

**Sensitivity of different species of field crops
to chilling temperature
Part III. ATP content and electrolyte leakage
from seedling leaves**

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Key words: Leakage, ATP content, chilling injury, seedling, bean, maize, soybean

Abstract

ATP content of leaves, electrolyte leakage and surface injuries to seedling leaves in bean, maize and soybean were determined after two, four and seven days of chilling at 6°C, 3°C and 0°C, and in pea, field bean, wheat and spring rape after seven days of chilling at 5°C and 3°C. When electrolyte leakage did not increase relatively to its initial value before chilling, then low temperature did not cause by itself any decrease, but on the contrary an increase of ATP content of leaves in species with both low and high chilling tolerance. Similarly, no decrease and even some increase of ATP content was observed when electrolyte leakage increased after two days at 3°C or 6°C in bean and at 6°C in maize. In all other cases, especially after prolonged chilling, higher rates of electrolyte leakage were accompanied by a significant decrease of ATP, which was a symptom of partial or complete disintegration of cell membranes. Though ATP content is related to membrane permeability, a decrease of its content cannot be regarded as the primary reason for membrane leakiness at low temperatures.

Introduction

The physiological mechanism involved in the damaging effects of chilling temperatures (above 0°C) in tissues of many plant species is not as yet fully understood. Descriptions of the many physical and metabolic symptoms associated with chilling have failed to establish definitively the question of what is the primary reason of tissue injuries. Are changes in the physical state and structure of membranes the first effect of chilling, having as their consequence metabolic changes caused by lower metabolic activity, or are the

primary metabolic changes the cause of disintegration of membrane structures, which leads to, among others, greater membrane permeability? An example of the differences of opinions referring to those questions is provided by investigations into the relations between greater membrane permeability, as measured by electrolyte leakage from tissues, and the changes of ATP content in leaves at chilling temperatures.

According to Wilson (1976, 1978), the leaf dehydration (at 5°C, 85% R. H) in *Phaseolus vulgaris* and not a fall of ATP supply that is the cause of cell death. Similarly, his experiments with *Episcia reptans*, a plant with very low chilling tolerance, lead to conclude that a reduced ATP supply is unlikely to be the cause of chilling injury. Other authors have studied the effects of chilling and freezing temperatures on membrane permeability and ATP content in leaves of cucumber and winter rape. Their conclusion, contrarily to Wilson's interpretation, was that in those leaves a decrease in ATP supply might have been the primary reason for the membrane leakiness at low temperature (Sobczyk *et al.* 1985).

In this paper the problem is considered in the light of determinations of leakage from membranes and of ATP contents in leaves of a number of cultivated plant species carried out under different conditions of chilling temperature and length of chilling.

Material and methods

The plants in the first experiment were of six species or cultivars:

1) Dwarf common bean (*Phaseolus vulgaris* L. var. *nanus*) cv. Sisal, 2) Maize (*Zea mays* L.) cv. Hybrids S72 x Cm7, 3) Field pea (*Pisum sativum* L.) cv. Kaliski, 4) Field bean (*Vicia faba* var. *minor*) cv. Nadwiślański, 5) Spring wheat (*Triticum vulgare*) cv. Jara, 6) Spring rape (*Brassica napus*) cv. Bronowicka.

In the second experiment there were three species and varieties: 1) Dwarf common bean, 2) Maize cv. Hybrids S72 x Co125, 3) Soybean (*Glycine max* L.) cv. Progres.

Seeds were planted in pots filled with 3:1 peat and sand mixture, kept in air conditioned glasshouse at 25°C for two days and then at 20/18°C day/night temperatures for 12 days, in natural light supplemented in a day/night cycle of 15/9 hours with ROF 400 W lamps (75 W/m²). After two weeks the pots were moved to separate air conditioned chambers with artificial light in a 15/9 hours day/night cycle, at temperatures of 3°C or 5°C for seven days in the first experiment and of 0°C, 3°C and 6°C for two, four and seven days in the second experiment. Air humidity was maintained at about 70% R. H. After the different periods of chilling plants were moved again to the glass house and four days later chilling injuries to plant surfaces were evaluated. The percentage share of the particular leaves in the total surface area of the whole plant and the percentage injury scale for the particular plant species or cultivars were determined in a preliminary experiment. Determinations referred to the area of necrotic spots, the withering of whole plants or their parts, and the cracking and dropping away of cotyledons. The method of evaluating injuries in soybean were described in an earlier report (Markowski and Grzesiak 1975). Electrolyte leakage from the leaf discs was determined according to Dexter *et al.* (1932). Details of measurements were described in a previous paper (Janowiak and Markowski 1987). In the first experiment (Fig. 1) electrolyte leakage from chilled tissue was calculated as percentage of the specific conductance of the leakage from first chilled then heat killed samples. In the second experiment (Fig. 2) leakage from chilled tissue was measured on the "index of injury" scale according to Flint's formula (Flint *et al.* 1967).

ATP determinations after extraction were by luciferin-luciferase assay according to Strehler (1962). Discs for ATP analyses and dry-weight determinations were cut out from fully developed leaves. Every treatment was analyzed in four repetitions, every repetition consisted of 12 disks collected from leaves of the same age of four plants. Tissue samples were weighed then frozen immediately in liquid nitrogen and crushed in 1.5 N HClO₄. After centrifuging the supernatant was neutralized with 5 N KOH to pH 7.4, centrifuged again, then the supernatant was diluted to vol. 20 cm³. Immediately after adding 0.4 cm³ of enzyme solution

to this solution the luminiscence of the sample was measured with a 1250 LKB Wallac luminometer. A calibration curve was plotted for every experimental series. To prepare the enzyme solution 100 mg of dried lanterns (Sigma Chemical Company) were ground with 20 ml of ice-cold 0.1 M sodium arsenate buffer at pH 7.4 in a hand homogenizer with an addition of 4 ml of buffer and the homogenate was centrifuged. The supernatant fraction was retained and mixed with 100 mg of $MgSO_4$. This mixture was used as the luciferin-luciferase preparation.

The statistical analysis of differences was evaluated by the variance analysis using the F-test, Duncan's multiple range test (experiment 1) and the Student t-test (experiment 2).

Results

Leakage of electrolytes after chilling during seven days at 5°C and 3°C and a drop of ATP content in leaves at both temperatures occurred only in maize (Fig. 1). In bean after seven days at 5°C leakage of electrolytes did not increase and the ATP content of leaves rose to a much higher level than before chilling. But in bean similarly as in maize after chilling for seven days at 3°C the leakage increased and the ATP content of leaves dropped. In the other four species — pea, field bean, wheat and rape — leakage did not rise after seven days of chilling at both temperatures but the ATP content greatly increased; the only exception was wheat chilled at 3°C, the ATP content of which did not change from its previous level (Fig. 1).

These results suggest that rather than dropping at chilling temperatures the ATP content in the dry weight of leaves increased in those cases when there was no rise of electrolyte leakage, which would have shown that chilling had disrupted the functioning of membranes.

The relationship between ATP and membrane permeability was studied in the second experiment with three chilling sensitive species — bean, maize and soybean. Three indexes of injuries were measured: leakage of electrolytes, surface injuries of leaves and ATP content of leaves. These determinations were made not only after seven days but also after two and four days of chilling at 6°C, 3°C and 0°C (Fig. 2).

In bean chilled at 6°C the leakage of electrolytes and surface injuries of leaves remained at about the same level even after prolonged exposure, but the ATP content of leaves after seven days of chilling was almost three times higher. After two days of chilling at 3°C the ATP content increased significantly even though there was a simultaneous rise of leakage; after four days injuries to seedlings continued to increase, but ATP remained at the initial level; not till after seven days at 3°C did the ATP content drop significantly and this was accompanied by much more extensive seedling injuries. In bean seedlings after two days of chilling at 0°C the leakage of electrolytes was more than 75% of their total content, but the ATP content still remained unchanged, to drop suddenly after four and seven days to almost total disappearance in the dry matter of seedlings (Fig. 2A).

In maize a similar tendency of growth or no change in the ATP content accompanied by a simultaneous rise of membrane permeability and surface injury of seedlings was observed only after two days at 6°C; after this time there was a drop of the ATP content and an increase of seedling injury. The same effects were observed already at the beginning of chilling at 3°C and 0°C (Fig. 2B).

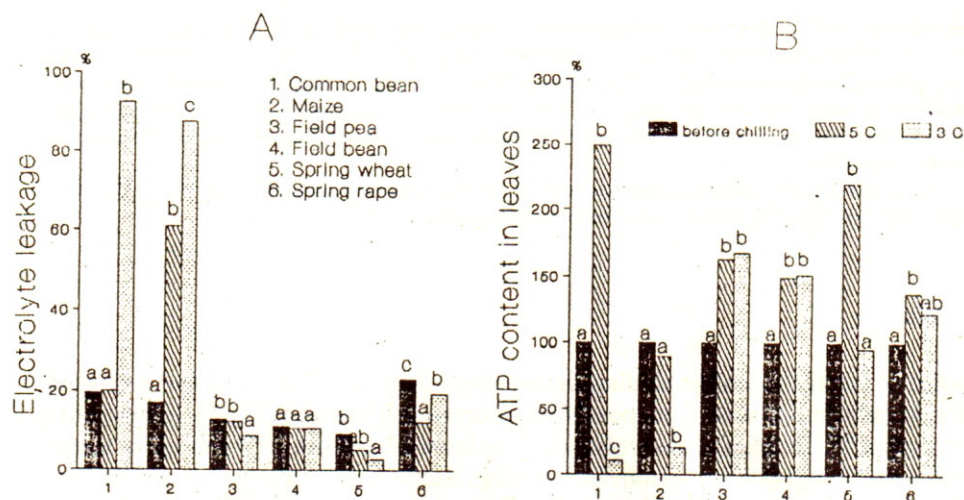


Fig. 1 (A) Leakage of electrolytes in % of their total content in leaves. (B) ATP content in leaves in % of initial values before chilling. (A) and (B) after seven days of chilling at 3°C and 5°C. Entries marked with the same letter within the same plant species did not differ significantly according to Duncan's multiple t-test at $p < 0.05$ (at the probability level of 0.95).

The investigated soybean variety had a greater chilling tolerance than maize and bean. Even after seven days of chilling at 6°C and 3°C leakage of electrolytes and injury of seedling leaves were small, but the ATP content increased markedly at both temperatures. On the other hand, from the beginning of chilling at 0°C both injuries and electrolyte leakage increased and at the same time the ATP content of leaves dropped (Fig. 2C).

Thus the second experiment confirmed the previous results. Smaller seedling injuries after chilling at higher temperatures and seedling injuries with greater electrolyte leakage after shorter chilling at lower temperatures were not accompanied by a decrease of ATP content or its content could even rise. ATP content dropped distinctly only after prolonged chilling at temperatures that had to be the lower the lower was the chilling sensitivity of a plant species. Such a drop of ATP content was always associated with greater injuries of seedlings and higher electrolyte leakage.

Discussion

The greater membrane permeability and the decrease of the ATP content of leaves are symptoms of intrinsic physico-chemical disfunctioning of the lipid-protein system of membranes caused by chilling. When there is no disfunctioning, then the low temperatures alone do not depress the ATP content, they even may have a positive effect on the relation between the phosphorylation rate and the ATP utilization rate, thereby increasing the ATP content of leaves in both chilling resistant (Fig. 1) and chilling sensitive species, *i. e.* bean, maize and soybean (Fig. 2). A similar ATP increase at 4°C was found

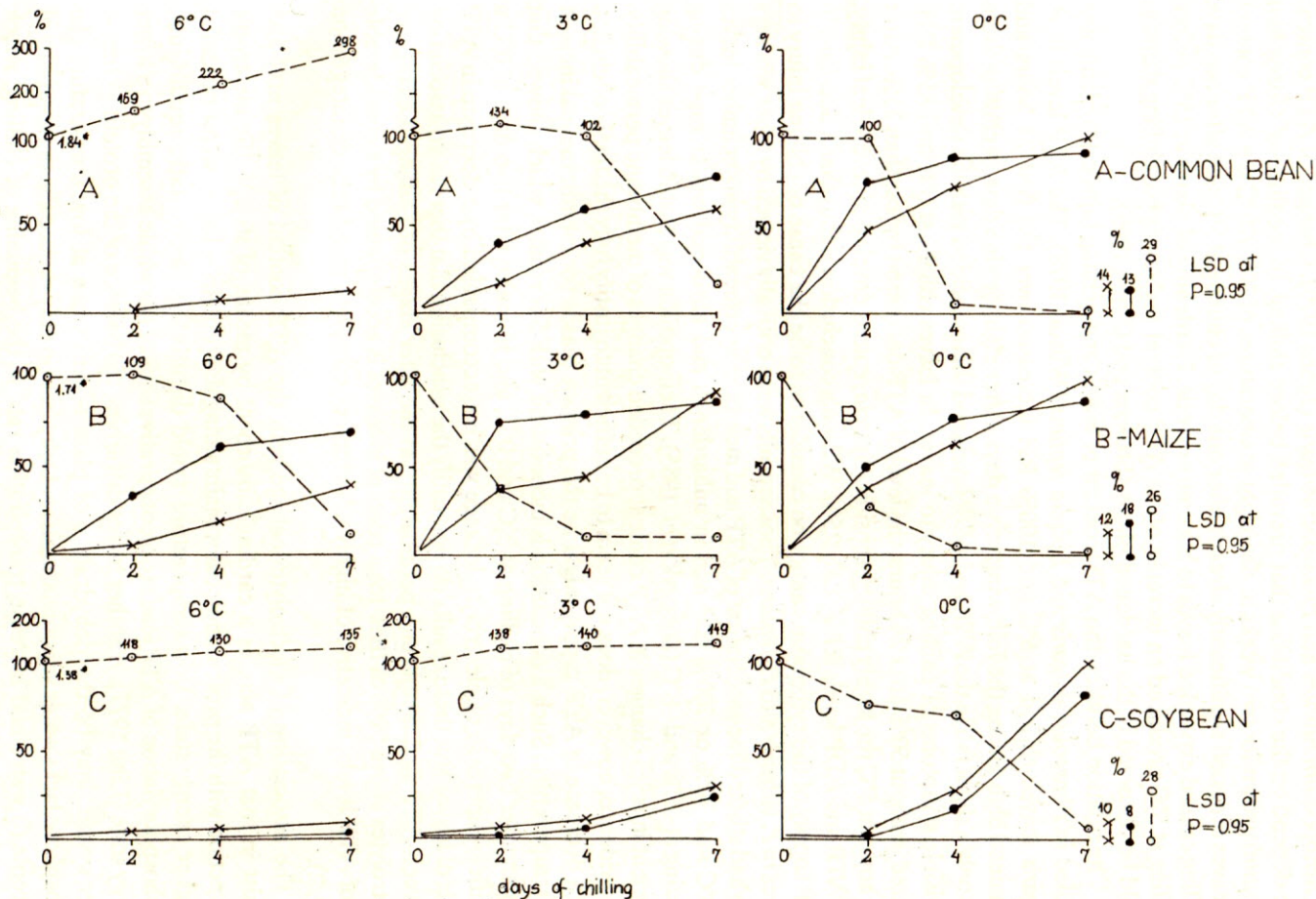


Fig 2. Effects of chilling at 6°C, 3°C and 0°C after 2, 4 and 7 days on seedling injury in % of total leaf area (x), electrolyte leakage on the "index of injury" scale (•), and ATP content in leaves in % of initial values before chilling (o). * ATP content before chilling in $\mu\text{mol (gDW}^{-1})$

in *Chenopodium rubrum* and *Phaseolus vulgaris* (Jones 1970). Changes of ATP content also depend on the conditions that prevailed before chilling. In seedlings of *Gossypium hirsutum* grown for two weeks at day/night temperatures of 30°C/20°C the ATP content of leaves dropped significantly already after one day of chilling at 5°C and after two days chilling caused expanded leaves to become necrotic. Hardening of plants at 15°C before chilling at 5°C produced an increase of the ATP content and no visible chilling damage could be observed in the hardened plants (Stewart and Quinn 1969).

The relation between the ATP content of leaves and chilling injuries to plants was studied in *Phaseolus vulgaris* and *Episcia reptans* (Wilson 1978). The ATP levels in *P. vulgaris* leaves chilled at 5°C and 100% RH increased over the first 24 hours and remained high during the following seven days. After eight or nine days of chilling there was only a slight fall in the ATP and ADP levels and this coincided with the development of visible symptoms of chilling injury to leaves. In leaves chilled at 5°C and 85% RH, injured in about 50% after 24 hours, the level of ATP decreased by less than 33%. After five hours at 5°C the highly chilling sensitive *Episcia reptans* developed severe leaf injury, but ATP and ADP levels fell by only 25%. Wilson's conclusion is thus that a decrease in ATP supply of the cytoplasm cannot be considered to be the cause of chilling injury to leaves in *P. vulgaris* and *E. reptans*. Those results were critically viewed by other workers who had studied the association of ATP and membrane permeability in cucumber leaves at 3°C and 70% or 100% RH and in unhardened and hardened winter rape during freezing at -5°C and -8°C (Sobczyk *et al.* 1985). Their opinion was that in leaves showing incipient injuries changes in ATP content preceded changes of membrane permeability. The evidence, however, does not seem to be sufficient to justify fully the final conclusion that a decrease in ATP supply might be the primary reason for membrane leakiness at low temperature. Such a conclusion is inconsistent with our results, which showed that in bean after two days of chilling at 3°C and 0°C, and in maize after two days at 6°C a significant increase of electrolyte leakage was not accompanied by a decrease in ATP levels of leaves. But these results do not justify the conclusion that there is no association between ATP and membrane permeability at the later stages of chilling. Leakage of electrolytes above 50% of their total content in leaves is accompanied by a considerable fall of ATP levels, indicative of highly advanced or complete membrane disintegration (Fig. 2).

The association of electrolyte leakage with the ATP content of leaves may also consist in that ATP acts as a cardinal absorbent by means of its specific electronic interaction with the protein molecules maintaining the protein — ion — water system at a higher energy state. Low temperature would disturb this electrostatic equilibrium. Dephosphorylation of ATP leads to a cooperative shift of the whole assembly to a lower energy state (Ling 1977). Another factor disturbing the balance of the protein — ion — water system may be the dehydration of plant tissues when at low temperature the atmosphere lacks water vapour saturation. Dehydration may reduce the permeability of the roots to water and increase transpiration rate at the beginning of chilling, which induces the opening of stomata (Wilson 1976). Because of this, when investigating the primary reason of chilling injury it is necessary to disassociate the temperature stress

from the drought stress (Wright and Simon 1973, Kabaki and Tajima 1981, Sobczyk *et al.* 1985).

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Received March 31, 1989; accepted March 19, 1990