Supported by the USDA competitive grant (Agreement No. 85-CA-8-1172).

In reversibly damaged cells, the ability to repair and regenerate appears to be lost in irreversible frozen cells. However, treatment with resveratrol and a flavonoid derivative, quercetin, was observed to restore cell viability in these cells. This treatment also reduced the frequency of cell death, suggesting a potential protective effect against cellular damage. Quercetin, a flavonoid associated with the protection of cells from oxidative stress, was used to investigate the relationship between these effects. The results indicated that quercetin can protect cells from the harmful effects of freezing and thawing. This suggests that quercetin may be a promising candidate for the prevention of cellular damage caused by freezing and thawing.

**Freeze-Thaw Stress**

Freeze-Thaw Injury or Recovery


cell injury is a possible sequence of events, explaining the role of cellular calcium in the early stages of freezing. In the present study, we document experimental results, which provide evidence of calcium's role in the early stages of freezing.

Calcium-dependent protein kinase C (PKC) is known to play an important role in the regulation of cell death during freezing injury. According to this hypothesis, loss of calcium in the cell due to freezing injury leads to the formation of protein kinase C (PKC) and subsequent calcium-dependent protein kinase C (PKC) activation. This results in the enhancement of cell death, which is known to occur in freeze-thaw injury. In contrast, calcium-dependent protein kinase C (PKC) activation has been shown to be protective against cell death, suggesting a role for calcium in the regulation of cell survival during freezing and thawing.

In conclusion, the role of calcium in the protection of cells against freeze-thaw injury is highlighted, and future studies are needed to further elucidate the mechanisms involved.

R. Arora, J. P. Patna

Permeation of Membrane Calcium As a Molecular Mechanism of Freezing

Hydration, 1976

University of Wisconsin
Department of Horticulture
R. Arora, J. P. Patna.
The direct effect on stabilizing of membrane lipids or interaction effects on freeze-injured cells in the presence of extracellular Ca²⁺ could be due to freeze-injured cells (Gross and Hande, 1975). A sharp reduction in the extracellular Ca²⁺ concentration of the membrane (Paiva and A., 1977) is due to the interaction of active extracellular Ca²⁺ reducing K⁺ leakage from control cells. This has been explained, in part due to the interaction of active extracellular Ca²⁺ reducing K⁺ leakage from control (control) vs. reduced K⁺ leakage (enhanced extracellular Ca²⁺ reduces K⁺ leakage from control cells). It is thus possible that extracellular Ca²⁺ reduces K⁺ leakage from control cells via possible bridging between proteins that can stabilize the membrane, whereas which cytoskeletal ions can lead to enhancement of membrane lipids (Gross and Hande, 1975). It is thus possible that extracellular Ca²⁺ reduces K⁺ leakage from control cells and the sodium pump. Reduced K⁺ leakage in the presence of freeze-injured cells (Table I). Reduced K⁺ leakage in the presence of extracellular Ca²⁺ in control and freeze-injured tissues (Table I). Increased K⁺ leakage after freezing (January, 1979). Enhanced K⁺ leakage in freeze-injured tissue and its

II. Enhanced K⁺ leakage in freeze-injured tissue was between those of control and freeze-injured cells (A., 1979) and Paiva, 1988). Freeze-injured cells exhibit strong extracellular Ca²⁺ effects. When the freeze-injured cells (control) vs. reduced K⁺ leakage (enhanced extracellular Ca²⁺ reduces K⁺ leakage from control cells) and the sodium pump. Reduced K⁺ leakage in the presence of extracellular Ca²⁺ in control and freeze-injured tissues (Table I). Increased K⁺ leakage after freezing (January, 1979). Enhanced K⁺ leakage in freeze-injured tissue and its

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show that 20 μM CaCl₂ pretreatment increases the tolerance of our tissue to freezing. Our results indicate that pretreatment with CaCl₂ enhances the freezing tolerance of the tissue. The tissue was examined for cell viability using a TTC (triphenyl tetrazolium chloride) reduction test (p. 198). Our results showed that pretreatment with CaCl₂ increased the viability of the tissue.

**Figure 1:** Photomicrographs of mouse epidermal cells of various pressures. (a) 0 MPa, (b) 2 MPa, (c) 4 MPa, (d) 6 MPa, (e) 8 MPa.
Table I. Effect of Extracellular Ca\(^2+\) on K\(^+\) Efflux/Transport as \& of Control

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Freeze-Injured Axon Scale Tissue</th>
<th>Control (uninjured)</th>
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<tbody>
<tr>
<td></td>
<td>Mean of three replications (\pm) S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7 (\pm) 0.3</td>
<td>4.7 (\pm) 0.3</td>
</tr>
<tr>
<td></td>
<td>4.4 (\pm) 0.3</td>
<td>4.4 (\pm) 0.3</td>
</tr>
<tr>
<td></td>
<td>2.4 (\pm) 0.3</td>
<td>2.4 (\pm) 0.3</td>
</tr>
<tr>
<td></td>
<td>0.9 (\pm) 0.3</td>
<td>0.9 (\pm) 0.3</td>
</tr>
</tbody>
</table>

Freeze-Injured Axon Scale Tissue

K\(^+\) Efflux (Cauntact + In the Presence of Extracellular Ca\(^2+\) on K\(^+\) Efflux/Transport as \& of Control...
A possible role of cytosolic and membrane calcium in freezing injury

Table 1, 1985

| Treatment | 0.7 mM CaCl₂ | 20 mM CaCl₂ | % Reduction (a) when pretreated with
<table>
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<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

(a) as assessed by TTC reduction method (Patton et al., 1985).

Table 1. II. Effect of CaCl₂ and 2 h pretreatment on cell viability of cotton seedling tissue after freeze-thaw stress. Cell viability was determined by TTC reduction method (Patton et al., 1985).
In Figure 2, the phosphor-etch technique of axial motion epidermal cell showing...
Figure 3. A Scheme for Ca2+ as a Second Messenger

For details see Pomeroy and Andrews, 1985.

Further experiments are needed to systematically document these events in freezing.

These alterations could result in irreversible injury (Fig. 4). Further alterations and/or activation of phospholipase A may cause injury (Fig. 4).

Severe injury (Ca2+ losses, 1) may lead to recruitment of Ca2+ storage and thus lead to recovery (Fig. 4). On the other hand, a severe mechanism (membrane bound H+-ATPase may get activated (Fig. 4) and thus lead to recovery (Fig. 4).
Recovery

Dissipation
Water soaking

H₂O taken up by the cell

Effluxed ions

(K⁺) pumped back

H⁺ ATPase activation of cytosolic membrane structure

Increased H⁺ ATPase

Scheme in Figure 3

Increased functions of enzyme

enzymes associated with membrane

Pigment structure weakened

Increased (above a threshold)
Loss of membrane calcium

Loss of membrane calcium (below a threshold)

Freeze-thaw stress

Figure 4. Possible sequence of events involving membrane calcium leakage to cell recovery or irreversible injury after freeze-thaw stress.
In conclusion, roots due to Ca2+ starvation, plant cell physiology 27:222.

272

In conclusion, roots due to Ca2+ starvation, plant cell physiology 27:222.

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Xu et al. (1996), changes of some membrane

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