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EFFECT OF CHILLING AND FREEZING STRESSES ON JASMONATE CONTENT IN *ARABIS ALPINA*

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Abstract

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Depending on the temperature involved, there are two types of cold stress: chilling stress exerted at low positive temperatures around +5°C and freezing stress when temperatures are below zero. As an adaptation to cold stress, plants have evolved multiple mechanisms for tolerance. These mechanisms can be mediated by a number of stress phytohormones and plant growth regulators, including ABA, JA, SA, ethylene. It is well known that stress phytohormones exert their action by triggering phosphoprotein cascade pathways, which in turn lead to expression of genes, involved in the acquisition of cold stress tolerance. The perennial plant *Arabis alpina* (Brassicaceae), closely related to *Arabidopsis* sp. was selected as a model plant in this study. *A. alpina* is wide spread in mountain areas of the northern hemisphere, some of its populations being tolerant to frost. Using HPLC-MS analysis, we represent experimental data on JA and JA-ile content in three *A. alpina* populations exposed to chilling or freezing stress: frost tolerant (T), non-tolerant (NT) and plants with a short hypocotyl (SH).

Key words: Arabis alpina, cold stress (chilling, freezing), jasmonic acid, jasmonate-isoleucine
Abbreviations: ABA – abscisic acid, JA – jasmonic acid, JA-ile – jasmonate isoleucine, SA – salicylic acid, T – tolerant plants, NT – non-tolerant plants, SH – plants with short hypocotyl, LOX – lipoxygenase, DW – dry weight, HPLC – high pressure liquid chromatography

Introduction

Abiotic stresses such as drought, cold or high salinity are among the major environmental factors that limit the productivity and growth potential of plants. Cold stress affects crop productivity in most parts of the world, as many crop cultures are intolerant to cold. Moreover, with the late global climate changes acute temperature variations may occur comprising short warm periods followed by subsequent freezing temperatures, the latter damaging the plants (Schwartz et al., 2006). Depending on temperature, there are two types of cold stress: chilling stress, which is exerted at low positive temperatures (around +5°C) and freezing stress arising at temperatures below zero. As an adaptation to cold stress, plants have evolved multiple tolerance mechanisms at physiological, biochemical, and molecular levels, thus enabling them to survive under unfavorable environmental conditions (Shinozaki et al., 2003). A number of stress phytohormones and plant stress regulators, including ABA, JA, SA and ethylene can mediate stress tolerance in plants (Wasternack, 2007). Thus, it was found that in wheat the content of JA and SA increased after cold stress (Kosová et al., 2012). In addition, JA content increased several times in a population of *Pinus pinaster*, when plants were exposed to low temperature and

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drought (Pedranzani et al., 2007). Moreover, the expression of LOX (one of the first enzymes in JA biosynthetic pathway) in kiwi (*Actinidia delico*sa) and *Caragana jubata* was regulated positively during cold stress (Zhang et al., 2006; Bharwaj et al., 2011). The object of this work was the perennial mountain plant *Arabis alpina* (*Brassicaceae*), which is a new model plant, relative to *Arabidopsis*, that has been recently used in cold stress adaptation research. Up to now, little is known about the changes in phytohormone content in *A. alpina* under conditions of chilling and freezing stress, as well as the mechanisms of hormone-mediated cold tolerance in this plant. The main objective of this work was to study the possible involvement of endogenous jasmonates (JA and JA-ile) in cold stress tolerance of *A. alpina*.

Materials and Methods

Seeds from three populations of *Arabis alpina* were selected according to their tolerance to frost: tolerant (T) collected in the French Alps (Col du Galibier), non-tolerant (NT) (harvested in the mountain of Vercors), and plants with a short hypocotyl (SH), considered as an adaptation feature to the snow coverage. The plants were grown for 2½ months in a cultivation chamber (Percival) at 22ºC, 12/12 h photoperiod, photon flux density of 220 µmol $m^{-2}s^{-1}$ and 70% relative humidity. Afterwards the plants were exposed to chilling stress (4ºC) for 4 days at the same conditions and freezing stress (–7ºC) for 12 h in darkness. Endogenous JA and JA-ile were extracted from 100–200 mg fresh weight in methanol/formic acid/water (15/1/4, v/v/v) and purified using a dual-mode solid phase extraction method, which allows separation of hormones of basic and acidic character by sequential elution from Oasis MCX column (Waters Co, Milford, MA, USA) (Dobrev and Kaminek, 2002). Detection and quantitation of JA and JA-ile was carried out using HPLC (Ultimate 3000, Dionex) coupled to hybrid triple quadrupole / linear ion trap mass spectrometer (3200 Q TRAP, Applied Biosystems) set in selected reaction monitoring mode. The first elute contained the acidic hormones (including JA and JA-ile), while the second consisted of the basic ones (cytokinins). The samples were dissolved into acetonitrile, centrifuged and analyzed by LC/MS.

Results and Discussion

Our results showed very high content of JA (4–6 nmol g^{-1}DW) in the three studied populations at control temperature of 22ºC (Figure 1A). After chilling stress at 4ºC,

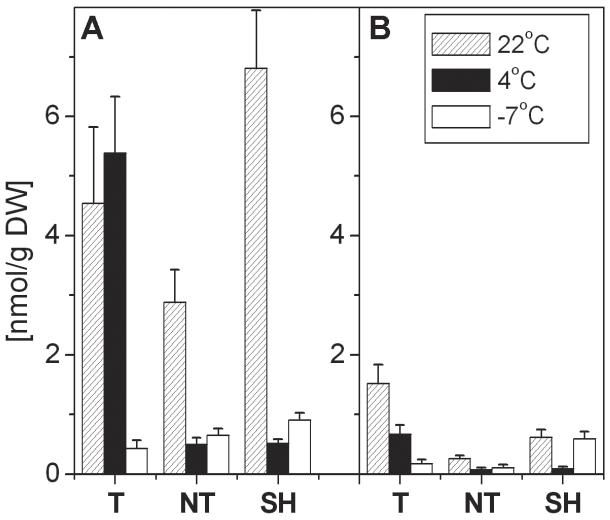

Fig. 1. Endogenous content of jasmonic acid (A) and the biologically active jasmonate-isoleucine (B) in three populations of *Arabis alpina*: frost tolerant (T), non-tolerant to frost (NT) and plants with a short hypocotyls (SH). Plants were grown at 22ºC (control conditions) for 2½ months, then exposed to chilling (4ºC) or freezing stress (–7ºC) for 12h in darkness

the content of JA in T-plants did not change considerably in contrast to the 10-fold reduction in NT and SH populations (mean value of 0.5 nmol g^{-1} DW). However, upon exposure to frost, JA content dropped in the T-population reaching the levels in the other two populations, which remained similar at both chilling and freezing temperatures. The content of the bioactive jasmonate compound JA-ile at control conditions (Figure 1B) was significantly higher (5-fold) in T-plants (1.5 nmol g^{-1} DW) compared to NT- and SH- plants (0.26 and 0.6 nmol g^{-1} DW, respectively). The subsequently exerted chilling stress led to a 2-fold reduction in the content of JA-ile in T-plants, the latter still being 10 times higher (0.67 nmol g^{-1} DW) compared with NT- and SH-plants (0.07 nmol g^{-1} DW and 0.09 nmol g^{-1} DW, respectively). JA-ile content in T dropped to a level comparable with that in NT-plants after freezing stress (0.18 nmol g^{-1} DW and 0.11 nmol g^{-1} DW, respectively) (Figure 1B). Our results indicate that JA content was much higher compared to its amino acid conjugate JA-ile in all studied *A. alpina* populations, thus suggesting that JA may play a principle role as an intracellular mediator of the environmental stress. Furthermore, the content of JA was much higher in T than in NT after chilling stress, which is in agreement with the data published

so far (Pedranzani et al., 2007; Kosová et al., 2012). In contrast to JA, the content of bioactive compound JA-ile decreased after the chilling stress thus indicating that in *A. alpina* JA may be the principle jasmonate mediator of the cold stress. Detailed analysis of jasmonate biosynthesis via investigation of specific genes expression is now in progress.

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