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ION UPTAKE AND CELL-MEMBRANE BEHAVIOUR OF SYNPRAN N AND DACTHAL HERBICIDE-TREATED RICE PLANTS

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Abstract

A study was made of the effects of Synpran N and Dacthal herbicides on the ion uptake, leakage and change of free amino acids of young rice plants in nutrient solution with the help of an isotope technique. The isotopically labelled solution contained different concentrations of Synpran N or Dacthal herbicides. Synpran N is 34% dichloropropion-anilide, while Dacthal is the dimethyl ester of tetrachloroterephthalic acid.

It was established by investigation of Synpran N that 10^{-5} M and 10^{-6} M concentrations do not give rise to unfavourable effects on the K-ion uptake compared to the control. At 10^{-4} M concentration a slight inhibitory effect can be observed, while at 10^{-3} M the ion uptake practically ceases. The situation is similar, to a certain extent, to the uptake of phosphate ion. In contrast with the Synpran N examinations the ion uptake is not inhibited markedly by Dacthal, even at a concentration of 10^{-3} M.

From the K-ion leakage experiment it can be established that the rate of efflux tends to inrease with the Synpran N concentration. The free amino acid content of the roots after a four hours' treatment with 10^{-3} M and 5×10^{-4} M Synpran N was very low due to damage to the cell-membranes.

General growthinhibition of the roots is caused by 10^{-3} M and 5×10^{-4} M Synpran N concentrations, while Dacthal causes a striking disturbance even at 10^{-6} M concentration.

Introduction

An investigation was made earlier to study the ion uptake of fungicidetreated rice seedlings. It was established that the fungicide Kitazin effectively inhibited the ion uptake and growth at higher fungicide concentrations, while at lower concentrations the effects exhibited were not injurious, but rather favourable (ZSOLDOS, 1973).

There is no clear understanding why biologically active compounds, among them herbicides, modify the uptake of mineral elements (AUDUS, 1964; FREAR and SHIMABUKURO, 1970). Changes in cell-membrane permeability and the rate of cell respiration of treated plants probably also play an essential role in this process.

It is clear that this question is very important from both theoretical and practical points of view, because of the toxic or enhancing effects of herbicides on mineral uptake. For this reason ion uptake experiments were carried out with rice plants with different herbicides in the expectation of clearing up some of the above problems.

Materials and Methods

Excised rice roots and intact rice plants *Oryza sativa* var. *japonica* were used throughout these experiments. Seeds were disinfected for one minute with 1% $HgCl_2$ solution, rinsed in running tap water for 4—5 hours and allowed to germinate on filter paper in Petri dishes. Following germination, the material was transferred to stainless steel screens supported in 15-litre polyethylene containers or one-litre glass containers filled with 5×10^{-4} M $CaSO_4$ solution. The entire container was covered by a sheet of nylon.

The seedlings were grown in the dark at 25 °C for 3 days. After this, the nylon was removed and the seedlings were exposed to artificial light at 10 thousand Lux for 16 hours a day, still at 25 °C. The plant were used for ion uptake experiments when they were 7—8 days old, the roots then being about 6 cm long.

Before the start of a short-time ion uptake experiment, the whole roots were excised just below the stainless steel screen, 3 g samples were transferred to 250 ml beakers and washed with distilled water. The samples were then placed in 500 ml aerated, isotopically labelled absorption solution containing different concentrations of Synpran N or Dacthal. The active ingredient of Synpran N is 34% dichloropropionanilide, while that of Dacthal is the dimethyl ester of tetrachloroterephthalic acid. In the following, the concentration values given refer to the pure active ingredient.

The K-ion uptake studies were carried out in 10^{-3} M KCl solution using Rb^{86} as tracer. The uptake of phosphate ion from 5×10^{-4} M KH_2PO_4 solution was studied with the help of P^{32}. Uptake vs. time graphs were obtained at different concentrations of herbicides.

The root samples were removed from the absorption solution at different intervals and rinsed three times in distilled water. The roots were then dried on filter paper for two hours at room temperature, and put into aluminium dishes for determination of the activity of the samples. The results are given in μM/g dry weight. The pH of the absorption solutions was adjusted to 6.3—6.4 by adding 0.1 N NaOH or HCl and after the absorption period it was again checked.

In the experiment on K-ion loss from tissues affected by treatment with different concentrations of herbicide, 3 g root samples were first left to stand 40 minutes at 23 °C in 600 ml 10^{-3} M KCl + $+5\times10^{-4}$ M $CaSO_4$ solution containing Rb^{86} as tracer. They were then rinsed in 3×350 ml distilled water and put into 500 ml 5×10^{-4} M $CaSO_4$ solution at 23° C containing herbicide in different concentrations. A root sample was taken from the various solutions every 10 minutes, washed as described above, and prepared for activity measurement.

The difference between the activities at the beginning and end of the leakage experiment was considered to be equivalent to the efflux. In our opinion the data obtained in this way also show the change in the permeability of the membranes. All experiments were repeated at least two or three times and the trends were indentical, showing the effects to be due to sample treatment and not sample difference.

In the experiments on the effects of different Synpran and Dacthal concentrations on the growth and the change of the free amino acid content of the roots, the plants were put in Hoagland nutrient solution containing herbicide in different concentrations. After 4—5 days' treatment the free amino acid content of the roots was determined by the standard paper-chromatographic method. The amino acid content of fresh roots represents the fraction soluble in 70% ethyl alcohol.

Results and discussion

1. Investigation with Synpran N

The K-ion uptake by excised roots in the presence of different concentrations of Synpran N is visible in the graphs of Fig. 1. The graphs clearly indicate that lower (10^{-5} M and 10^{-6} M) Synpran N concentrations do not give rise to unfavourable effects compared to the control. At higher concentrations, e.g. 10^{-4} M, a slight inhibitory effect can be observed, while at 10^{-3} M the ion uptake practically ceases.

As seen in Fig. 2. the situation is similar to a certain extent with the uptake of phosphate ion; the difference is that a slight inhibitory effect is found at a slow as 10^{-5} M, while, in marked contrast, at 10^{-6} M the herbicide exhibits a stimulatory effect.

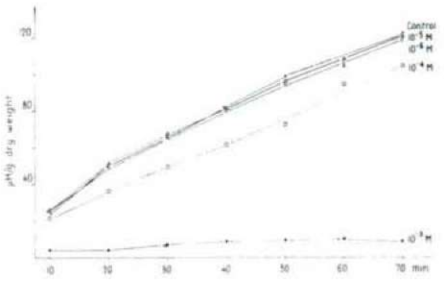

Fig. 1. K-ion uptake from 10^{-3} M KCl solution at different Synpran N concentrations.

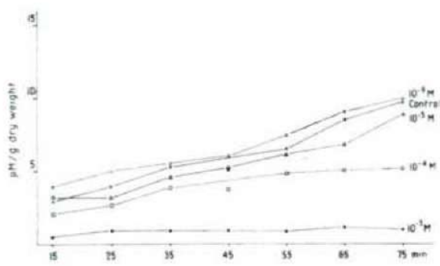

Fig. 2. Phosphate ion uptake from 5×10^{-4} M KH_2PO_4 solution at different Synpran N concentrations.

With Synpran N too, a study was made of the effects of herbicide treatment on the ion leakage. The results are illustrated by the data of Fig. 3. It can be seen that the rate of efflux tends to increase with the Synpran N concentration, a definite increase of rate being found for 5×10^{-4} M Synpran N, and a much more marked one for 10^{-3} M Synpran N.

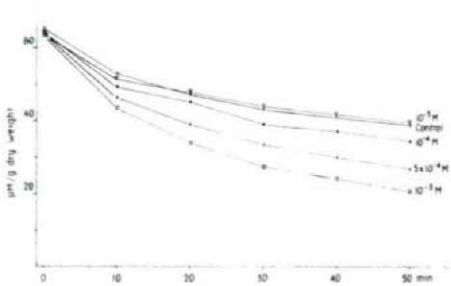

Fig. 3. K-ion leakage by roots on different Synpran N treatments.

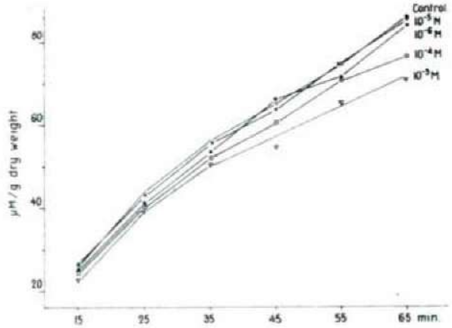

Fig. 6. K-ion uptake from 10^{-3} M KCl solution at different Dacthal concentrations.

At lower Synpran N concentrations the ion-leakage is not significantly different from that of the control. That is to say, in the presence of 10^{-4} M, or 10^{-5} M Synpran N, where the roots could absorb ions essentially in a unidirectional fashion, there is little if any measurable efflux. Thus, the membrane separating the cell interior from the external medium, the plasmalemma, is highly impermeable to diffusive permeation by inorganic ions.

In a normal healthy tissue, the membrane acts as a barrier to free diffusion and exhange of ions (EPSTEIN, 1972). However, when the membranes responsible for this retention are injured, as was found at high Synpran N concentrations, there is a rapid leakage of ions and even other compounds out of the tissue following their own diffusion gradients. From the above findings, the conclusion can be drawn that the effects of biologically active compounds in high concentrations result in an extensive disorganisation of the cell membranes and the root tissue becomes "leaky".

In addition to the ion uptake experiments, a study was also made of the effect of Synpran N on the free amino acid content of the roots. Fig. 4 a shows a chromatogram obtained from alcoholic extracts of roots treated with various concentrations of herbicide.

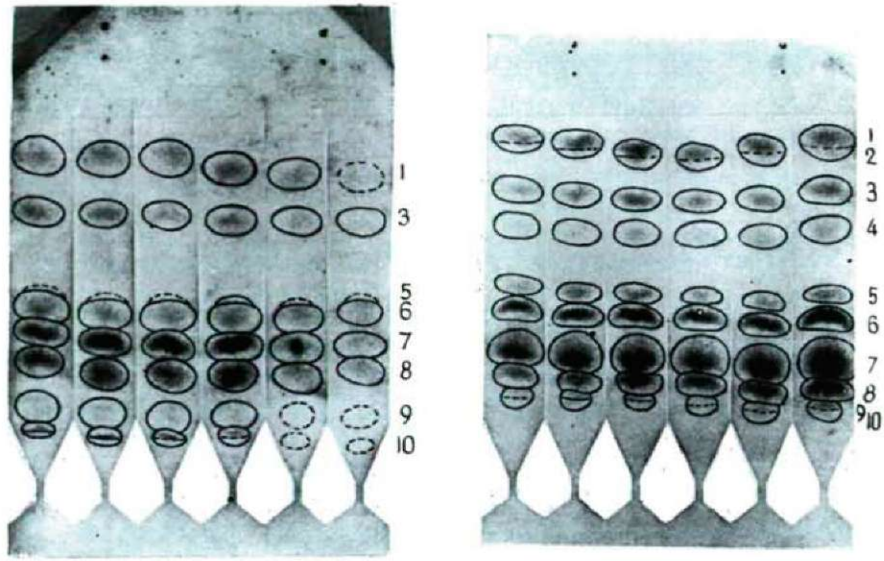

Fig. 4. a. The effect of a four hours' treatment with Synpran N on the free amino acid content of roots. From left: control (at the beginning of the investigation), control, 10^{-5} M, 10^{-4} M, 5×10^{-4} M and 10^{-3} M Synpran N. (1. leucine, 3. valine, 5. tyrosine, 6. alanine, 7. glutamic acid, 8. glutamine+aspartic acid+glycine, 9. asparagine, 10. histidine, lysine).

b. The effect of a four days' treatment with Dacthal on the free amino acid content of roots. From left: control, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M Dacthal (1. leucine, 2. isoleucine, 3. valine, 4. γ-aminobutyric acid, 5. alanine, 6. glutamic acid, 7. glutamine+aspartic acid+glycine, 8. asparagine, 9. histidine, 10. lysine).

It can clearly be seen that at 10^{-3} M and 5×10^{-4} M Synpran N concentrations there is a very marked decrease of the free amino acid content, presumably due to damage increasing the cell membrane permeability, resulting in leakage of the free amino acids from cells.

It is remarkable that at 10^{-4} M Synpran N concentration some of the free amino acids are higher than in the control, probably due to herbicide treatment resulting in an unfavourable effect on the nitrogen metabolism leading to a lower formation of protein.

The ion uptake experiments were supplemented with growth experiment, the results of which are shown in Fig. 5. It can be seen that a general growth inhibition is caused only by the 10^{-3} M and 5×10^{-4} M Synpran N treatments. It must be emphasized that the length of the roots at 10^{-3} M and 5×10^{-4} M Synpran N

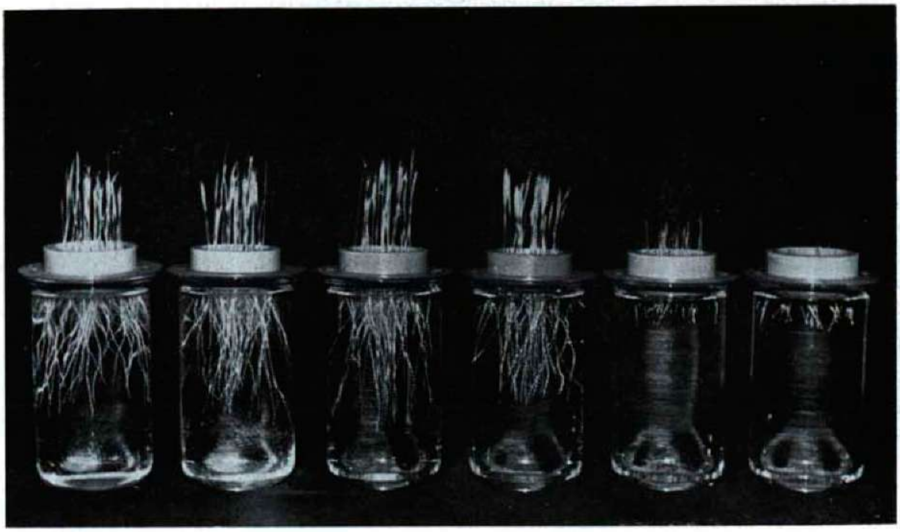

Fig. 5. The effects of a four days' treatment with Synpran N on the growth of rice plants. From left: control, 10^{-6} M, 10^{-5} M, 10^{-4} M, 5×10^{-4} M and 10^{-3} M Synpran N

were the same at the beginning of the experiments as at the end of them, i.e. no change took place during the 4—5 days.

The formation of new roots is prevented at 10^{-3} M and 5×10^{-4} M Synpran N concentrations, thereby demonstrating the influence on the morphogenesis. STRUBBE and FELLENBERG, (1972) have reported the inhibition effects of some herbicides on root formation. It is noteworthy that although 10^{-3} and 5×10^{-4} M Synpran is indisputably toxic, a herbicide treatment of 4—5 days does not destroy rice plants.

2. Investigation with Dacthal

Dacthal, a pre-emergent herbicide, kills many weeds and annual grasses in rice fields. This herbicide is not used extensively, but experiments have recently been made with it on rice since it appears to be effective againts *Echinochloa crus galli*. It is a root herbicide, and thus seemed very suitable for our ion uptake studies.

Fig. 6 shows K-ion uptake in the presence of various concentrations of Dacthal. It is immediately obvious that in contrast with the Synpran examinations the ion uptake is not inhibited markedly, even at a Dacthal concentration of 10^{-3} M. This is all the more surprising, for at the same time there is a striking disturbance of the root growth at very low (10^{-6} M) concentration (Fig. 7).

In our opinion the effect of Dacthal on root elongation can be explained in two ways. First, on Dacthal treatment an endogenous production of ethylene can occur and inhibits auxin transport and root elongation. Secondly, Dacthal treatment itself may inhibit auxin transport. Naturally, the role of Dacthal in the inhibition of root growth is subject to further investigation. It is known, however, that the endogenous production of ethylene causes root elongation disturbances (CHADWICK and BURG, 1970; PRATT and GOESCHL, 1969).

From the above-mentioned facts it appears that Dacthal, though having a solubility in water of less than 0.5 ppm, still causes a remarkable physiological

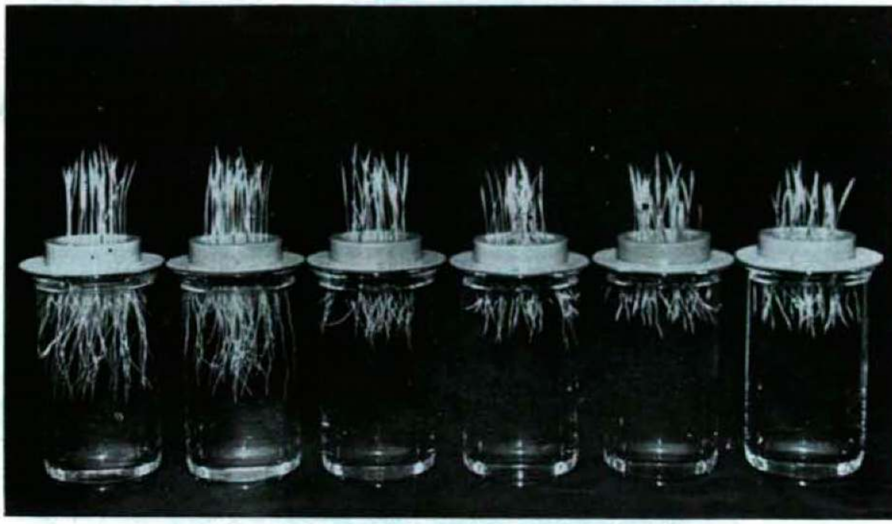

Fig. 7. The effect of a four days' treatment with Dacthal on the growth of rice plants. From left: control, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M Dacthal.

disturbance. At the same time it is noteworthy that Dacthal, in contrast with Synpran N, does not cause any membrane damage, as proved by the free amino acid investigations too (Fig. 4b.)

The free amino acid examinations demonstrated that some of them increased, especially at 10^{-3} M Dacthal concentration. In our opinion this result can be explained in that Dacthal does not disturb the uptake of nitrogen particularly. Only nitrogen transport and the synthesis of some nitrogen compounds may suffer damage, resulting in an increase of free amino acids in the roots.

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