

CHANGES INDUCED BY CHILLING IN THE ION UPTAKE, GROWTH AND ANATOMICAL STRUCTURE OF RICE ROOTS

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Abstract

The after-effect of a brief cold treatment on the ion uptake, growth and the anatomical structure of thermophilic rice (*Oryza sativa* L.) and non-thermophilic wheat (*Triticum aestivum* L.) roots was investigated. It was established that in rice an unexpectedly large K-ion uptake occurred following the cold stress. In the case of wheat under similar experimental conditions neither the ion uptake nor growth showed any irregularity. The anomalous ion transport in thermophilic rice was considered a passive influx (or exchange), made possible by the change in permeability of cell membranes as a result of cold stress. The anatomical investigations confirmed that cold treatment resulted in the destruction of the root hairs and 4—5 cell layers of the primary cortex. The meristematic cells of the root apex were disorganized to such an extent after chilling that no root elongation could be detected. Vigorous side rooting from the pericycle, about 2—3 cms from the tips of roots (still in the root hairs zone) occurred 6—7 days after the chilling. Full regeneration of the roots followed on the 8—9th days after the brief cold treatment when the basiton type root was detected.

Introduction

During our earlier ion uptake experiments it was established that the roots of thermophilic plants, such as rice, cucumber, sorghum, melon, etc. show an anomaly in K-ion uptake after a sudden change in temperature (ZSOLDOS, 1968). A considerably larger K-ion influx than expected was observed at 0 °C or near it. A close connection can be detected between the degree of anomalous influx and the length (age) of the root: the shorter (younger) the root, the more pronounced is the K-ion uptake anomaly (ZSOLDOS, 1975).

It was also established that the K-ion uptake anomaly belongs to determined root zones. In the case of rice, e.g., it occurred mainly in the one centimeter segment of the tip, while with the cucumber it was detected in the second 1 cm segment counted from the tips on account of the fairly long calyptra (ZSOLDOS—KARVALY, 1978).

Better understanding of the K-ion uptake anomaly was gained from examinations dealing with the K-content of root segments. At low temperature the K-content of the thermophilic rice roots decreased considerably as a result of the increased permeability of cell membranes, occurring mainly in the apical zone of the roots (ZSOLDOS—KARVALY, 1978).

Related to the important metabolic role of the K-ion, other symptoms occur after cold treatment, too. It is important to notice that under completely similar experimental conditions with non-thermophilic wheat, neither the ion uptake ano-

maly, nor the K-ion loss could be detected in the root tips. From this we supposed that there may be an important difference in the compositions of the cell walls and membranes of root cells of thermophilic and non-thermophilic plants.

It is well known that many plant species of tropical and subtropical origin are subject to chilling injury when exposed to temperatures below about 10 °C, but above the freezing point (LYONS, 1973; LEWIS—WORKMAN, 1964). Chilling injury is one limiting factor in crop production that has received increased attention. Although the importance of low temperature to the life of the plants has been known for a long time the problems relating to the effects of cold stress that are important for both practical and theoretical reasons, are still not clear.

Though most plants survive the cold stress, later (under normal temperature conditions) disturbances in metabolism and growth and consequently decrease in production can be expected. It follows that the examination of the so-called after effects must be considered very important, too.

Thus we here give an account of our investigations on the ion uptake, and on the growth structural changes in roots of rice and wheat seedlings exposed to cold stress.

Materials and Methods

1. Growth of seedlings: Rice (*Oryza sativa* L. cv. *Dunghan Shali*) and winter wheat (*Triticum aestivum* L. cv. *Jubilejnaja*) seedlings, grown in 5×10^{-4} M CaSO_4 solution, were used in our experiments. Surface sterilized seeds were rinsed in running tap-water for 6 hours (rice) and 3 hours (wheat) respectively, and then allowed to germinate on filter paper for 2 days (rice) and 1 day (wheat), respectively. The seedlings were grown in 5×10^{-4} M CaSO_4 solution under standard conditions in a phytotron (CONVIRON, Cabinet Model EF7) at 25/30 °C day/night temperatures with 16-hour illumination of 10^4 lux and with 65% relative humidity.

2. Ion uptake experiments: The potassium uptake studies were carried out in a 5×10^{-4} M K^{86}RbCl solution. The roots of 6–8 day old plants were excised and immediately washed for 10 minutes in distilled water at room temperature. After a 30 minute chilling pretreatment at 0, 10 and 22 °C about 2 g of the root material was immersed in 500 ml of aerated uptake solution at room temperature. The pH of the uptake solution was adjusted to 6.3–6.4 by adding 0.1 N HCl, and was checked again after the absorption period. For technical reasons ^{86}Rb was to monitor the K^+ ion movement; the suitability of this method was carefully checked before the experiments were conducted. The uptake medium contained 15 μCi ^{86}Rb per litre in each case. Root samples were removed from the absorption solution at 10 minute intervals, rinsed three times in distilled water at 22 to 23 °C, and left to dry for thirty minutes on filter paper. The isotope contents of experimental material were measured with a scintillation counter as described earlier (ZSOLDOS—KARVALY, 1978).

3. Chilling treatment of intact plants: The seedlings were chilled for five hours at 0 °C on the sixth day after the start of germination, after which they were cultivated in Hoagland stock solution diluted thirty times and/or in tapwater. The growth of plants, the pH value of the water culture, etc. was systematically checked and photographed.

4. Root anatomical investigations: Preparation for light microscopy was carried out as follows. Specimens of the roots taken on the third and sixth days after the brief 5 hour chilling treatment, were fixed in 40% ethanol. Fixed tissue was dehydrated in a graded series of alcohol (40, 50, 70, 96 and 100% alcohol). For microtome sectioning a 2–3 cm root tip and that portion of the root tip where the side branching starts were then embedded in celloidine. Then 10–15 μ thick sections were prepared with a sliding microtome, stained with Erlich-type acidic haematoxyline, fixed in Canadian balsam, and used for light microscope and photograph investigations.

Results and Discussion

1. Ion uptake investigations: Fig. 1 shows the K^+ -ion uptake under normal ($22^\circ C$) temperature conditions of rice roots, previously exposed to 30 minutes cold stress. The time curves clearly demonstrate that the K^+ ion uptake as an after-effect of the cold injury differs from the normal: the lower the temperature of the water used for the precooling, the higher the K^+ -ion uptake at $22^\circ C$. Because according to our earlier investigations the permeability of cell membranes considerably increases and a passive $^{86}Rb-K^+$ -ion exchange (or leakage) occurs especially in the apical zone of root after $0^\circ C$ chilling (ZSOLDOS—KARVALY, 1978), we can say that the ion influx (or exchange) described above is not connected to metabolism. This is justified by uptake experiments done with different root segments of thermophilic plants under cold stress (ZSOLDOS—KARVALY, 1978a).

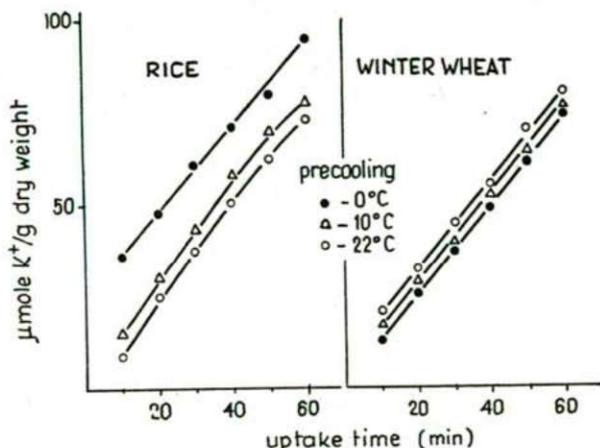


Fig. 1. The after-effect of precooling on the potassium uptake of rice and winter wheat roots. Uptake solution: 5×10^{-4} M $K(^{86}Rb)Cl$; precooling time: 30 min.

Not only is it true that non-thermophilic plants, such as winter wheat, do not show any K^+ -ion uptake anomaly under the same experimental conditions, it is true that after cold treatment unfavourable metabolic effects result in a moderately decreased ion uptake as may be seen in Fig. 2. Because the cold treatments were brief, it is obvious that in thermophilic plants under such conditions only the change in membrane permeability (damage) makes possible the K^+ influx anomaly (or ion exchange) and the considerable K^+ -ion leakage of the apical root-zone during the cold treatment, respectively (ZSOLDOS—KARVALY, 1978).

2. Growth experiments: Fig. 3 shows the growth of rice and winter wheat seedlings after 5 hours cold treatment. A growth disturbance in both rice roots and shoots can be detected as soon as two days after the relatively brief low temperature treatment. At the same time, under completely identical experimental conditions no damage was detected in non-thermophilic winter wheat.

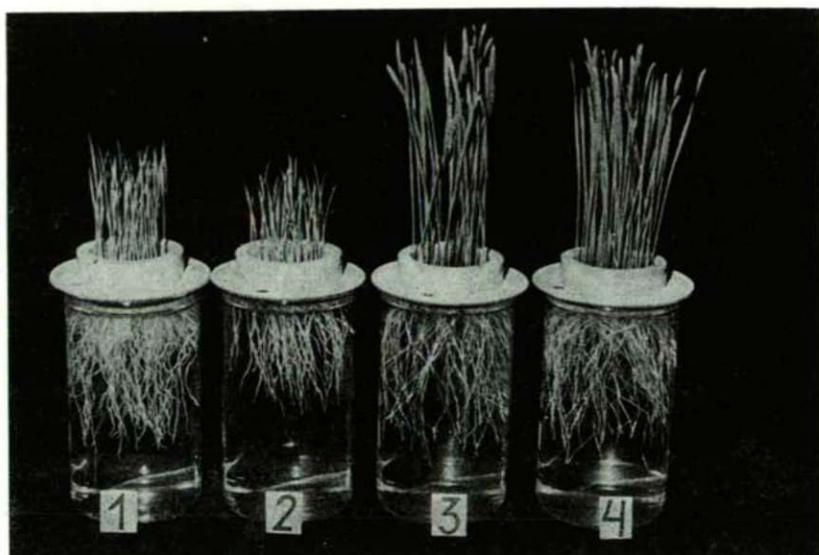
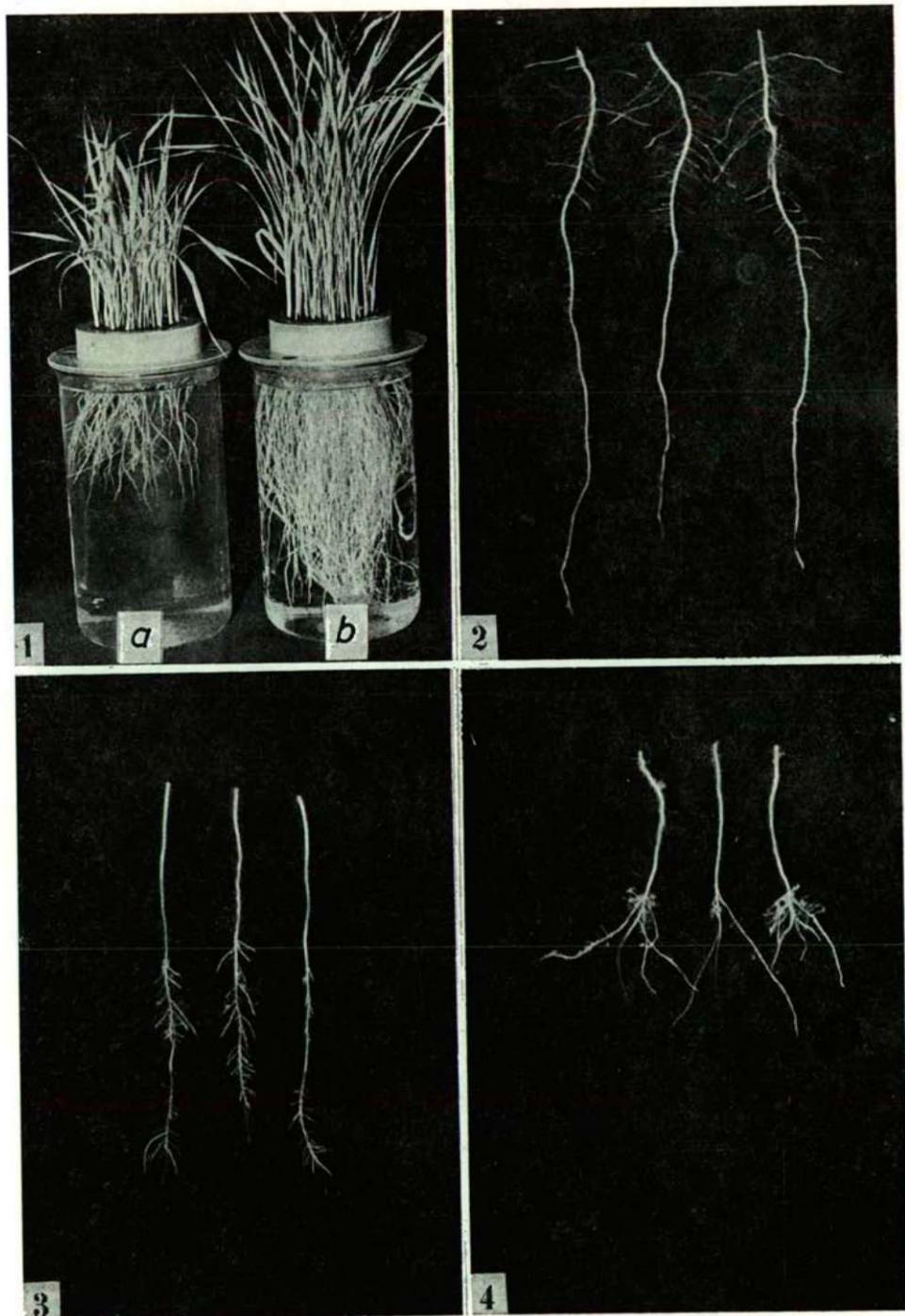


Fig. 2. The manifestation of brief chilling treatment in the growth of rice and winter wheat seedlings on the second day after treatment. From left to right: 1. rice (control); 2. rice (5-hours chilling treatment at 0 °C); 3. winter wheat (control); 4. winter wheat (5-hours chilling treatment at 0 °C).

Plate 1 shows rice plants on the ninth day following cold treatment. Comparing the photos seen in Fig. 3 and Plate 1 it is very clear that: 1) the roots of cold-treated rice plants remained practically the same length (no elongation growth); 2) as a result of low temperature stress an intensive development of side-roots occurred.

In 8–9 days after the cold treatment we detected that the roots having a length of 9–10 cm and that the tips of the side roots became discolored to a pale yellow, and later gradually decayed. From this fact we can conclude that there may be considerable differences between the different root segments (organizational conditions) as regards cold sensitivity. In connection with this we must draw the attention to the changes occurring in the fine structure of the cell membranes (permeability) after the cold stress. This is considerably influenced by its chemical composition e.g. the proportion of saturated and unsaturated lipids and fatty acids (FARKAS et al. 1975, FARKAS et al. 1977, LYONS 1973, ROCHE et al. 1975).

- Plate 1. Fig. 1. The manifestation of brief chilling on the growth of rice seedlings on the fourteenth day after treatment. From left to right: a) untreated (control); b) chill-treated. Fig. 2. Untreated rice roots, age: 21 days, growing in water culture. Fig. 3. Chill-treated rice roots on the ninth day after treatment with intense side-root formation above the root tips. (After chilling treatment seedlings were cultivated in a diluted Hoagland solution, i.e. at a "low-salt condition"). Fig. 4. Chill-treated rice roots on the fourteenth day after treatment with strong side-root formation above the destroyed root tips. (After chilling treatment seedlings were cultivated in tapwater, i.e. at a "high-salt" condition)



3. Anatomical studies: Microscopic investigations demonstrated that 2 or 3 days after the cold treatment a considerable change in construction and disorganization occurred in the roots (Plate 2). The hexarch type rice roots, secondarily still unthickened showed the changes in the following ways:

a) as an effect of cold treatment the root hairs, and the underlying 4–5 cell-layers of the primary cortex, died. At the same time we could not detect changes in the stele of the roots. The primary cortex seemingly protected the interior tissue-layers from being damaged;

b) besides this, the meristem layers of the root tips also showed some disorganization.

After the mentioned tissue-decay the stele of the roots behaved as it does when the roots begin thickening under optimal conditions. At the distance 2–3 cm from the root tips — as it was mentioned earlier — still in zone of root hairs, an intensive side-root development started. This process could be detected later in zones farther from the tip too. The newly developed side-roots which were nearer to the tips developed more vigorously.

On the third day after the cold treatment the development of side-roots from the one layer pericycle started at many places. This development of side-roots, however, differed from normal side-root development in the following:

a) the supplementation of the decayed tips began in the root-hair zone;

b) the cell-division started at more places at the same time than under normal conditions;

c) in spite of the fact that the side-roots formation can be detected almost the whole length of the cold-treated roots, they formed not basiton system, but temporarily an acroton type root system because the side-roots formed closer to the tip and grew faster. One of these (Plate 1) later usually overgrew the others and somehow took the guiding role. Thus the destroyed root tip became regenerated;

d) the above side-root formation was strongly influenced through the salt concentration of the cultura medium too. (Compare Figs. 3 and 4 in Plate 1).

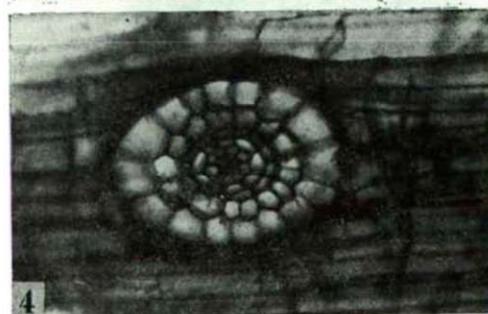
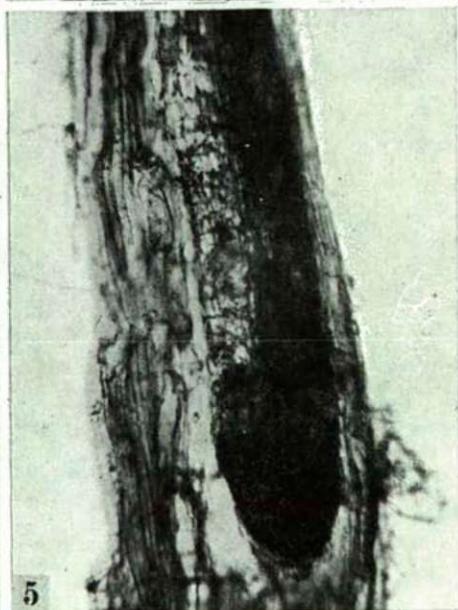
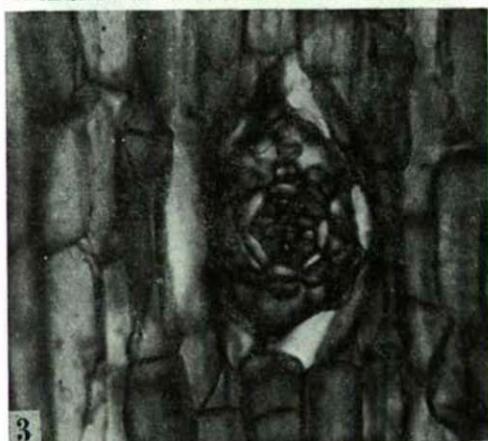
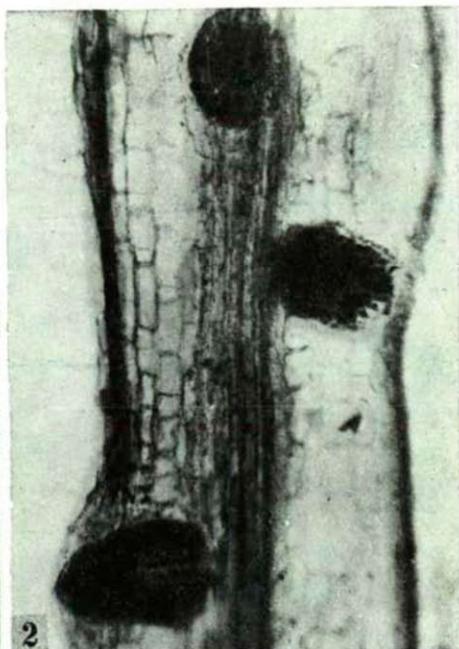
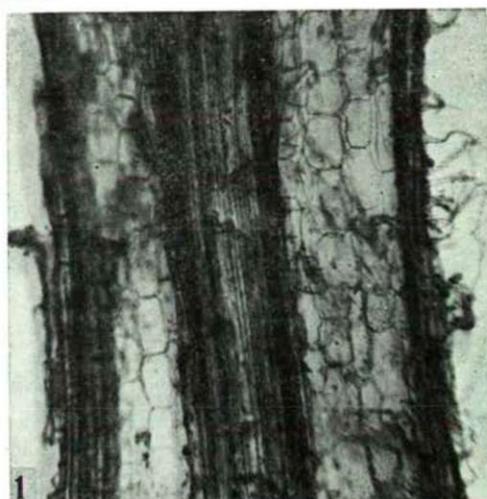
An interesting observation is that the substitution of the "lost" root tip can be so intensive that some of the newly developed roots, immediately after their formation, grow not in a sidewise direction but perpendicularly downward directly into the decayed cortex of the old roots. The regeneration of the cold-treated roots is completed when we observe basiton type roots externally, too, about 14–16 days after cold stress.

Conclusions

1. On the basis of K^+ uptake and efflux after a sudden fall in temperature, i.e. cold stress, plants can be divided into two large groups. One group (the thermophilic rice belongs into this) show a K^+ uptake anomaly and a large scale structural change

- Plate 2. Fig. 1. The external layers of rice cells, exposed to cold stress, began disorganizing (x370).
Fig. 2. The side-root formation begins at many places from the pericycle on the third day after cold treatment (x370).
Fig. 3. Cross-section of the tip of a young side-root (x370).
Fig. 4. Cross-section of an older side-root (x370).
Fig. 5. Side-root developing the closest to the decayed apical meristem grows downwards through the decayed tissues (310)

Plate 2



and disorganization in the roots after cold stress. This phenomenon cannot be observed with the other group (in our case winter wheat) under the same experimental conditions.

2. The after-effects of the cold-stress, that is the changes in the ion uptake and especially in the root structure draw attention to the important role played by cell membranes in the course of cold resistance (damage).

3. Important differences appeared in the cold damage of some of the root segments (zones). On account of this it is necessary to do some comparative examinations of the segments in the course of which we can get more concrete information about the chemical composition of cell membranes (segments) and in this way about the biochemical background of cold resistance.

4. The disorganization of the apical meristem is explained by the considerable change in membrane permeability accompanying cold sensitivity and by the K^+ leakage afterwards. Under such conditions the K^+ concentration, having crucial importance to the metabolic processes, cannot be provided just in the apical meristem.

5. As it is well known that auxin plays a chief role in the initiation of roots, we must not forget about the possible role of growth-regulators in connection with the irregular side-root formation as a result of cold stress. Obviously it is not by chance that after the cold stress of the apical meristem (i.e. the supposed place of auxin synthesis), elongation growth ceases and that after this the structural changes leading to side-root formation occur and are preceded by the occurrence of a convenient hormone level.

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