# Effect of Chilling and Freezing Stresses on Phytohormone Content in *Arabis alpina*

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## Summary

Depending on the temperature involved, there are two types of cold stress: chilling stress (low positive temperatures around +5°C) and freezing stress (temperatures below zero). As an adaptation to cold stress, plants have evolved multiple mechanisms for cold tolerance. These mechanisms are mediated by a number of stress phytohormones, including ABA, JA, SA, Ethylene. It is well known, that stress phytohormones trigger phosphoprotein cascade pathways, which in turn lead to expression of genes, needed for acquiring the cold stress tolerance. The perennial *Arabis alpina*, related to *Brassica* sp. and *Arabidopsis* sp. has been selected for this study, because of its wide spreading in mountain areas of the northern hemisphere, at elevations ranging from 500 to 3200 m. We represent experimental data obtained by HPLC-MS-MS on phytohormone content in three *A. alpina* genotypes: frost tolerant (T), non-tolerant (NT) and plants with short hypocotyl (SH), exposed to chilling and freezing stress.

## Experimental scheme

Growing of T, NT and SH plants at 22°C (control), followed by acclimation to chilling stress (4°C) for 4 days and exposure to freezing stress (-7°C, 12h, darkness)

| | 22°C | | 4°C | | -7°C |
|---|---|---|---|---|---|
| T | **T22** | ⟷ | **T4** | ⟷ | **T-7** |
| NT | **NT22** | ⟷ | **NT4** | ⟷ | **NT-7** |
| SH | **SH22** | ⟷ | **SH4** | ⟷ | **SH-7** |

## Materials and Methods

The endogenous phytohormones in *A. alpina* were extracted according to Dobrev and Kaminek 2002 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). Fresh plant material of about 100-200mg FW was homogenized with Retsch MM301 Ball mill. Detection and quantitation of phytohormones 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 fractions were then dissolved into 1M of HCOOH and eluted in MCX column. The first elute contained the acidic hormones (ABA, JA, GA), and the second – the basic ones – CKs. The samples were dissolved into acetonitrile, centrifuged and analyzed by LC/MS (mass spectrometer).

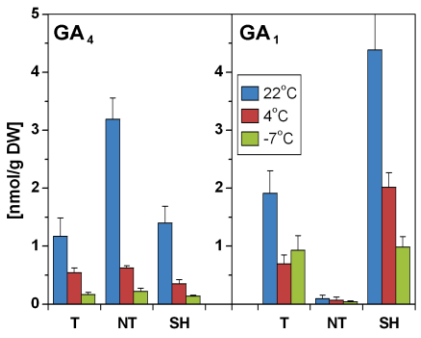
Net photosynthetic rate was estimated by means of LiCor LC Pro+ (ADC Bioscientific, England).

## *A. alpina* plants in controlled conditions

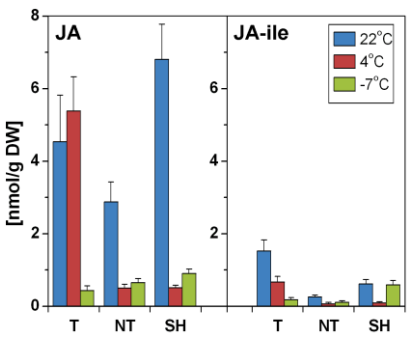
GAL T-(tolerant) F005 NT-(non-tolerant) F109 SH-(short hypocotyl)

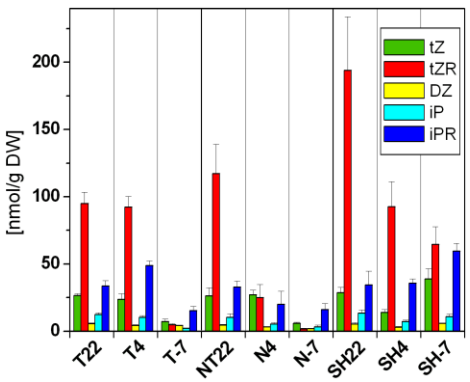
photoperiod 12/12h (220 µmol), 22-23°C, age 2½ months

## Content of $GA_4$ and $GA_1$



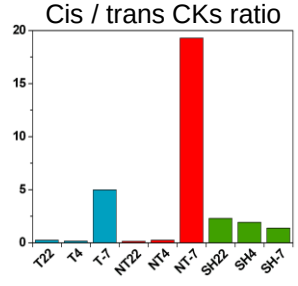

## Content of JA and JA-ile



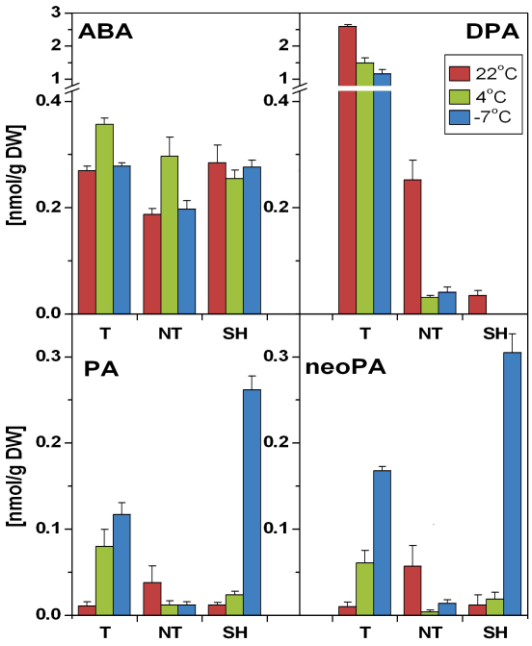




## Biologically active CKs

## Cis / trans CKs ratio

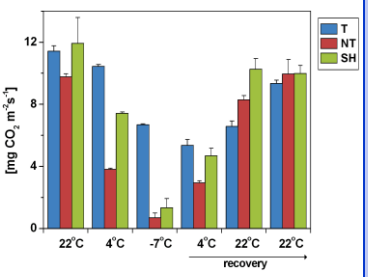



## Content of ABA and its degradation products




ABA – abscisic acid; DPA – dihydrophaseic acid
PA – phaseic acid; neoPA – neophaseic acid

## Net Photosynthetic Rate




Recovery was applied at 4°C for 4 days followed by transfer at 22°C for the same periods of time

## Conclusions

- ABA and the products of its degradation (PA and DPA) maintained high content in T, while being almost absent in NT. This indicates a possible role of ABA in regulation of plant cold stress tolerance.
- The content of biologically active CKs remained higher in T after chilling stress, in contrast to NT. Cis/trans CKs ratio increased in NT after the freezing stress, thus indicating a higher proportion of biologically active trans- CKs in T.
- The level of biologically active $GA_4$ in *Brassicaceae* was much higher in NT, as compared with T and SH-populations. $GA_1$ was almost absent in NT, but its content was higher in T and SH.
- After chilling stress, JA and JA-ile were present in higher concentration in T.
- These results suggest that phytohormones can play an important role in cold stress tolerance in *A. alpina*.