Acta Biologica Szeged 18 (1—4), pp. 121—129 (1972)

INFLUENCE OF CHILLING ON ION UPTAKE BY ROOTS

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(Received November 20, 1971)

Introduction

Many investigators have studied the influence of low temperature on ion uptake in different plants. It is a general opinion that the intensity of ion uptake varies according to the temperature (CSEH and BÖSZÖRMÉNYI, 1964; KOROVIN et al., 1963; OBERLÄNDER, 1963; SARIC and CURIC, 1963; SUTCLIFFE, 1962; ZHURBITZKY and SHTRAUSBERG, 1958).

Ion uptake by different plants increases considerably with a rise in temperature. The Q_{10} value of mediated absorption is generally above 2. Detailed examinations, however, have shown that in the case of certain thermophilic plants with a sudden fall in temperature, a so-called cold shock effect (uptake anomaly) can be detected. The result of this effect is to cause the uptake of Rb ion to drop to a minimum at some temperature above 0 °C and the rate of uptake increases as the temperature is further reduced to 0 °C from the critical temperature (ZSOLDOS, 1967).

In connection with the cold shock effect further studies have been carried out under IAEA contract. The programme of our work is as follows: *a)* To complete studies on the investigation of the physiological basis of cold shock; *b)* Ion-uptake under cold shock influenced by inhibitors; *c)* To complete studies on induction of cold shock phenomena in cold-sensitive plants using different pH values; *d)* Attention has been focused on some environmental factors such as the presence of bivalent cations, the concentration of the absorption solution and the duration of the experiment; *e)* In addition, to carry out experiments in which inorganic compounds of nitrogen labelled with N—15 are used to study the uptake of nitrogen by roots subjected to varying degrees of cold shock.

Materials and Methods

The experiments were made with young (6—8 day) plants. Seedlings were grown in 5×10^{-4} M $CaSO_4$ solution under wellcontrolled conditions. Before the ion-absorption period the roots were excised, washed with distilled water for 10 minutes at room temperature and placed in 500 ml of aerated absorption solution at different temperatures. The fresh weight of the root material was about 3 g in each experiment. The pH was adjusted when necessary with 0,1 N NaOH or 0,1 N HCl.

The uptake studies connected with the cold shock effect were carried out in RbCl or in KCl solutions always using Rb—86 as tracer. It was observed that though the cold shock effect can be detected by the uptake of other ions too the half-life and counting technique of Rb—86 proved to be the most adventageous. Hence in our experiments in the main this isotope was used.

Uptake vs. time graphs were obtained at different temperatures. The root samples were removed from the absorption solution at different intervals and rinsed three times in distilled water for 1 minute at room temperature. Then the roots were dried for two hours on filter paper at room temperature and put into aluminium dishes to determine the activity. The dry weight per sample was about 35—40 mg. Results are given in μM/g dry weight.

In the experiments on the exchange of Rb ion by K ion the Rb ion-uptake at room temperature lasted 35 minutes in 5×10^{-4} RbCl solution labelled with Rb—86. The active roots were then rinsed three times in distilled water for one minute. Subsequently they were put into a solution of 10^{-3} M inactive KCl solution at different temperatures and pH values. A sample was taken from the KCl solution every 12 minutes during this procedure, and the activities of the samples were measured and plotted as functions of time and Rb concentration, that is, the rate of exchange.

The uptake of nitrogen was studied with the help of N—15 using a mass spectrometer for the determination, in the IAEA Laboratory at Seibersdorf (Austria).

Results and discussion

1. Ion-uptake in different plants exposed to a sudden fall in temperature

It was mentioned in the Introduction that unusual results may be detected in the ion-uptake as a result of cold shock. Since the ion-uptake in rice roots is rather surprising at low temperature, we continued our investigations with various species of plants. The results are demonstrated in Fig. 1.

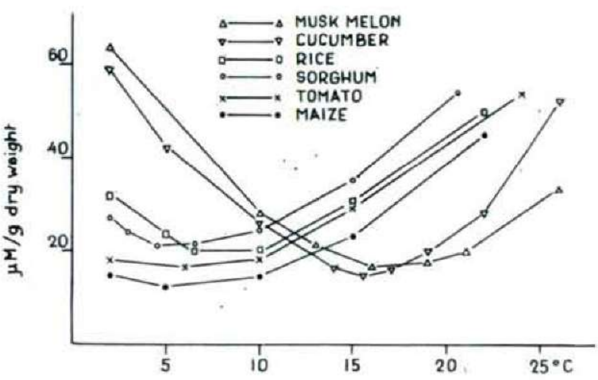

Fig. 1. K-ion uptake from 5×10^{-4} M K(Rb)Cl solution at different temperatures by cold sensitive plant roots. Uptake time: 50 minutes

From the curves it can be stated that in the plants examined the critical temperature at which the cold shock effect appears varies considerably. Thus for example it is 5—6 °C in sorghum, maize and tomato, about 9 °C in rice, 15—16 °C in cucumber and 18—19 °C in musk-melon. The ion-uptake minimum may be obtained at a higher temperature than 0 °C, depending on the temperature-sensitivity of the plant. This remarkable difference is in full aggreement with the coldsensitivity of these plants that is noted in practice too.

Two important deductions can be made from these experiments: (i) The more cold-sensitive a plant the higher the critical value on the temperature scale; (ii) The more cold-resistant the plant the less the uptake anomaly at 0 °C.

It is remarkable that the cold shock effect can always be demonstrated in the first minutes of the uptake period. Under similar conditions with cold-resistant plants like wheat a normal ion-uptake occurs in the vicinity of 0°C and the rate of ion-uptake is proportional to the temperature (ZSOLDOS, 1968a).

2. The effect of some external factors on the ion-uptake by roots at low temperature

The ion-uptake characteristic of the cold shock effect is substantially influenced by external factors. For instance, the intense passive Rb-ion uptake which is observable at 0 °C may be decreased strongly by Ca-ion. At the same time the presence of Ca ion in the absorption solution enhanced the uptake of Rb ion at higher temperature bringing about the Viets-effect (ZSOLDOS, 1968b). Viets-effect is a stimulation of absorption of some monovalent ions by Ca or other polyvalent cations (ELZAM, 1967; WALLACE, 1971). It is remarkable that the effect of Ca especially at low temperature till now goes unexplained (WALLACE, 1971).

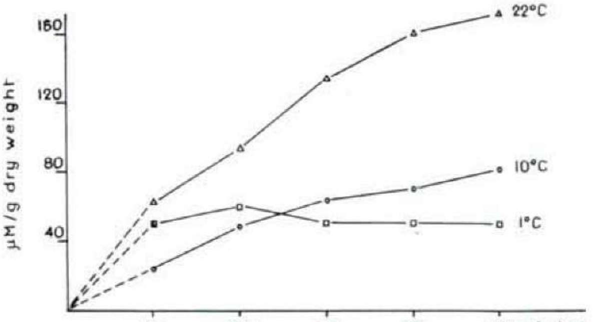

Fig. 2. Effect of longer experiment times on the uptake anomaly by intact rice plant roots. Uptake solution: 5×10^{-4} M K(Rb)Cl.

These experimental results with Ca ions demonstrated that the membranes (cell surfaces) play a very essential role in the uptake anomaly caused by the cold shock effect. As is well known, calcium plays an important role in the structure and in the stabilization of membranes and is also essential in the maintenance of the normal physiological conditions of cells (MARSCHNER and MENGEL, 1966; MARSCHNER et al., 1966; EPSTEIN, 1961).

The unusual effect of the sudden fall of temperature did not occur if the duration of the experiment was longer, or at very low concentration of absorption solution (Fig. 2). Similarly the cold shock effect could not be detected in the case of phosphate, nitrate or ammonium ions (Fig. 3). From the curves of Fig. 3. it can also be seen that Viets-effect was not at all observed for NH_4-ion.

In this process the sudden fall of temperature also plays a major part. This is indicated by the fact that gradual temperature cooling for 2—3 hours could reduce the effect. It is to be noted that the short period of 4—6 hours of cold treatment used, was not lethal for the coldsensitive plants, although a few days later a reduction in growth, especially in root, was detectable in them (ZSOLDOS, 1969).

3. Ion-uptake as a result of cold shock influenced by inhibitors

It is assumed from the above data, that as a result of cold shock the permeability of the cell membranes (surfaces) increases considerably and the absorption curve has a form reminiscent of passive diffusion.

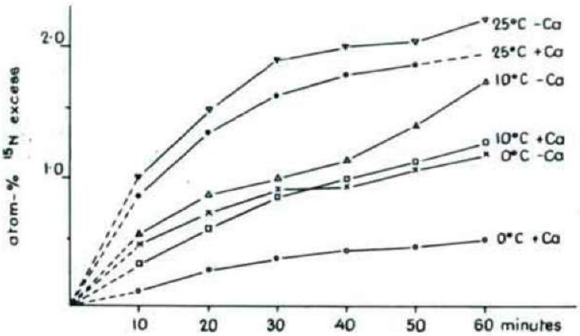

Fig. 3. NH_4-ion uptake from 5×10^{-4} M $^{15}NH_4NO_3$ solution at different temperatures in the presence and absence of 10^{-3} M $CaCl_2$ by rice roots.

The additional of 2,4—DNP (dinitrophenol) to the absorption solution did not appreciably influence the ion-uptake of the sorghum roots as a result of cold chock (Fig. 4). Consequently, with this effect the ion-uptake is independent of the root metabolism.

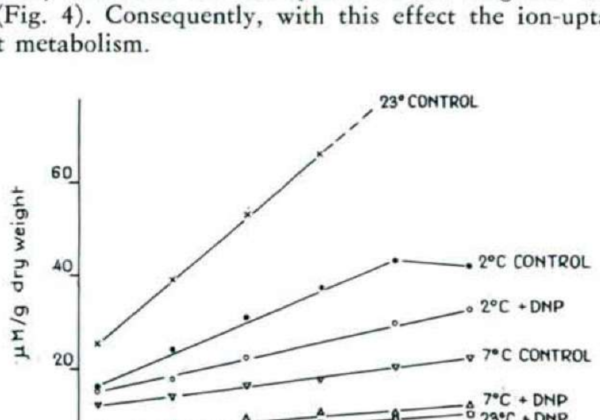

Fig. 4. K-ion uptake by sorghum roots from 5×10^{-4} M K(Rb)Cl solution at different temperatures in the presence and absence of 2,4—DNP

It should be noted that similar results have been achieved in the presence of KCN, but NaF did not influence the Rb ion-uptake (ZSOLDOS, 1969).

Our results led to the conclusion that a sudden fall of temperature changes the permeability of the cell membranes, thus bringing about an important passive influx and efflux. At higher temperature, on reaching the critical temperature value of the plant, however, the membranes are capable of retaining their semi-permeability and as a result a slow mediated uptake occurs, its rate depending on the actual temperature.

The subsequent objective therefore was to elucidate the role and the effect of factors already known to influence the conditon of the cell membranes as well as the ion-uptake. This stressed the importance of examining the pH effect of the external medium which was known to play an important role in the ion-uptake (FRIED and NOGGLE 1958, JACOBSON et al. 1957, KERNAN 1965, MARSCHNER et al, 1966, RAINS et al. 1964).

4. The influence of pH and temperature on ion-absorption and exchange by roots

Figure 5 shows Rb-ion uptake of excised rice roots at various temperatures and pH values. As shown by the diagrams, Rb-ion absorption influenced by hydrogen ion concentration is temperature dependent too. Whereas the „cold shock" effect described in earlier experiments is readily demonstrable at pH 6,5 it cannot be observed at all at pH 5.

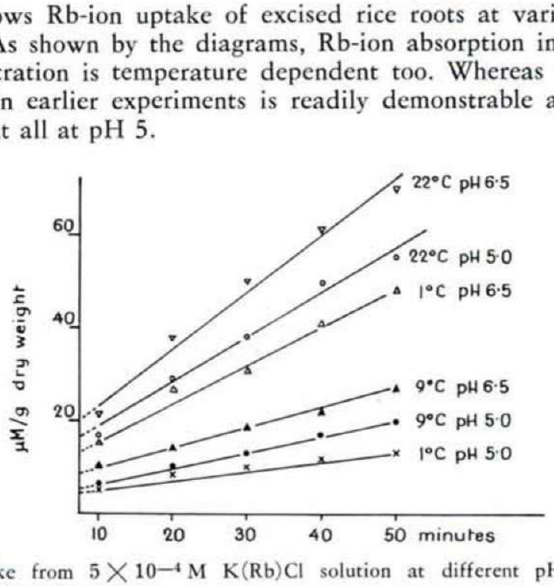

Fig. 5. K-ion uptake from 5×10^{-4} M K(Rb)Cl solution at different pH-values and temperatures by rice roots.

A substantial difference can be noticed with sorghum. In the case of this plant the cold shock effect is still visible at 5×10^{-4} M RbCl concentration even at low pH values. However, the ion-uptake anomaly also ceases gradually at lower RbCl concentration (5×10^{-5} M) and the Rb ion-absorption seemingly complies with the temperature, that is, the uptake anomaly is determined by some external factors, however, the physiological properties of the plants should be taken into consideration too (ZSOLDOS, 1970).

On the basis of the results we suppose that the „physiological pH range" may be wider in the case of sorghum than in that of rice. On the results we may state without exaggeration that the rice plant is more sensitive to environmental factors than sorghum.

It was also presumed that the ion exchange caused by the cold shock effect is considerably influenced by the pH value. This hypothesis was supported by the results of the examination of the exchange between Rb and K ions at various temperatures and hydrogen ion concentration. As shown by the data of Figure 6,

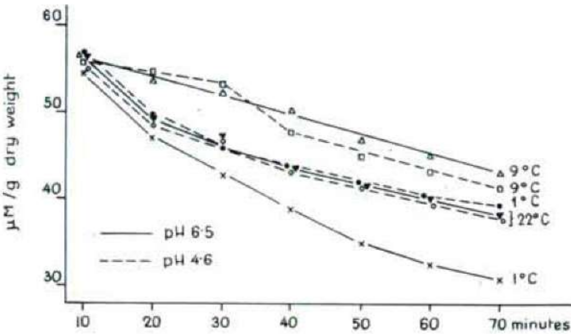

Fig. 6. Exchange of Rb-ion in 10^{-3} M KCl solution at different temperatures and pH-values by rice roots.

this process is the most intensive around O °C and at pH 6.5. t should be noted that Ca-ion in the uptake and exchange solution has essentially affect the efflux of Rb-ion first of all at low and high temperatures (Fig. 7).

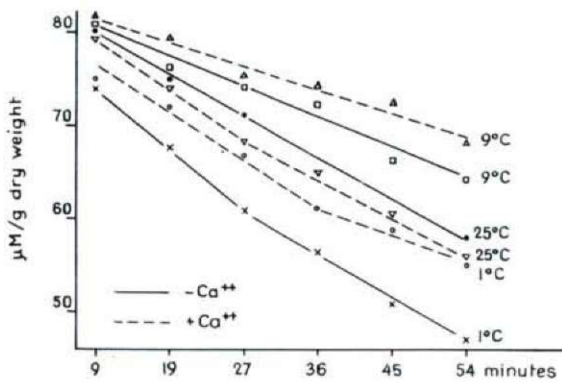

Fig. 7. Exchange of Rb-ion in 10^{-3} M KCl solution at different temperatures in the presence and absence of Ca ions.

The above-mentioned experimental data indicated that in several plant roots under low-temperature conditions a passive exchange diffusion takes place. This is supported by BARBER and SHONE (1967) who also found that at low pH the H-ions suppressed the dissociation of carboxyl groups so reducing the number of exchangeable cations.

These results with cold shock served as a basis for our further investigations connected with the cold-resistance of plants, the main purpose of which was the development of a procedure suitable for the quick assessment of possible differences of cold-resistance between species and varieties.

5. Investigation of cold-resistance in rice varieties

Cold-resistance, as is well known, plays an important role in the cultivation of certain plants. With rice plants, which are very cold-sensitive, it decides the frontiers of its cultivation in temperate zones. Therefore, researchers in many countries try to develop varieties of rice that are less cold-sensitive, thus providing more reliable production in the temperate zones and also the possibility of expanding the northern and southern frontiers of production (IRRI Report, 1970).

For the investigations of the cold-resistance of plants numerous involved methods have been developed (e. g. investigations of the intensity of nutrient transport, germination in a refrigerator, establishment of „cold-garden") which are thus not very advantagecus from the point of view of selection work.

The recognition of this, and the results connected with the uptake anomaly, led us to undertake the comparative investigation of the cold-resistance of some rice varieties.

It was mentioned earlier in this paper that a connection can be observed between the ion-uptake by plants under cold shock and the cold-resistance. On this basis the Rb ion uptake was studied of some rice varieties at 0°C and at room temperature. It was established there is a considerable difference between rice varieties in the Rb ion-uptake (Zsoldos, 1971). For instance, the uptake at 0 °C of the considerably cold-resistant Dunghan Shali and Kákai—203 varieties was always lower than that of the Dubovsky variety, which is less cold-resistant. At the same time, practically no difference could be observed in the Rb ion uptake of the varieties at room temperature.

These results totally supported our supposition that the measurement of uptake anomaly (passive influx) can be used to datermine not only the species but also the varietal differences in cold-resistance. Indeed our experiments with other rice varieties, shown in the graphs of Fig. 8, are very encouraging.

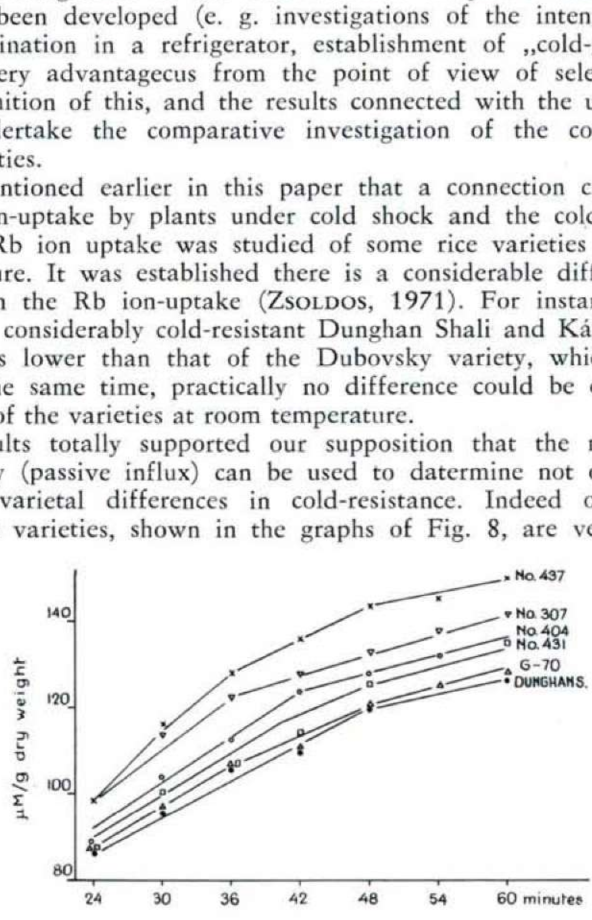

Fig. 8. Rb-ion uptake by roots of different rice varieties from 10^{-3} M RbCl solution at 0 °C.

On the basis of earlier results, the variety Dunghan Shali and G—70 appears to be the most coldresistant, while No. 437 is the least cold-resistant.

Though the purpose of the above investigations was to compare the cold-resistances of different rice varieties, in our opinion such a method will be convenient for the determination of the varietal differences in cold-resistance in other thermophilic plants too.

Cold-sensitivity could be related to membrane proporties which result in an excessive ion influx and efflux. Therefore measurements of changes in ion influx effected by exposure to low temperature can be used to estimate varietal differences in cold-resistance.

Conclusions drawn

The work done during the contract period has shown that:

1. In the case of certain thermophilic plants (e. g. musk-melon, rice, cucumber, sorghum, maize, tomato) with a sudden fall of temperature a cold-shock effect (uptake anomaly) can be detected. As a result of this effect the concentration curves of the uptake of certain ions in short period experiments show a shape which is characteristic of diffusion, and so the uptake observed near 0°C is higher than expected for the actual temperature.

2. The ion-uptake and exchange characteristic of the cold-shock effect is substantially influenced by external factors (e. g. presence of bivalent cations in the absorption solution, change in the concentration of the absorption solution, uptake time, pH, etc.). Viets effect was observed at high temperature for K(Rb)-ion but not for NH_4-ion.

3. Ion-absorption inhibitors (2,4—DNP and KCN) inhibited K(Rb)-ion absorption, but had no effect under cold shock. Consequently, under these conditions the ion-uptake is independent of the root metabolism.

4. The work has shown that the hydrogen ion concentration of the culture solution plays an important role in the sensitivity of the tested plants to cold shock. With increasing hydrogen ion concentration the anomalous pattern of ion-uptake caused by low temperatures disappears. The anomaly of ion-uptake is determined by the concentration of the absorption solution and by the pH, but the physiological properties of the plants should be taken into consideration too. The experiment data indicated that under low temperature conditions a passive exchange diffusion takes place in several plant roots.

5. A test procedure has benn developed for the investigation of the cold-resistance of rice varieties. It appears that this procedurs can be of help in early and efficient screening of rice cultures in selection programmes for cold-resistance, especially in the temperate zones.

Acknowledgements

This work was supported in part by a grant under Contract No. 561/R2/RB of the International Atomic Energy Agency (Vienna, Austria), and part by the Research Institute for Irrigation (Szarvas, Hungary), to whom the author indebted.

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