BIOLOGIA PLANTARUM (PRAHA)

7(4) : 285—292, 1965

The Uptake of Mannitol by Higher Plants

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Received November 30, 1964

Abstract. Experiments with young *Hordeum sativum* and *Helianthus annus* plants showed that in the excretion of mannitol in the guttation liquid observed by GROENEWEGEN and MILLS (1960) after uptake by the root system of plants, the osmotic concentration of mannitol in the nutrient medium and the temperature are significant. The beginning of mannitol excretion during guttation is accelerated considerably by the increase of the osmotic concentration of mannitol in the nutrient medium and the rising temperature. The osmotic concentration of mannitol is also important for the duration of mannitol excretion in the guttation liquid after transfer of the plants into a nutrient medium without mannitol. In the presence of mannitol in the nutrient medium water uptake by the root system and growth are inhibited and the tissues of the organs above ground and of the root system are dehydrated. The inhibitory effect of mannitol on the water uptake by the root system is immediate.

Excretion of mannitol, taken up by the root system of intact plants, by the guttation liquid was first reported by GROENEWEGEN and MILLS (1960). Their experimental results were confirmed by STROGANOV and LAPINA (1964), who simultaneously extended the number of plants, taking up mannitol and excreting it in the guttation liquid. These authors emphasized also the ease and rapidity of mannitol penetration into the organs of intact plants.

The possibility of the uptake of mannitol by plant cells has been assumed by BURSTRÖM (1953) and by ORDIN, APPLEWHITE and BONNER (1956), when mannitol was used as an osmotic in the work with tissue cultures. THIMANN, LOOS and SAMUEL (1960) reported a very slow uptake of mannitol by the cells of potato discs. It appears doubtful, that mannitol could penetrate the vacuoles. The authors assume that mannitol gets into an apparently free space, from where it can easily disappear.

Some authors pointed out some special properties of mannitol as compared to isoosmotic concentrations of other osmotics (SLATYER 1961, WALLACE, SADEK and SUFI 1962, LINSER and HERWIG 1963).

In connection with the results of the experimental observations of GROENEWEGEN and MILLS (1960) and of STROGANOV and LAPINA (1964) we became

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interested in the problem of the velocity of the transport of mannitol taken up by the root system of intact plants into the organs above ground. The changes of the velocity of the appearance of mannitol taken up by the root system in the guttation liquid were studied at various osmotic concentrations of mannitol in the root medium at various temperatures and in the presence of a respiration inhibitor. The capacity of mannitol uptake by the root system of dicotyledons was also investigated.

Material and Methods

Experimental plants: *Hordeum sativum distichum* HACK., variety Dunajský trh, 4—8 days old seedlings in the investigations of the velocity of appearance of mannitol in the guttation liquid and of the effects of mannitol on plant growth; *Helianthus annus* L., 5—8 days old seedlings for investigations of the velocity of the appearance of mannitol in the guttation liquid and 15 days old plants, when the effect of mannitol on the water uptake by the root system was investigated; *Pisum sativum* L. variety Židlochovická perla, 2—3 weeks old plants for the investigation of the effect of mannitol on water uptake by the root system of intact plants. Of the varieties used peas are mentioned among the plants with which the capacity for mannitol synthesis was observed (BOURNE 1958).

The germinated plants were cultivated in water culture with aeration. After asepticity of the root medium was attained, the solutions were poured through a bacteriological filter into sterilized vessels. When the solutions were replaced every day, the root system was rinsed with sterile solution (GROENEWEGEN and MILLS).

Solutions: 1/2 Knop nutrient medium (OP = 0·26 atm.) was the basic solution serving as the solvent for preparing the mannitol solutions. In the investigations of mannitol excretion in the guttation liquid, osmotic concentrations of mannitol of 0·5—1·0—2·0 and 5·0 atm. were used. In the studies of the effect of mannitol on the water uptake by the root system lower concentrations of 0·5 and 1·0 atm. were used. In the experiments with the growth of barley increasing osmotic concentrations of mannitol of 2·17—4·9—6·5 atm. were used, which were changed in intervals of 24 hrs. The osmotic pressure of the experimental solutions was determined by calculation and simultaneously estimated cryoscopically. The changes of OP of the employed solutions were also determined experimentally after 24 hrs. In order to enhance guttation, the experimental plants were kept under conditions of high relative humidity. The respiration inhibitor was DNP in a final volumetric concentration of 10^{-6}M (TAKAOKI 1956).

Methods: Mannitol was determined in the guttation liquid by paper chromatography according to HAIS and MACEK (1959). Solvent system: n-butanol--ethanol-water (4 : 1 : 5); detection with silver nitrate. The guttation liquid in quantities of 0·01 ml. was spotted directly on Whatman No. 1 paper.

The water uptake by the root system was determined potometrically at constant temperature of the root system (23 $\pm$ 0·5° C) and of the shoot parts (25 $\pm$ 1° C). The microsolution was injected directly into the potometric

vessels. To the root system of the control plants an equal volume of nutrient solution was added simultaneously.

Results

a) Velocity of the appearance of mannitol in the guttation liquid

In all experiments with different concentrations the presence of mannitol in the guttation liquid of barley and sun-flowers was determined at 18—20° C 24 hrs. after adding mannitol to the nutrient medium. It was observed that the osmotic concentration of mannitol in the nutrient medium significantly affects the time of appearance of mannitol in the guttation liquid at this temperature. Mannitol was determined in the guttation liquid at an osmotic concentration of 2 atm after 17 hrs and at 5 atm already after 13 hrs (Tab. I.). The higher osmotic concentrations significantly inhibited the guttation intensity (OERTLI 1962).

An increase of the temperature caused considerable shortening of the time of mannitol appearance in the guttation liquid with barley and sun-flowers. This time was shortened to 1—2 hrs with barley at a temperature of 26° C and with sun-flowers at 30° C (the osmotic concentration of mannitol in the nutrient medium was 1 atm.).

Addition of DNP to the nutrient medium affected the time of mannitol appearance in the guttation liquid in dependence on temperature. At a temperature of 18—20° C the time interval before the start of mannitol excretion in the guttation liquid showed a tendency of prolongation. With sun-flowers mannitol was not determined in the guttation liquid even after 48 hrs. However, the guttation intensity was very low and did not facilitate continuous observations.

At higher temperatures mannitol appeared in the guttation liquid of barley and sun-flowers after 1—2 hrs, as with the control plants without DNP. The guttation intensity at these temperatures was initially high, it stopped, however, completely after several hours.

Table 1

Effect of the osmotic concentration of mannitol in the nutrient medium on the velocity of the appearance of mannitol in the guttation liquid of barley seedlings at 19 ± 1° C temperature

Osmotic mannitol concentration (atm.)	0·5	1·0	2·0	5·0
Time of mannitol appearance (hrs)	24	$x > 17$	17	13

b) Disappearance of mannitol from the guttation liquid of plants after their transfer into pure nutrient medium

THIMANN, LOOS and SAMUEL (1960) observed a comparatively rapid disappearance of mannitol from the tissues of potato discs after their transfer into distilled water. In our experiments with whole plants the disappearance time

Table 2

Effect of the osmotic concentration of mannitol in the nutrient medium on the velocity of the disappearance of mannitol from the guttation liquid of barley seedlings after transfer of the plants into a nutrient medium without mannitol at 19 ± 1° C

Osmotic mannitol concentration (atm.)	0·5	1·0	2·0	5·0
Time of mannitol appearance (hrs)	48	$x < 48$	68	$x < 68$

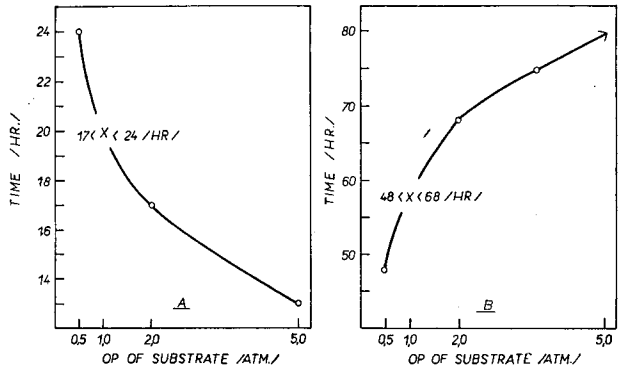

Fig. 1. Relationship between the osmotic concentration of mannitol in the nutrient medium and the velocity of its appearance (A) disappearance (B) from the guttation liquid of barley seedlings at 19 ± 1° C.

of mannitol in the guttation liquid was affected by the osmotic concentration of mannitol in the nutrient medium, in which the root system of the plants grew previously. The disappearance of mannitol from the guttation liquid in dependence on the osmotic concentration proceeded in the opposite sequence than its appearance. Even 60 hrs after the transfer of the plants into a nutrient medium without mannitol its presence in the guttation liquid was observed at an osmotic concentration of mannitol of 5 atm. (Tab. 2, Fig. 1).

c) Effect of mannitol on the water uptake by the root system of intact plants

It has been mentioned in the section on methods that in the potometric measurements of the water uptake by the root system the mannitol solution was injected directly into the potometric vessels without interruption of continuous observation of the water uptake. This technique facilitated the identification of the immediate effect of mannitol on the water uptake. The values obtained by these measurements show that the changes of the water uptake occurred very rapidly and the osmotic concentration of mannitol in the nutrient medium was important (Figs 2 and 3).

d) Effect of mannitol on plant growth

In a short-term experiment the changes of the fresh weight and of the water content of the organs above ground and of the root system of 5-day-old

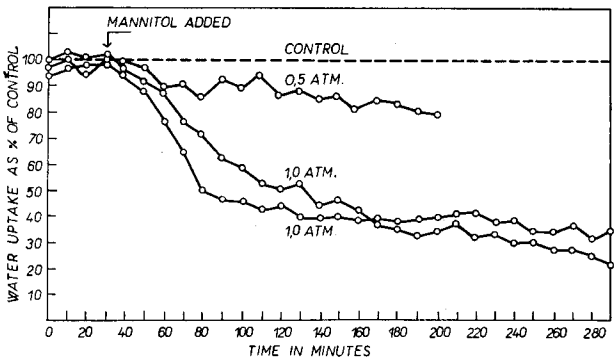

Fig. 2. Water uptake by the root system of *Pisum sativum* after addition of mannitol to the nutrient medium at a constant temperature of the root system and at the organs above ground.

barley seedlings were determined at an increasing osmotic concentration of mannitol in the root medium (intervals of 24 h). The data reported by SLATYER (1961) and by STROGANOV and LAPINA (1964) on the rapid dehydrating effect of mannitol were confirmed. This effect was made still more significant in our experiments by its increasing osmotic concentration in the nutrient medium (Figs. 4 and 5).

With the experimental plants the presence of mannitol in the guttation liquid was observed after 24 h. After transfer of the plants into pure nutrient medium mannitol was present in the guttation liquid even after 48 hrs. At this time necrosis could be clearly observed on the ends of the leaves. Total growth was also inhibited.

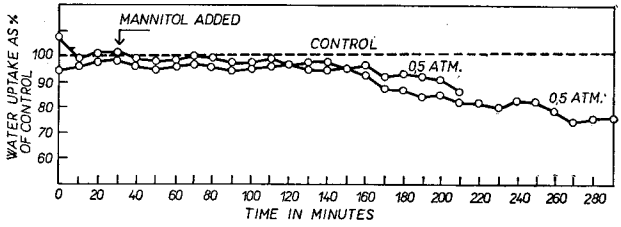

Fig. 3. Water uptake by the root system of *Helianthus annus* after addition of mannitol to the nutrient medium at a constant temperature of the root system and with the organs above ground.

Discussion

The application of mannitol as a physiologically indifferent osmotic, not sorbed, is becoming increasingly questionable. Some authors pointed out the special behaviour of mannitol as compared to other osmotic agents. SLATYER (1961) emphasized the different response of plants to isoosmotic concentrations of mannitol and to freely diffusing solutions of KNO_3, NaCl. JACKSON (1962) reported a higher osmotic effect of mannitol as compared to poly-

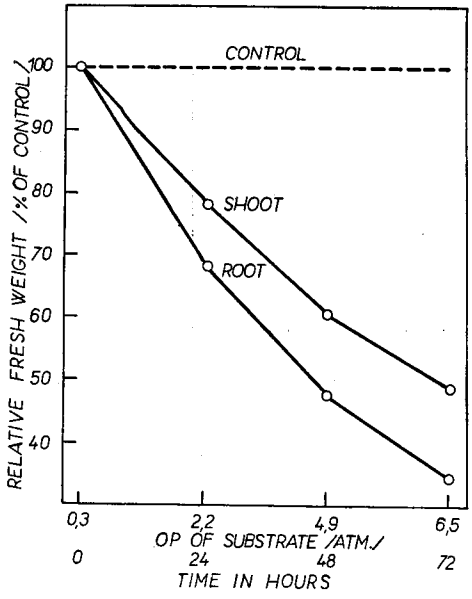

Fig. 4. Changes in fresh weight of the organs above ground and of the root system of barley seedlings, caused by the increasing osmotic concentration of mannitol in the nutrient medium.

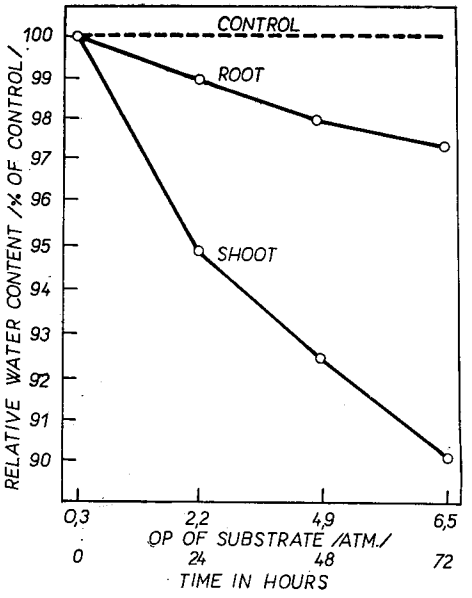

Fig. 5. Changes in water content of the organs above ground and of the root system of barley seedlings, caused by the increasing osmotic concentration of mannitol in the nutrient medium.

ethylene oxide, when their effects on the growth of root hairs were tested. LINSER and HERWIG (1963) reported stronger plasmolytic effects of mannitol as compared to polyethylene oxide at isoosmotic pressures. KAHN (1960) in experiments with germination of seeds differentiates a primary osmotic and secondary non-osmotic inhibition of germination by mannitol. MANOHAR and HEYDECKER (1964) observed the penetration of mannitol into the micropyle and its deposition in the parenchyme of the testa, where it causes swelling, with seeds germinating in solutions of high mannitol concentrations. WEST (1962) also assumed that mannitol penetrates the seeds during germination.

GROENEWEGEN and MILLS (1960) conclude, from the rapid transport of mannitol taken up by the root system of intact plants into the guttation liquid, that mannitol is transported by the xyleme and they doubt that it permeates into the cell vacuoles. The results of our experiments indicate the significance of the osmotic concentration of mannitol in the medium of the root and of the temperature for the transport of mannitol in the plant. A temperature increase into the range of the temperature optimum for water uptake by the root system, i.e. to 25—30° C (KUIPER 1964), and for the translocation processes in the plant (JENSEN and TAYLOR 1961, WHITTE 1964), and especially of the control activity of the root endodermis (WEATHERLEY 1963), led to considerable shortening of the time of the mannitol appearance in the guttation liquid.

Shortening of the appearance time of mannitol in the guttation liquid at

higher osmotic concentrations of mannitol in the root medium may indicate a disturbance of the control system of the endodermis, due to damage to the root, as assumed by GROENEWEGEN and MILLS.

A negative effect of DNP on the velocity of mannitol appearance in the guttation liquid became manifest only at temperatures beyond the range of the temperature optimum for absorption and translocation processes. However, more complete observations were impossible because of the strong inhibitory effect of DNP on guttation.

In this paper results were presented which widen the problem of the mannitol disappearance excreted by the guttation liquid after the transfer of the plant into a pure nutrient medium. The significance of the osmotic concentration of mannitol in the nutrient medium and of the temperature for the velocity of its disappearance was emphasized. GROENEWEGEN and MILLS pointed out another undesirable property of mannitol, i.e. its toxicity, becoming manifest in their experiments by necrotic drying of the leaf apices of barley. The toxicity of mannitol has been especially emphasized by STROGANOV and LAPINA. This property of mannitol was observed also in our short-term experiments.

The problem of the possibility that mannitol exogenously taken up is metabolized by higher plants remains open. TRIP, NELSON and KROTKOV (1963) recently reported the dissimilation of mannitol taken up by isolated leaves of some higher plants. Several higher plants have been found to synthesize mannitol (BOURNE 1963). These include also dicotyledons.

Acknowledgements

We want to express our gratitude to Miss T. Franková for her collaboration in the determination of mannitol.

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V práci sa zisťujú podmienky dôležité pre GROENEWEGENOM a MILLSOM (1960) prvý krát pozorované vylučovanie koreňovým systémom prijatého manitolu gutačnou tekutinou.

V pokusoch s mladými rastlinami *Hordeum sativum distichum* a *Helianthus annus* sa zistilo, že na rýchlosť objavenia koreňovým systémom prijatého manitolu v gutačnej tekutine vplýva osmotická koncentrácia manitolu v živnom roztoku a teplota prostredia koreňa. Pri narastaní osmotickej koncentrácie manitolu z 0,5 na 1,0—2,0—5,0 atm., časový interval objavenia manitolu v gutačnej tekutine sa znížil z 24 hodín na menej ako 24—17 a 13 hodín (tab. 1; graf 1). Pri zvýšení teploty prostredia koreňa, u jačmeňa na 26° C a slnečnice na 30° C, sa manitol objavil v gutačnej tekutine už po 1—2 hodinách (OP živného roztoku 1 atm.).

Osmotická koncentrácia manitolu v živnom roztoku je významná aj pre vymiznutie manitolu z gutačnej tekutiny, po preložení rastlín do živného roztoku bez manitolu. Vyššie koncentrácie predlžujú vylučovanie manitolu (tab. 2; graf 1).

Prítomnosť manitolu v živnom roztoku inhibuje časove bezprostredne príjem vody koreňovým systémom. Sila inhibície závisí na osmotickej koncentrácii manitolu. Inhibíciu príjmu vody doprevádza inhibícia rastu a rýchle odvodňovanie pletív koreňového systému a nadzemných orgánov rastlín (graf 4 a 5).

В. Козинка и Кленовска С., Отделение физиологии растений, Ботанический институт САН, Братислава: **О приеме манитола высшими растениями.** — Biol. Plant. **7** : 285—292, 1965.

В работе изучаются условия, важные для Греневегсном и Майлсом (Groenewegen, Mills 1960) впервые наблюдаемое выделение гуттационной жидкостью манитола, принятого корневой системой. В опытах с молодыми растениями *Hordeum sativum distichum* и *Helianthus annuus* установлено, что на скорость появления манитола, принятого корневой системой, в гутационной жидкости оказывает влияние осмотическая концентрация манитола в питательном растворе и температура среды корня. При нарастании осмотической концентрации манитола с 0,5 на 1,0—2,0—5,0 атм. интервал времени появления манитола в гутационной жидкости снизился с 24 часов на 17 и 13 часов (таб. № 1, гр. № 1). При повышении температуры среды корня у ячменя на 26° Ц и у подсолнечника на 30° Ц манитол появился в гутационной жидкости уже 1—2 часа спустя (ОР питательного раствора 1 атм.). Осмотическая концентрация манитола в питательном растворе имеет значение также для исчезновения манитола из гутационной жидкости, после перемещения растений в питательный раствор без манитола. Повышенные концентрации удлиняют выделение манитола (таб. № 2, гр. № 1). Присутствие манитола в питательном растворе ингибирует непосредственно прием воды корневой системой. Интенсивность ингибирования зависит от осмотической концентрации манитола. Торможение приема воды сопровождается торможением роста и быстрым обезвоживанием тканей корневой системы и надземных органов растений (гр. № 3 и 5).