and polyamine metabolism.

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Materials and Methods

Arabidopsis thaliana cv. Columbia (wild-type) and Atnoa1 mutant plants were grown in growth chambers at 21°C with a photosynthetic photon flux density (PPDF) of 150 µmol m^{-2} s^{-1}. Temperature acclimation treatments for the chlorophyll fluorescence investigations were carried out in a plant growth cabinet either at 30°C for 3 d at 80 µmol m^{-2} s^{-1} (heat acclimation) or in a cold chamber at 4°C for 3 d at 30 µmol m^{-2} s^{-1} (cold acclimation). The corresponding control plants were maintained at 80 µmol m^{-2} s^{-1} for 6 d. For the frost acclimation experiments the control plants were kept continuously at 21°C at 150 µmol m^{-2} s^{-1} PPFD; cold-hardened plants were kept at 4°C for 4 d under the same light conditions.

Freezing tolerance was tested by electrolyte leakage measurements were out according to Szalai et al. (1996).

A commercial SPAD-502DL chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) was used to estimate the chlorophyll content. Twenty readings were taken on the middle of the fully expanded leaves.

The chlorophyll-a fluorescence induction parameters were measured using a Handy FluorCam system (Photon System Instruments, Brno, CR) at room temperature after at least 20 min dark adaptation. Data were evaluated using the FluorCam7 software (Photon system Instruments, Brno, CR).

Antioxidant enzyme activities were estimated as described earlier (Janda et al. 1999).

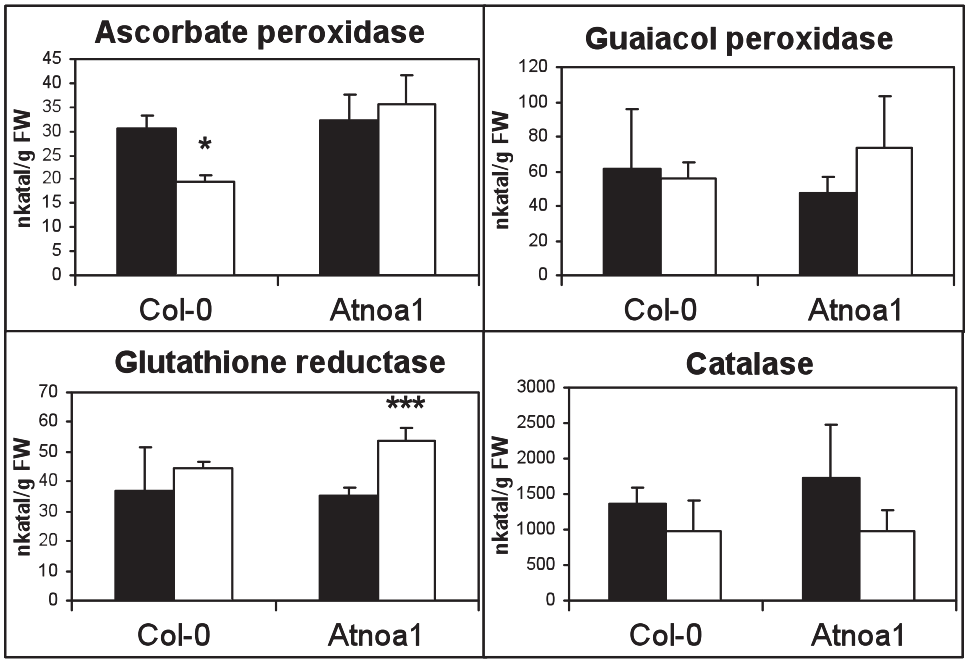

Figure 1. Effect of cold treatment at 4°C on antioxidant enzyme actvities in wild type (Col-0) and Atnoa1 mutant plants. Black bars: control plants (21 °C), white bars: cold-treated plants. * and *** represent significant differences between the control and cold-treated plants at the 0.05 and 0.001 levels, respectively.

Polyamine determination from leaf extracts was carried out according to Smith and Davies (1985) using HPLC. SA was measured according to Pál et al. (2005). The results were the means of 5 individual measurements and were statistically evaluated using the standard deviation and T-test methods.

Results and Discussion

The Atnoa1 mutation phenotypically causes reduced shoot and root growth rates and yeloowish leaves (Guo et al. 2003, Moreau et al. 2008). In order to characterise the effect of the Atnoa1 mutation on the adaptation processes of the photosynthetic machinery, certain chlorophyll-a fluorescence induction parameters were determined in control plants and in those pre-adapted to low (4°C) or high (30°C) temperatures for 3 d. In order to reduce the effects of low temperature stress-induced photoinhibition the low temperature treatment was carried out under reduced PPFD (30 μmol m^{-2} s^{-1}), while the heat acclimated and control plants were kept at 80 μmol m^{-2} s^{-1}. The F_v/F_m chlorophyll-a fluorescence induction parameter, which represents the maximum quantum yield of PS 2, was significantly lower in the Atnoa1 mutant than in the wild-type plants the actual quantum efficiency of PS 2 was also slightly, but statistically significantly lower in Atnoa1 than in Col-0. Non-photochemical quenching (NPQ) showed not only a substantial increase in the light-adapted state, but also more rapid light induction after the dark-adapted state in the Atnoa1 mutant than in the wild type Col-0.

Freezing tests were performed to reveal differences in the damage caused by freezing between the wild-type Col-0 and the Atnoa1 genotypes under cold hardened and non-hardened conditions. The difference in electrolyte leakage, characteristic of membrane destruction, between cold-hardened and unhardened plants was highly significant in both genotypes. There was no statistically significant difference between the Col-0 and Atnoa1 plants, under either hardened or unhardened conditions.

Exposure of plants to unfavourable environmental conditions is usually accompanied with the production of reactive oxygen species (ROS). In order to keep the amount of ROS in balance plants have evolved several enzymatic and non-enzymatic antioxidant systems. Results suggest that while under control conditions Atnoa1 mutation does not affect significantly the antioxidant enzymes in Arabidopsis plants, the cold induced increase in the activity of glutathione reductase was more pronounced in Atnoa1 than in the wild-type (Fig. 1). Changes in the main polyamines, agmatine, putrescine, spermidine and spermine were also measured in unhardened and cold hardened wild-type and Atnoa1 plants. The level of putrescine increased substantially, while that of spermine decreased by the end of the cold-hardening period. The quantity of spermidine in Atnoa1 was significantly higher than in Col-0, at both control and cold-hardening temperatures. A similar trend could be observed in the case of spermine, but only under control conditions. The level of putrescine was

higher in the mutant than in the wild-type after cold hardening, but due to the greater variability for this polyamine, the difference was not statistically significant. The contents of free and bound forms of SA were determined in control and cold-hardened wild-type and Atnoa1 plants. In the wild-type Col-0, cold hardening only caused a significant increase in the bound forms of SA. The mutant plants showed substantially higher SA contents for both the free and bound forms. This difference was significant not only in the control, but also in the cold-hardened plants.

In conclusion, these results suggest that Atnoa1 mutant Arabidopsis plants try to compensate for the negative effects of this mutation. These adaptation processes include the stimulation of photoprotection and alterations in the SA and polyamine compositions.

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References

Guo FQ, Okamoto M, Crawford NM (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signalling. Science 302:100-103.

Horváth E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid signaling. J Plant Growth Regul 26:290-300.

Janda T, Szalai G, Tari I, Páldi E (1999) Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. Planta 208:175-180.

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 45:712-722.

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF (2008) AtNOS/AtNOA1 is a functional *Arabidopsis thaliana* cGTPase and not a nitric-oxide synthase. J Biol Chem 83:32957-32967.

Pál M, Horváth E, Janda T, Páldi E, Szalai G (2005) Cadmium stimulates the accumulation of salicylic acid and its putative precursors in maize (*Zea mays* L.) plants. Physiol Plant 125:356-364.

Smith MA, Davies PJ (1985) Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. Plant Physiol 78:89-91.

Szalai G, Janda T, Páldi E, Szigeti Z (1996) Role of light in the development of post-chilling symptoms in maize. J Plant Physiol 148:378-383.