Monitoring freezing cytorrhysis and PS II efficiency in leaves using a temperature controlled microscope stage

Othmar Buchner[1], Gilbert Neuner[1]

1. Institute of Botany, Sternwartestrasse 15, A-6020 Innsbruck, Austria

Othmar.Buchner@uibk.ac.at
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Leaves of *Sphagnum capillifolium* were exposed to freezing temperatures under controlled conditions while mounted on a microscope stage [1]. The resultant cytological response to the freezing treatment was successfully monitored using light- and fluorescence microscope techniques. In addition to the observable cytological changes during freezing cytorrhysis, the concomitant critical low temperature threshold for inactivation of Photosystem II (PS II) was studied using a micro fibre optic and a chlorophyll fluorometer mounted to the microscope stage. Furthermore, tissue freezing tolerance and the influence of the phase transition from water to ice on the level of the detected fluorescence signal was determined.

Chlorophyllous cells of *S. capillifolium* showed extended freezing cytorrhysis immediately after ice nucleation at -1.1°C in the water in which the leaves were submersed during the measurement (Fig. 1). The occurrence of freezing cytorrhysis, which was visually manifested by significant cell shrinkage, was highly dynamic and was completed within 2s. A total reduction of the mean projected diameter of the chloroplast containing area during freezing cytorrhysis from 8.9 to 3.8 µm indicates a cell volume reduction of approximately -82%. Simultaneous measurements of basic chlorophyll fluorescence, F_0, of PS II were possible even through the frozen water in which the leaf samples were enclosed. Freezing cytorrhysis was accompanied by a sudden rise of F_0. The critical freezing temperature threshold of PS II (T_{CF}) was identical to the ice nucleation temperature (-1.1°C). This is significantly above the temperature threshold at which frost damage to *S. capillifolium* leaves occurs (-16.1°C; LT_{50}).

It is demonstrated that, under certain preconditions, light- and fluorescence microscopic techniques combined with simultaneous chlorophyll fluorescence measurements may act as a useful tool to study low temperature and ice-encasement effects on the cellular structure and primary photosynthetic processes of intact leaf tissues.

1. Buchner O., Lütz C., Holzinger A. Design and construction of a new temperature controlled chamber for light- and confocal microscopy under monitored conditions: biological application for plant samples, J. Microsc. 225 (2007) p183-191.

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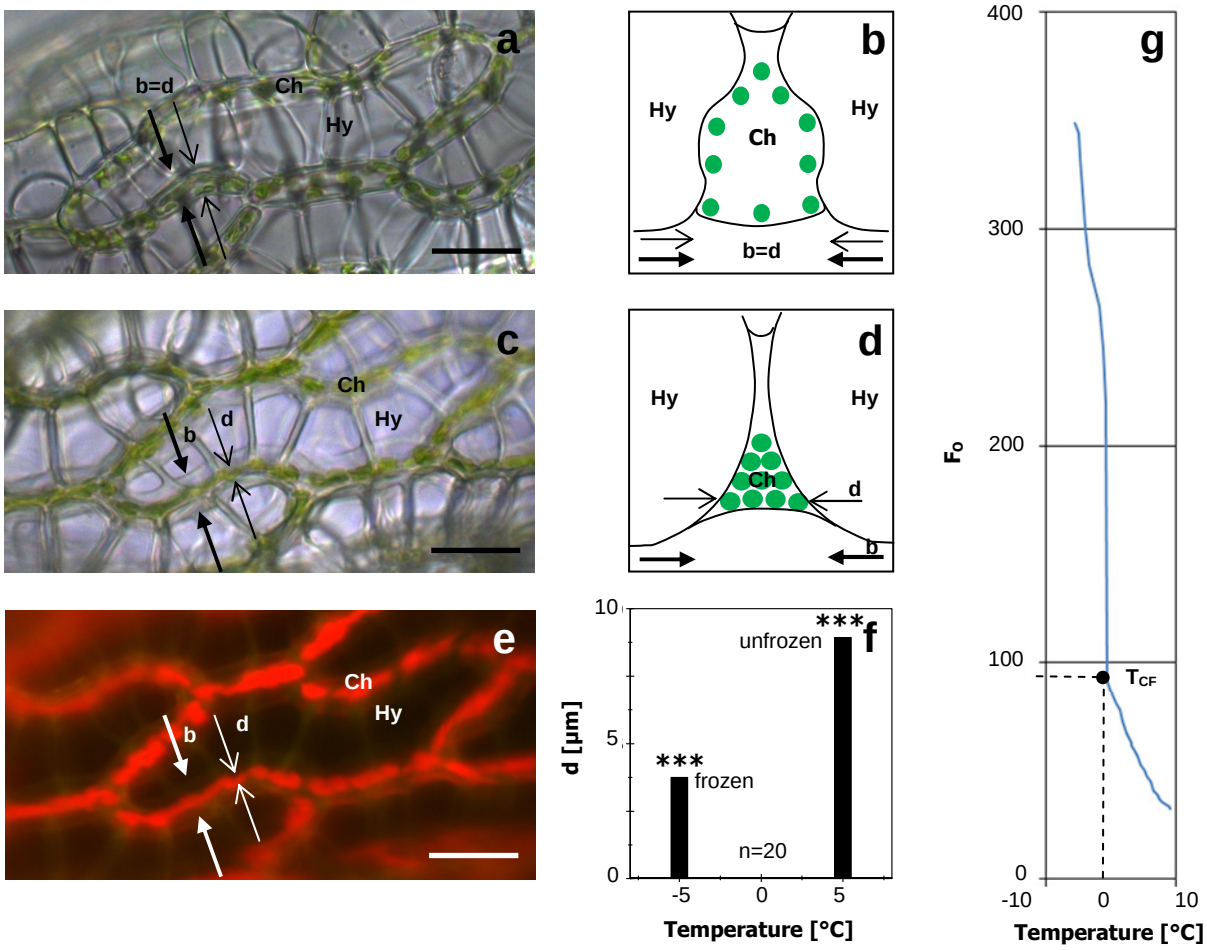

Figure 1. Freezing cytorrhysis and basic chlorophyll fluorescence, F_0, of chlorophyllous cells of a *S. capillifolium* leaf (adaxial view). **a-b** unfrozen leaf tissue (+5°C), **c-e** frozen tissue (-5°C). **Ch** chlorophyllous cells, **Hy** hyaline cells. Arrows indicate the projected diameter (**d**) of the chloroplasts containing area of the chlorophyllous cells. In the frozen state (**c-e**) the chlorophyllous cells dehydrate rapidly due to the high water potential gradient between the extracellularly and inside the hyaline cells formed ice which results in reversible freezing cytorrhysis of the chlorophyllous cells. Due to the cytorrhysis the chloroplasts become compressed while the projected diameter of the chlorophyllous cell (**b**, bold arrows) stays largely unchanged. The diameter of the chloroplast containing area **d** significantly ($p<0.001$) decreases from 8.9±0.9 to 3.8±0.5 µm (mean values±SD) during freezing (**f**). **a, c** light microscopic micrograph, **e** fluorescence microscopic micrograph (546 nm) emphasizing the compression of the chloroplasts inside the chlorophyllous cells during freezing. (Bar width 20 µm); **g** F_0 during controlled freezing (-2 K h^{-1}): In the temperature range -1.1°C < T < 8°C F_0 exhibited a slight, nearly linear increase with falling temperature. Freezing occurred at -1.1°C and caused an isochronal, sudden rise of F_0 (T_{CF}; critical freezing temperature threshold of PS II) while at the same time the chlorophyllous cells showed an extended freezing cytorrhysis.

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