

VLIV CHLADU A MRAZU

EFFECTS OF LOW AND FREEZING TEMPERATURE

MILOŠ BARTÁK

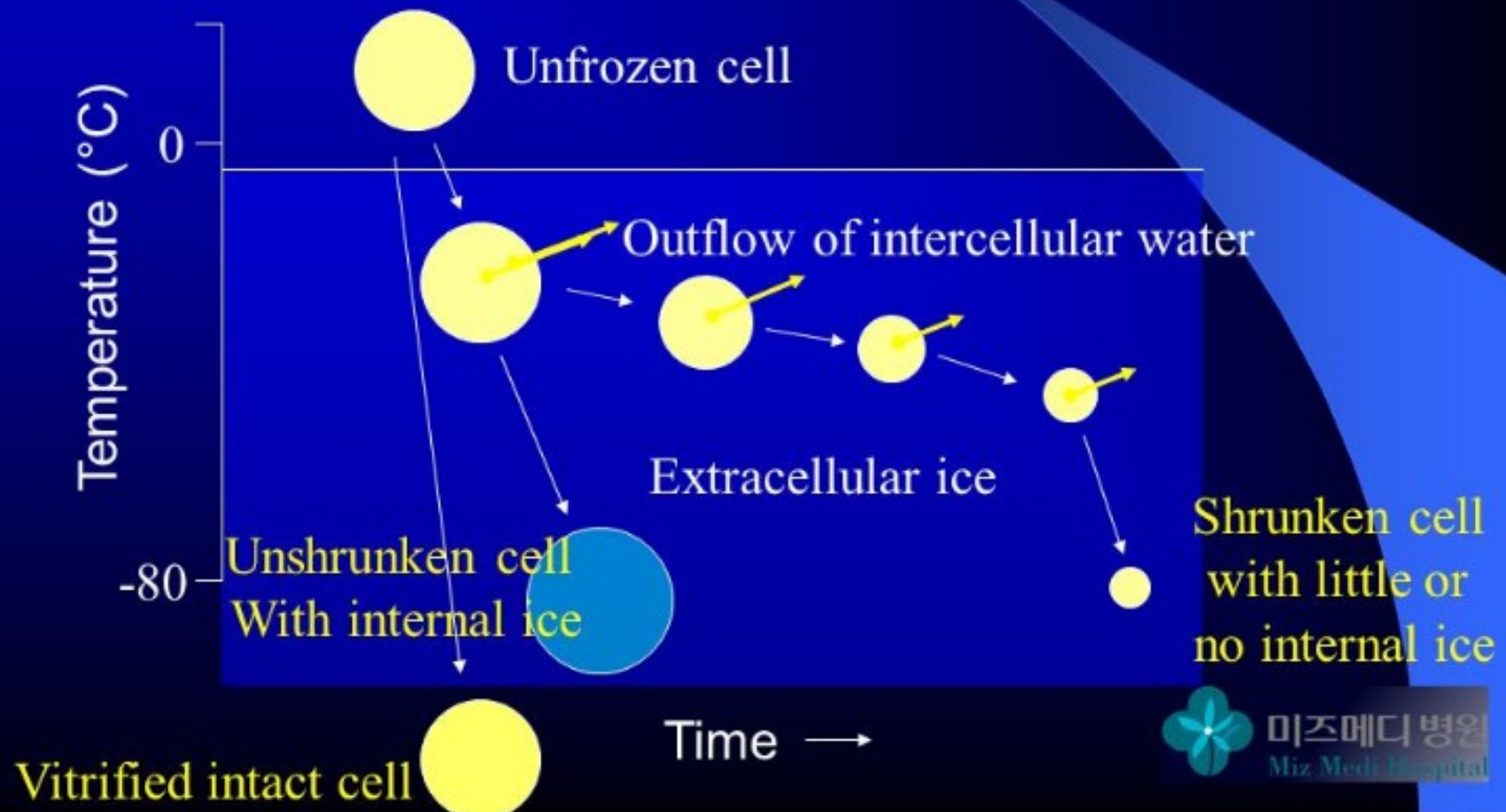
OFAR ÚEB PŘF MU,
EXTREME ENVIROMENTS LIFE LABORATORY

fdroje obrázků a informací v prezentaci uvedených: ac.ksu.edu.sa/sites, Schaberg 2010 (USDA Forest Service), Pearce 2001(Ann. Bot.), MIZ MEDI Hosp. Preservation of Germs, Jiří Zámečník, Iva Faberová (VÚRV), Lukatkin et al. (2012), Kawahara (INTECH Open)

× Teplotní stress / temperature stress

- × **Temperature stress: Low or high temperature, called frost injury or heat injury, respectively.**
- × **2.1 Frost (freezing)injury**
- × **The injury is caused by low temperature below freezing point ($< 0^{\circ}\text{C}$), accompanied with frost.**

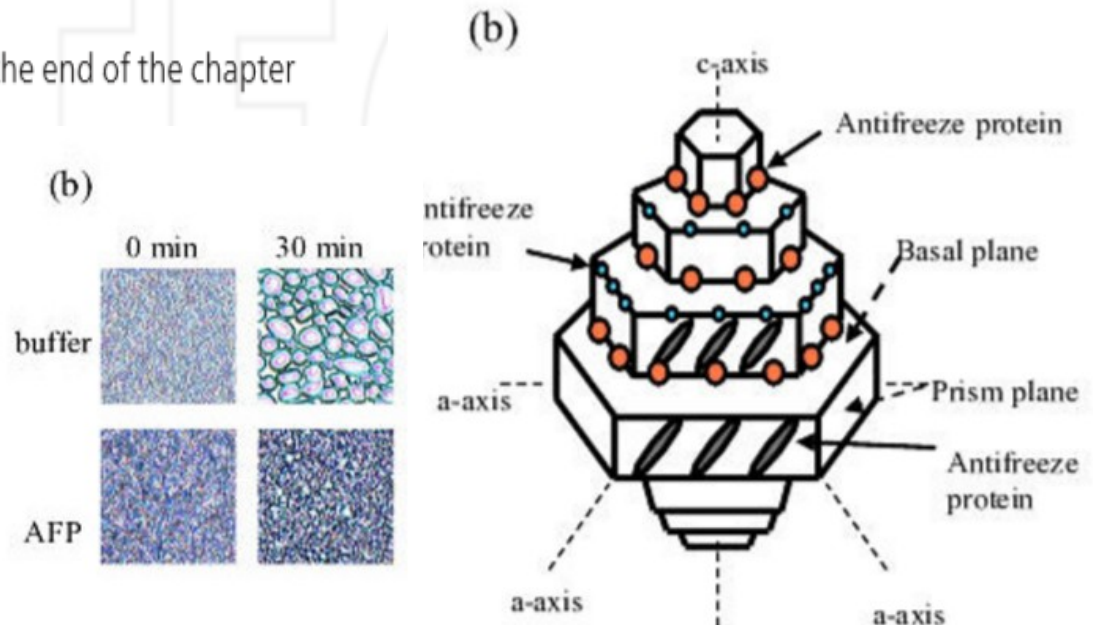
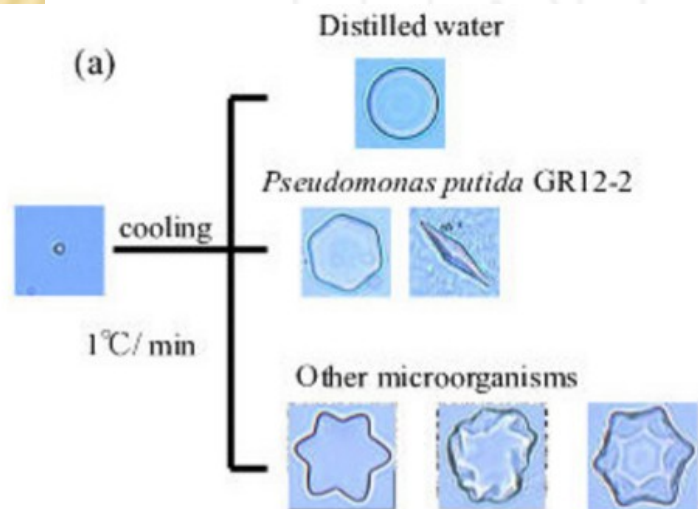
Procedure of Slow Freezing



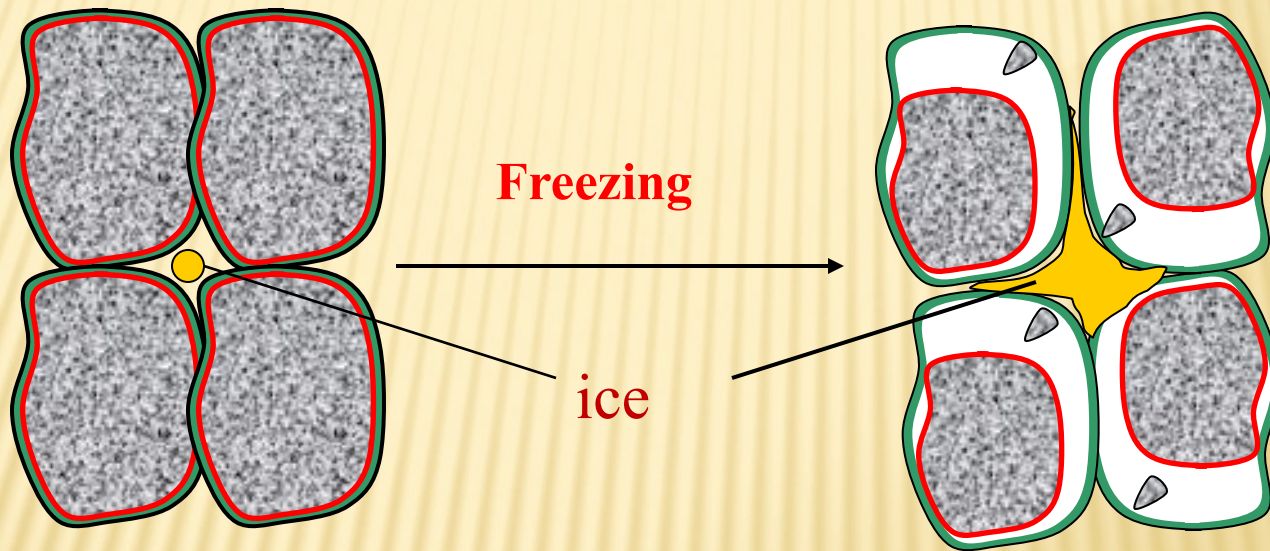
Characterizations of Functions of Biological Materials Having Controlling-Ability Against Ice Crystal Growth

Hidehisa Kawahara

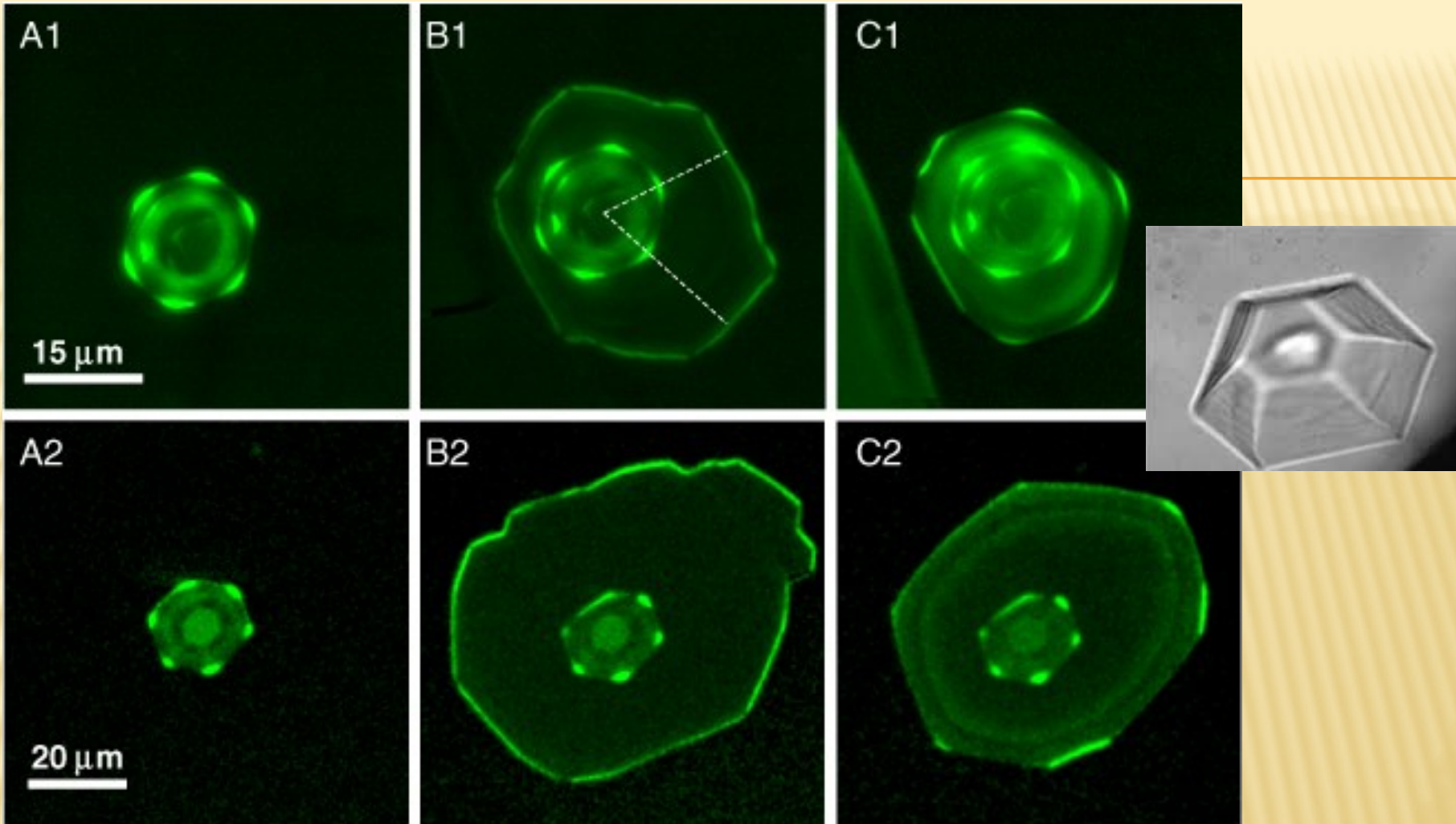
Additional information is available at the end of the chapter



- × **2.1.1 Mechanism of freezing (frost)injury**
- × **2.1.1.1.Freezing:(intercellular and intracellular freezing)**
- × **(1) Intercellular freezing**



Intercellular freezing occurs when temperature falls gradually.



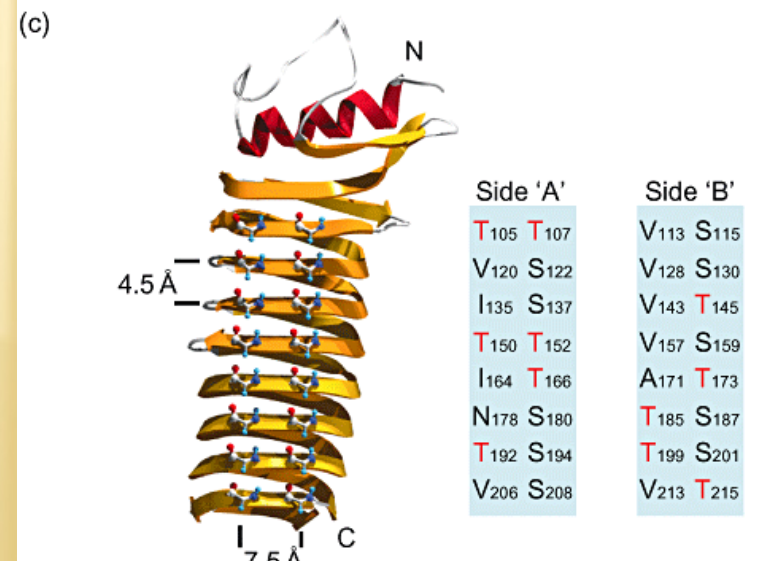
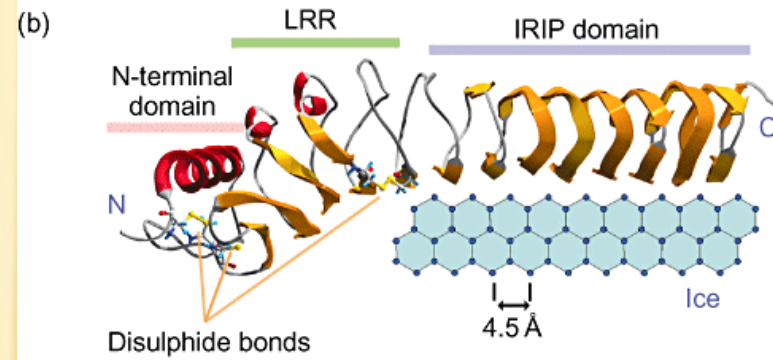
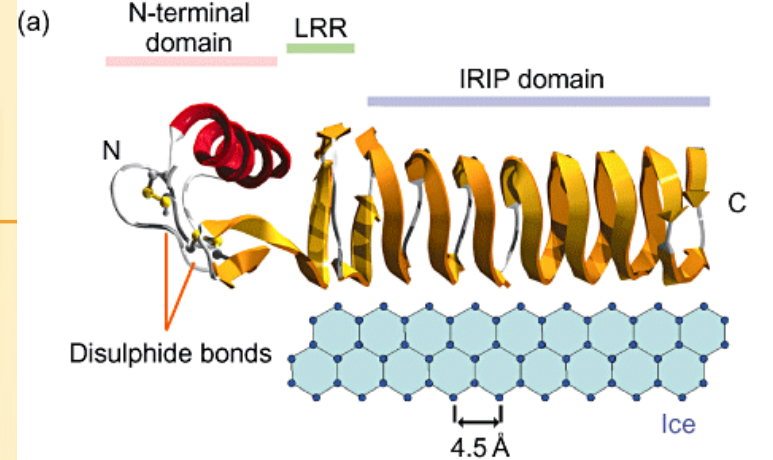
- ✘ **Growth–melt asymmetry in ice crystals under the influence of spruce budworm antifreeze protein**
- ✘ Natalya Pertaya¹, Yeliz Celik¹, Carlos L DiPrinzio¹, J S Wettlaufer^{2,3}, Peter L Davies⁴ and Ido Braslavsky^{1,5}
- ✘ Published 10 September 2007

Rapid Freezing

- **Advantages of vitrification**

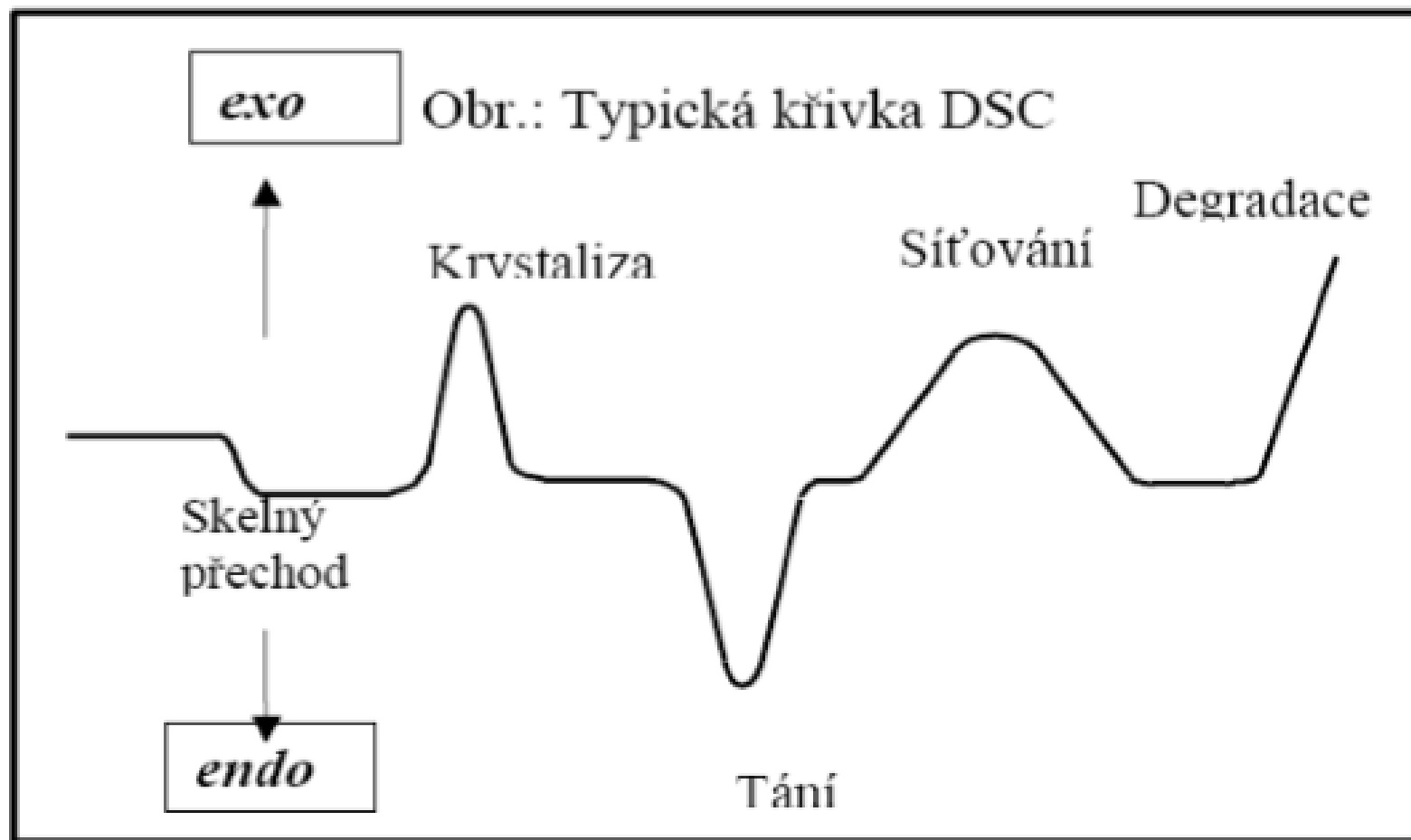
- No ice crystal formation
- Rapid equilibrium
- Absence of Water leak after equilibrium
- Decreased osmotic shock
- Short time required
- Minimum damage of membrane lipid
- Simple procedure
- No equipment require



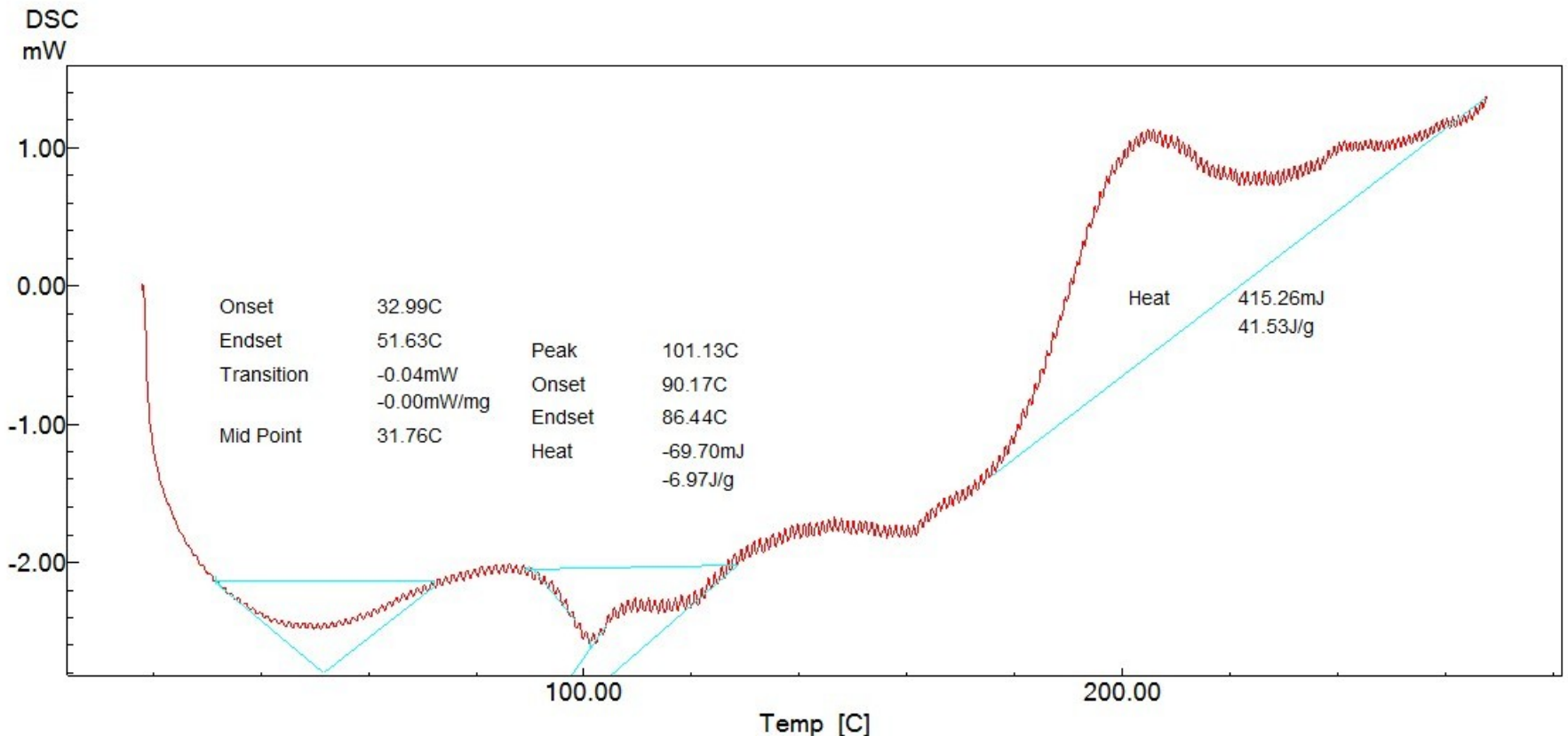


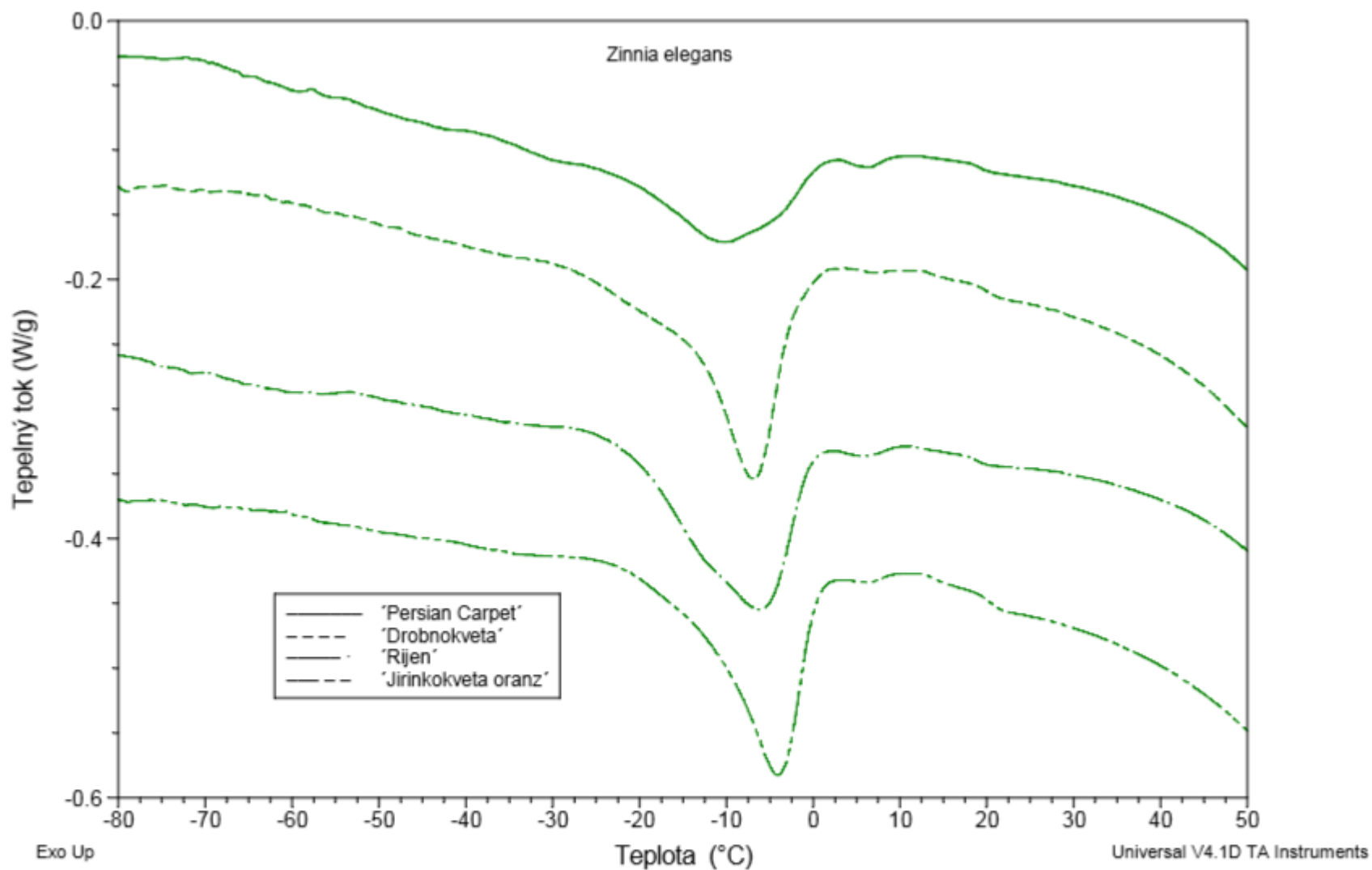
John et al. (2009): Ice recrystallization inhibition proteins (IRIPs) and freeze tolerance in the cryophilic Antarctic hair grass *Deschampsia antarctica* E. Desv.

DIFERENČNÍ SKANOVACÍ KALORIMETRIE



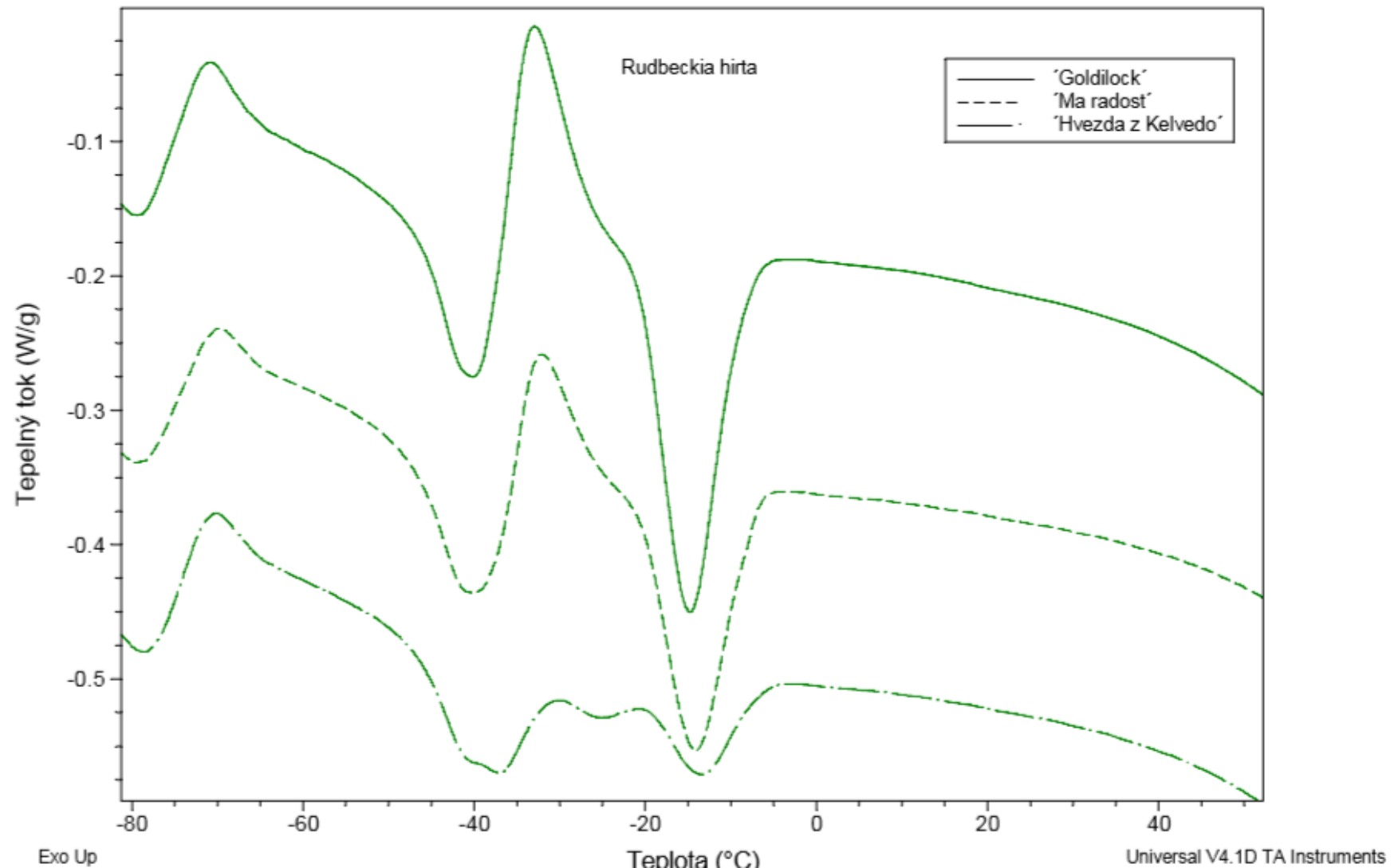
- ✘ Diferenční skenující kalorimetr (DSC) je vhodný přístroj pro měření a interpretaci fázových přechodů vody v rostlinách. DSC se většinou používá pro přímé měření skelných přechodů a teploty ledové nukleace s následným vyhodnocením pomocí termické analýzy. Teplotní podmínky a úrovně hydratace při kterých se začíná tvořit sklo byly porovnány na základě rozdílných mechanismů tolerance nízkých teplot rostlin z přirozených podmínek.





Pozn. Křivky pro jednotlivé vzorky jsou posunuty podél osy y z důvodu přehlednosti. Hodnoty tepelného toku platí pouze relativně.

Obr. 2: Termické odezvy semen tří kultivarů *Rudbeckia hirta* s dvojitou endotermou, které předcházejí skelný přechod, v závislosti na teplotě při ohřevu $10\text{ }^{\circ}\text{C min}^{-1}$.



- × **(2) Intracellular Freezing :**

- × **Intracellular freezing** often occurs when temperature falls suddenly.
- × Ice results in the direct injury in cytoplasm, biomembrane and organelle, and damages to cell compartmentation and metabolic disorder.
- × **Much more serious damage is caused by Intracellular Freezing than by Intercellular Freezing.**
- × **2.1.1.2 damage of protein:**
- × Sulfhydryl group hypothesis (disulfide bridge hypothesis)

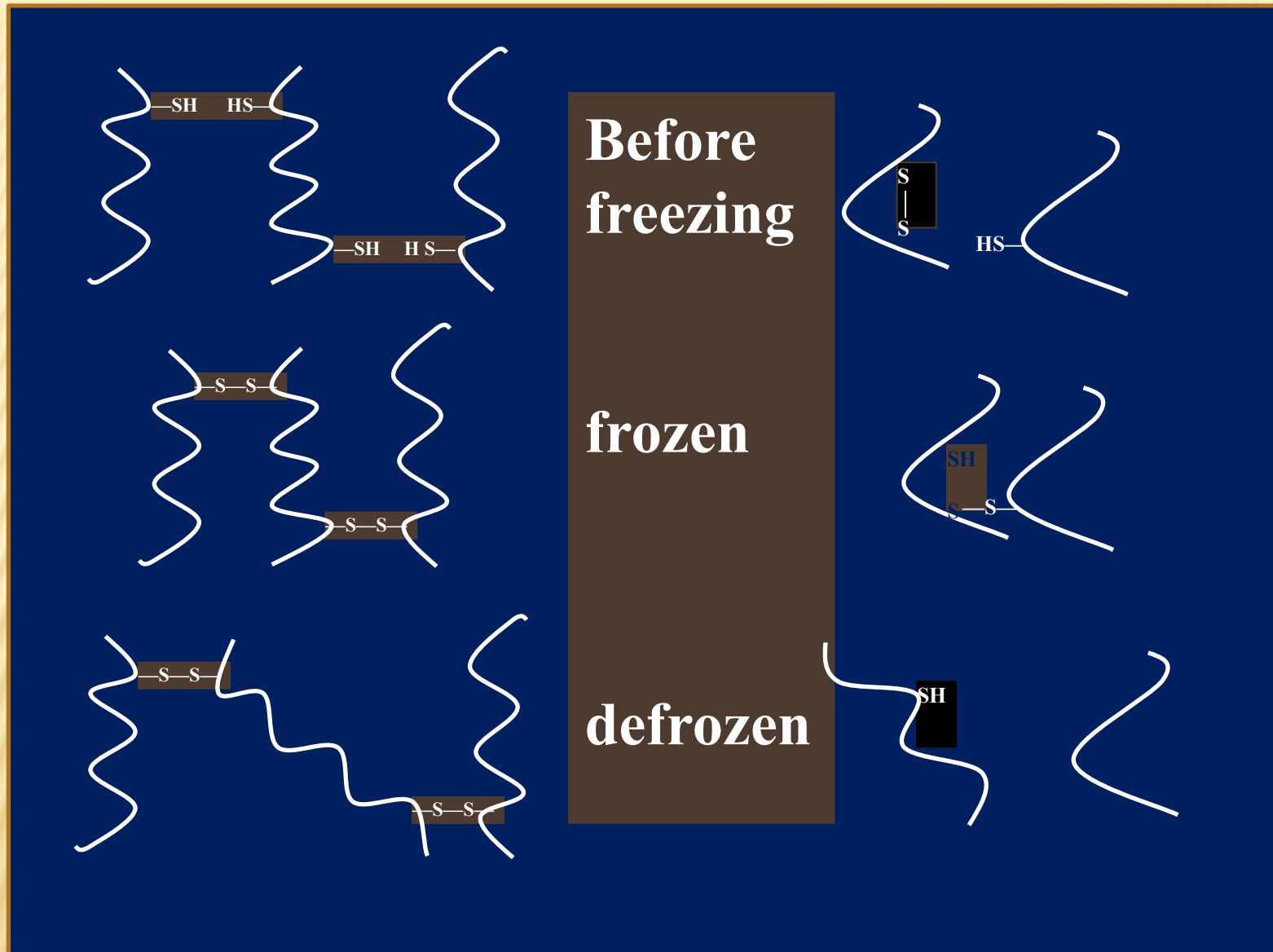


Illustration of sulfhydryl group hypothesis

× Supported Exp :

- × (1) —S—S— increase and soluble —SH decrease after plant tissue faces to freezing.
- × (2) Less —S—S— and —SH of protein in the resistant-freeze plants.
- × (3) The plant with free —SH, glutathione, is more resistant to freeze.
- × (4) **Artificial** —SH, mercapthanol increases resistance of plant to low temperature.

× 2.1.1.3. Damage of biomembrane

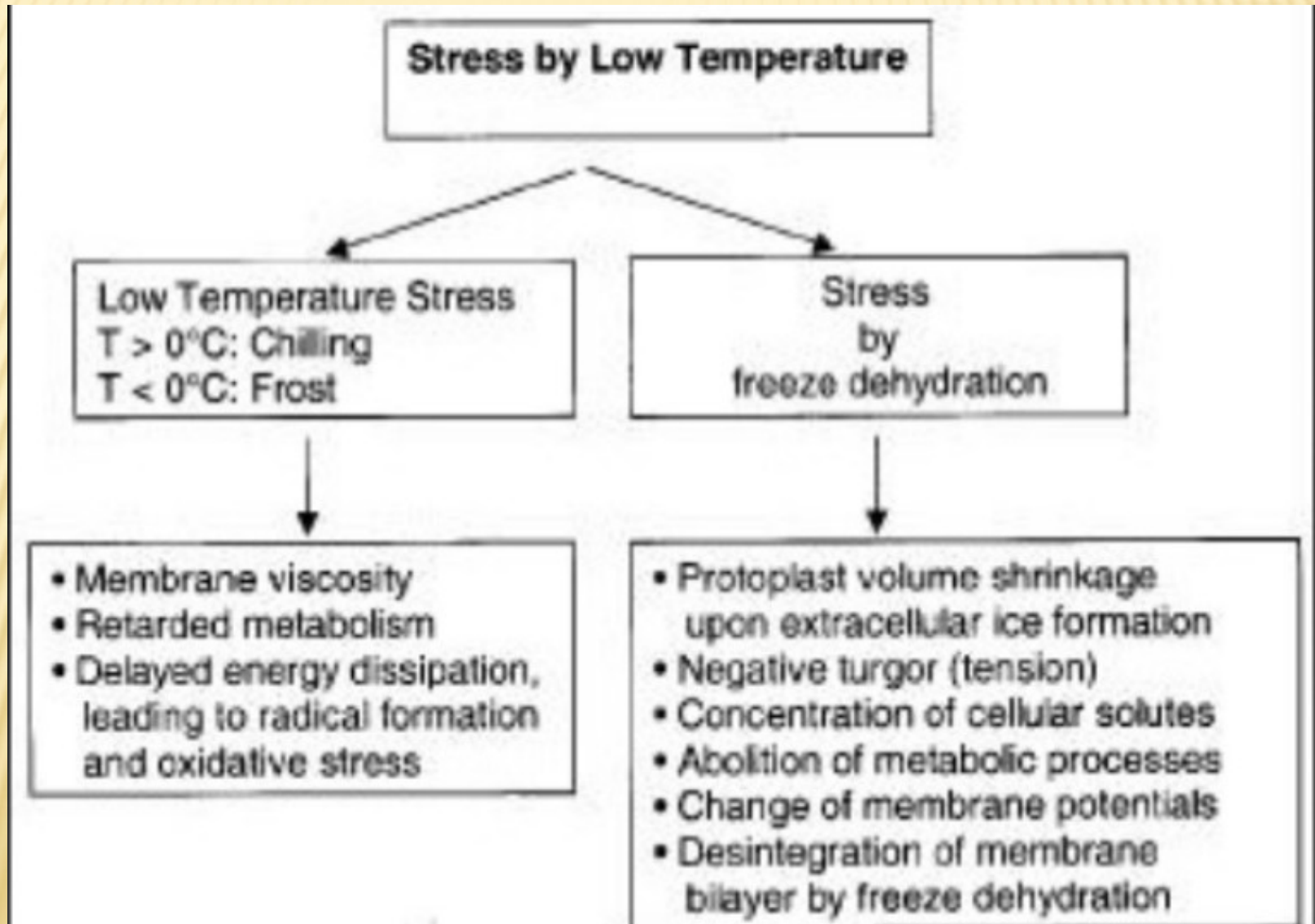
- × Electric conductivity↑, cell material leakage↑, photochemical activity and ATP production ↓, while photoinhibition ↑, CF1 and PC depart from membrane.
- × Change in state of lipid and protein denaturation

× 2.1.2 Chilling injury

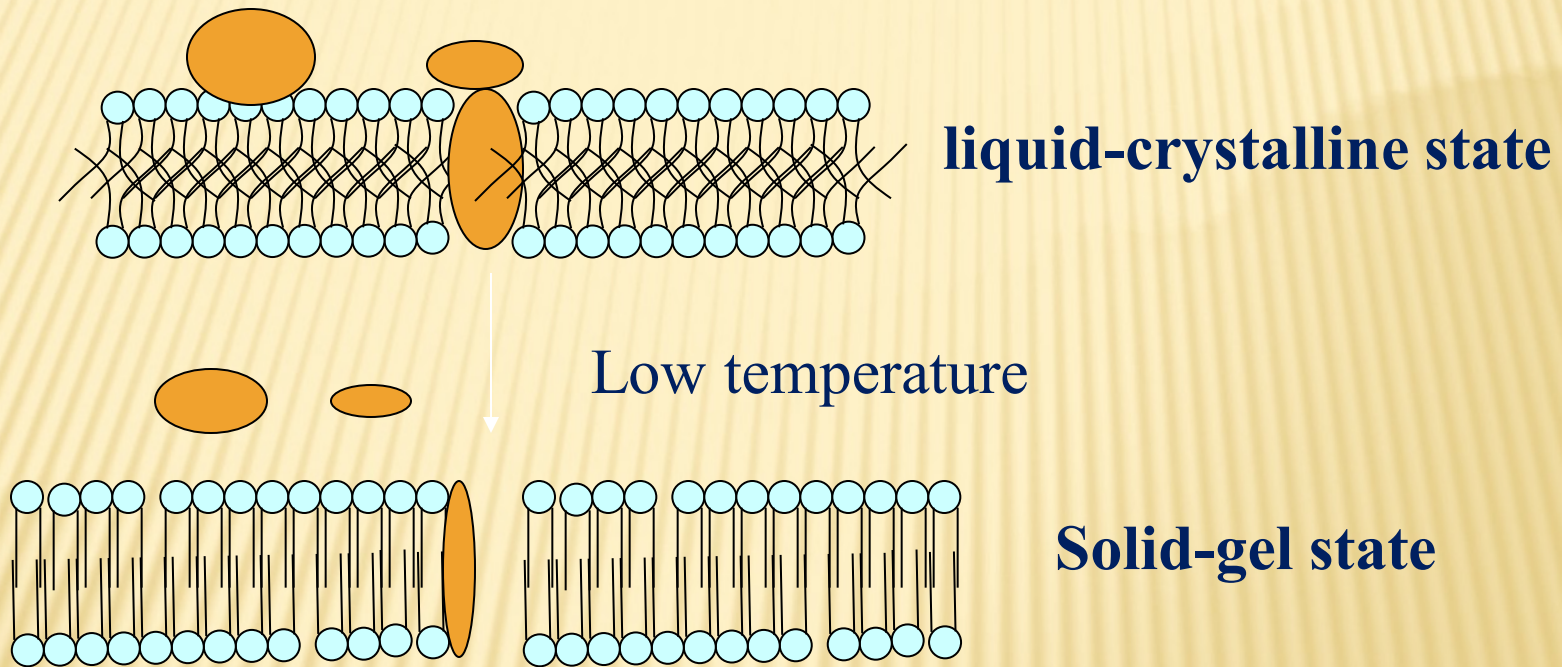
- × **Chilling injury** in tropical or subtropical plants is caused by temperature above 0°C (freezing point)..
- × Maize, cotton rice seedling——10°C。
- × Rice pollen-mother cell division, 23°C for *O. sativa* and 20°C for *O. japonica*.
- × Banana tree——13°C。
- × Oak tree——5°C。

Chilling stress

Freezing stress



× 2.1.2.1. Change in state of lipid



Electric conductivity as an index for resistance to low temperature in production

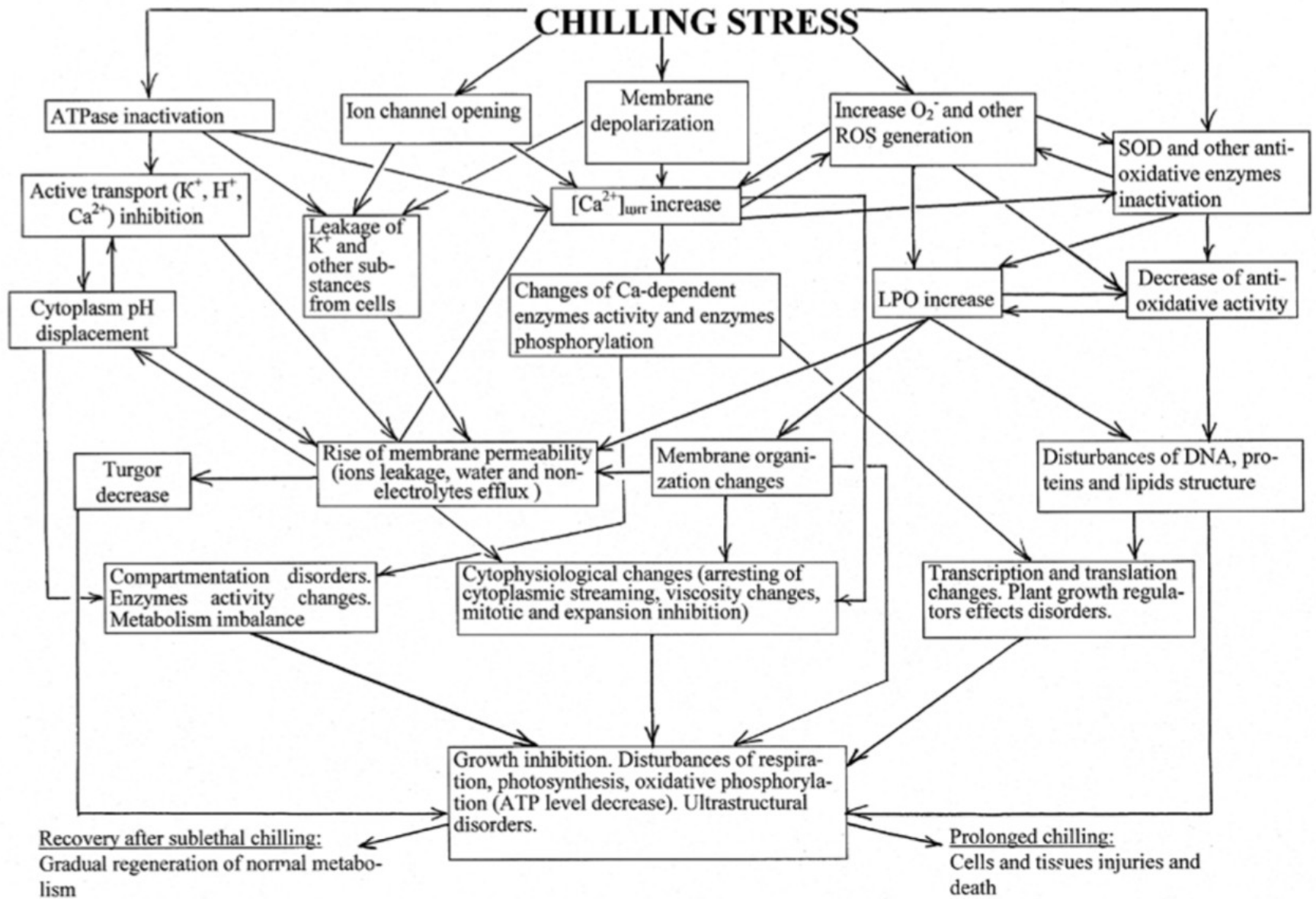


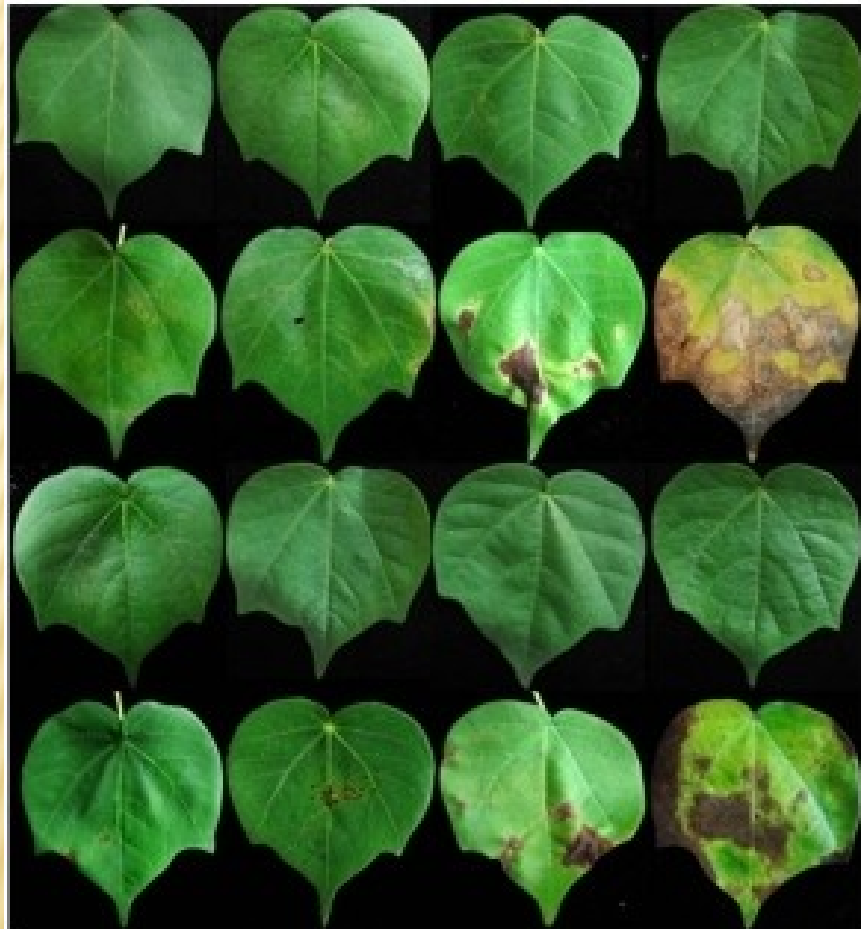
Figure. Scheme of the initiation and development of chilling injury in the cells of chilling-sensitive plants

× Metabolism disorder

- × (1) Uptake function of roots declines and water balance disorders
- × Transpiration > water absorption. The plant loses water and leaf curl ◦
- × (2) Photosynthetic rate lowers ◦
- × Photosynthesis < respiration, starvation to death
- × Rubisco loses activity under low temperature, PSP uncouples and free radicals break suddenly.

- ✘ (3) Aerobic respiration decreases and anaerobic respiration increases。
- ✘ Cytaa₃ activity ↓, respiratory electron transport and phosphorylation activities ↓. Ethanol poison.
- ✘ (4) Organic substance degrades。
- ✘ protease↑, protein↓, RNA、ATP ↓.

Control	Pre-chilling stress temperatures		
	28/20°C	20/16°C	16/12°C



XLZ13 mock inoculated with sterilized water

XLZ13 inoculated with *A. alternata*

XLZ33 mock inoculated with sterilized water

XLZ33 inoculated with *A. alternata*



Čerň střídavá je druh vřekovýtusné houby, způsobující skvrny na listech nebo poškození různých orgánů u celé řady druhů rostlin

Chilling stress--the key predisposing factor for causing *Alternaria alternata* infection and leading to cotton (*Gossypium hirsutum* L.) leaf senescence.

Zhao J, Li S, Jiang T, Liu Z, Zhang W, Jian G, Qi F - PLoS ONE (2012)

✘ **Physiological reaction of plant to low temperature**

✘ (1) Water content, metabolism, growth decrease .

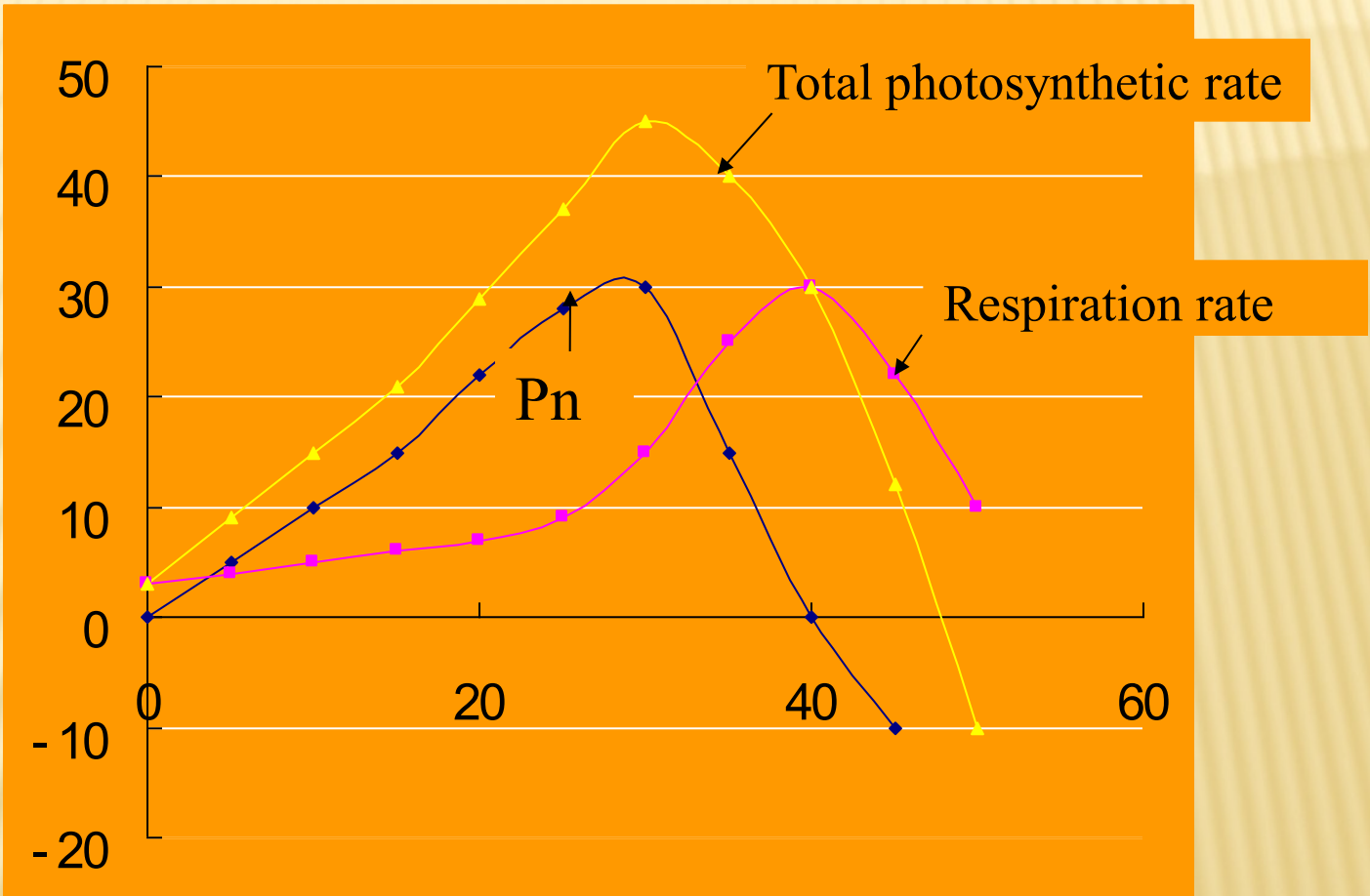
✘ Total water content↓, bound water↑, free water and ratio (free water/bound water) ↓。

✘ **(2) Protective substances increase。**

✘ NADPH——reduces —S—S— to —SH,
ATP and sugar↑, bound water↑.

- × **(3) Unsaturated fatty acid increase in membrane.**
- × **Unsaturated fatty acid↑ and saturated one ↓.**
- × **(4) ABA↑, GA↓, dormancy appears.**
- × **(5) Proteins-resistant to freezing accumulations.**
- × **Freezing resistant protein — Ice-Box—
The genes expression induced by freeze—
—freeze-resistant protein.**

- × **Methods to increase the resistance to low temperature.**
- × **(1) The resistant cultivars.**
- × **(2) Low temperature hardening.**
- × **(3) Chemical control.**
- × **ABA ,CCC, PP₃₃₀, Amo-1618).**
- × **(4) Others.**
- × **PK application, keep warm with artificial things.**



Zimní poškození



Cold tolerance of several north temperate conifers (sampled in Wolcott, VT on March 3, 1998)

Species	Mean cold tolerance (°C)
Red spruce	-38.1
White pine	-59.5
Eastern hemlock	-61.0
White spruce	-90.0
Red pine	-90.0



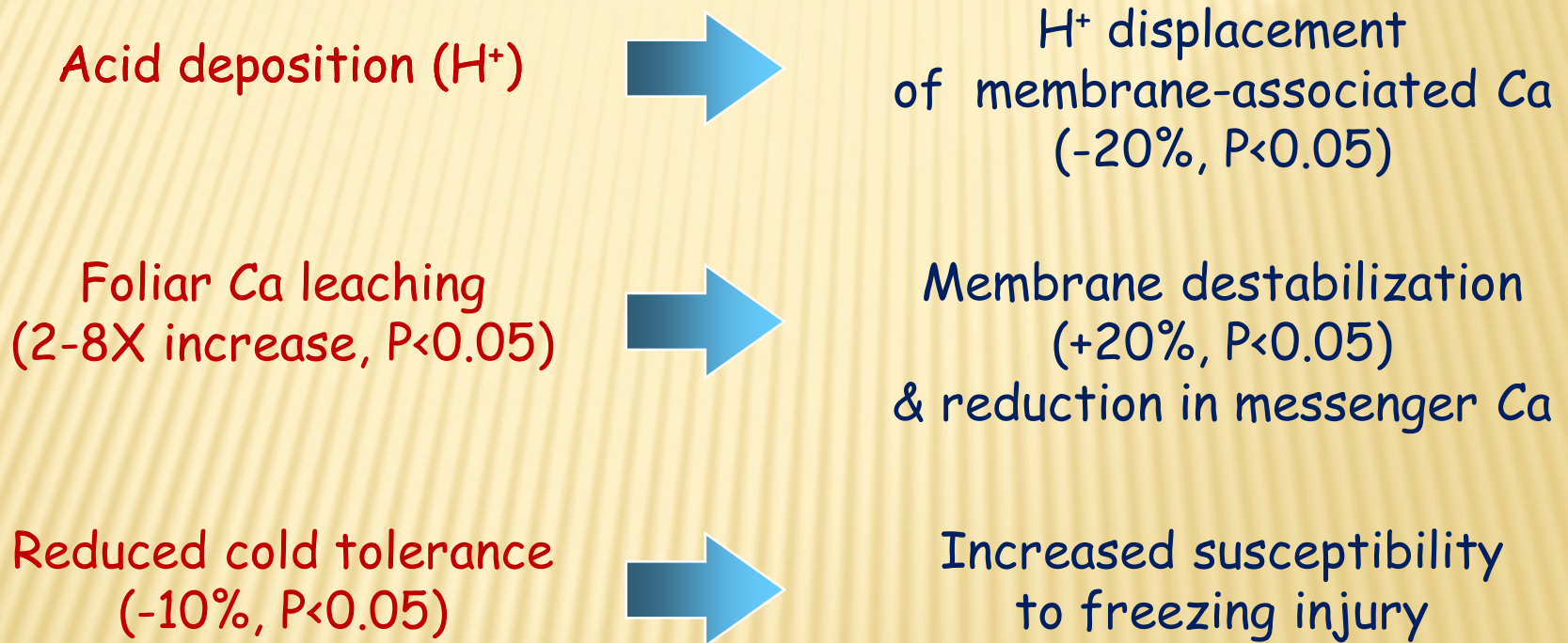
DeHayes et al. 2001

2003 – high winter injury year

Picea rubra



Mechanisms for acid-induced freezing injury of red spruce



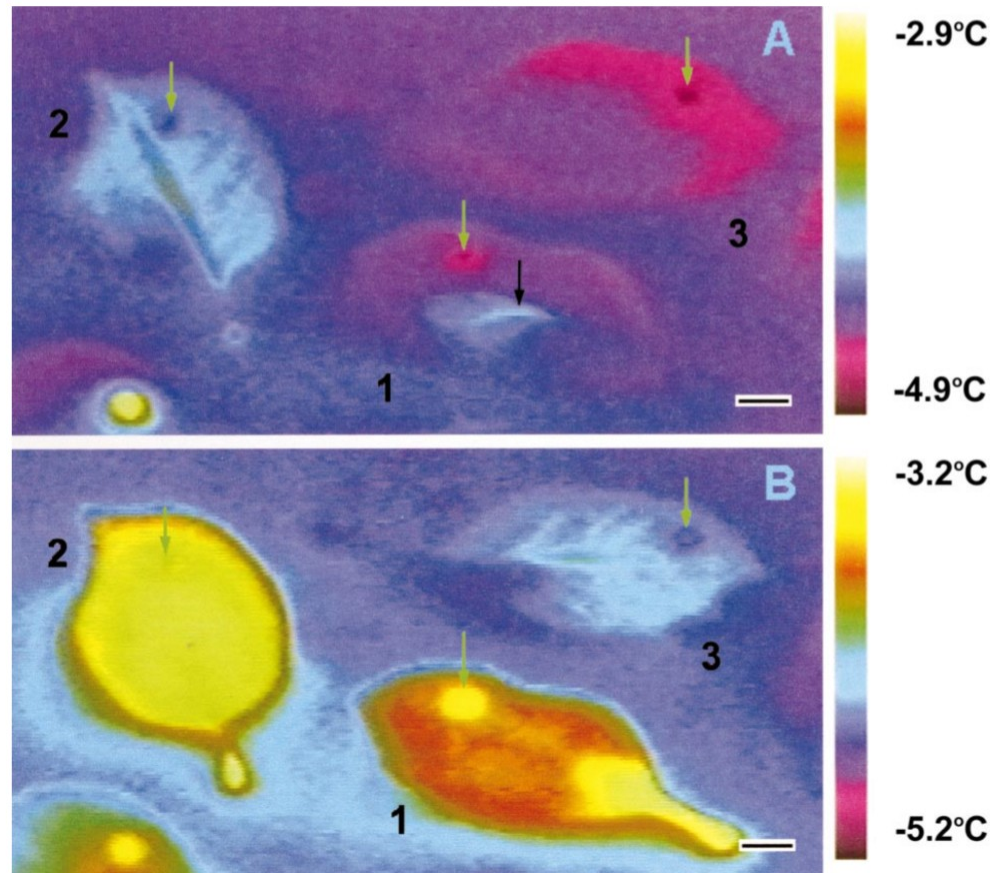


FIG. 1. Infrared video thermography of holly leaves during freezing in a cooling controlled environment chamber (Atherton, Pearce and Fuller, unpubl. res.). Methods were as described in Pearce and Fuller (2001). Freezing is exothermic and thus can be detected by detecting warming. An infrared imaging camera was used to monitor exothermic events in the freezing leaves. The image displays temperature as false-colours, running from 'cold' colours (pink and blue) to 'warm' colours (yellow and red). The scale to the right of each image shows the temperatures that correspond to the false colours. The temperature range detected was adjusted during cooling to maintain a 2°C window to include the current

RESPONSES TO CHILLING

- ✗ Lipid composition of a plant's membranes can help predict whether the plant will be sensitive or resistant to chilling
 - + The more unsaturated the membrane lipids are, the more resistant the plant is to chilling
- ✗ Supercooling – survive as low as -40°C
 - + Limits ice crystal formation to extracellular spaces
- ✗ Antifreeze proteins

**Dámy a pánové,
Děkuji Vám za
pozornost**