

# Preferential freezing avoidance localized in anthers and embryo sacs in wintering *Daphne kamtschatica* var. *jezoensis* flower buds visualized by MRI

Masaya Ishikawa<sup>1</sup>, Hiroyuki Ide<sup>2</sup>, Tetsuya Tsujii<sup>3</sup>, Timothy Stait-Gardner<sup>4</sup>, Hikaru Kubo<sup>1</sup>, Norihisa Matsushita<sup>1</sup>, Kenji Fukuda<sup>1</sup>, William Price<sup>4</sup>, and Yoji Arata<sup>5</sup>

<sup>1</sup>The University of Tokyo Graduate School of Agricultural and Life Sciences Faculty of Agriculture Department of Forest Science

<sup>2</sup>Ajinomoto Co Inc Institute for Innovation

<sup>3</sup>PerkinElmer Japan Co., Ltd.

<sup>4</sup>Western Sydney University

<sup>5</sup>Water Research Institute

September 24, 2021

## Abstract

To explore diversity in cold hardiness mechanisms, high resolution magnetic resonance imaging (MRI) was used to visualize freezing behaviors in wintering flower buds of *Daphne kamtschatica* var. *jezoensis*, which have no bud scales surrounding well-developed florets. MRI images showed that anthers remained stably supercooled to -14 -21°C or lower whilst most other tissues froze by -7°C. Freezing of some anthers detected in MRI images at -21degC corresponded with numerous low temperature exotherms and also with the “all-or-nothing” type of anther injuries. In ovules/pistils, only embryo sacs remained supercooled at -7degC or lower, but slowly dehydrated during further cooling. Cryomicroscopic observation revealed ice formation in the cavities of calyx tubes and pistils but detected no ice in embryo sacs or in anthers. The distribution of ice nucleation activity in floral tissues corroborated the tissue freezing behaviors. Filaments likely work as the ice blocking barrier that prevents ice intrusion from extracellularly frozen calyx tubes to connecting unfrozen anthers. Unique freezing behaviors were demonstrated in *Daphne* flower buds: preferential freezing avoidance in male and female gametophytes and their surrounding tissues (by stable supercooling in anthers and by supercooling with slow dehydration in embryo sacs) whilst the remaining tissues tolerate extracellular freezing.

## Hosted file

daphne27 v2 PCE text only.doc available at <https://authorea.com/users/435451/articles/538466-preferential-freezing-avoidance-localized-in-anthers-and-embryo-sacs-in-wintering-daphne-kamtschatica-var-jezoensis-flower-buds-visualized-by-mri>

## Hosted file

daphne24 PCE figures only +legends.pdf available at <https://authorea.com/users/435451/articles/538466-preferential-freezing-avoidance-localized-in-anthers-and-embryo-sacs-in-wintering-daphne-kamtschatica-var-jezoensis-flower-buds-visualized-by-mri>