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Metabolism of excised embryos of *Lupinus luteus* L.

II. The water uptake as influenced by external concentration

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The first work of this series (Czosnowski 1962) revealed that metabolic inhibitors check water uptake of excised, cotyledon — free lupin embryos in a much more efficient manner than their respiratory processes. The present announcement gives a report on the action of osmotic concentration of the external medium on growth of excised, cotyledonless lupin embryos.

MATERIAL AND METHODS

Seeds of *Lupinus luteus* L. var. "Express" were obtained from the Research Centre of the Institute of Soil Science, Cultivation and Manuring of Plants at Przebędowo.

The solutions were prepared with the use of distilled water, without any buffers. The pH ranged between 5.4 and 5.9. Embryos were placed on Petri dishes, 6 cm in diameter, and supplied with the tested solutions in quantities permitting them to break the surface. Details concerning the time and technique of the excision performance, as well as data on the quantity of examined embryos are given in the descriptions of individual tests.

Measurements of embryo respiration were carried out according to Warburg's standard method. The osmotic value of juice pressed out of the embryo tissues was measured after the microcryoscopic method described by Obuchowicz (1956).

RESULTS

1. Effect of different concentrations of mannitol on water uptake

250 mg lots of embryos isolated from seeds presoaked for 24 hrs were placed in mannitol solutions of graded concentrations. Fig. 1. shows the water uptake of embryos incubated for 24 hrs (mean values from

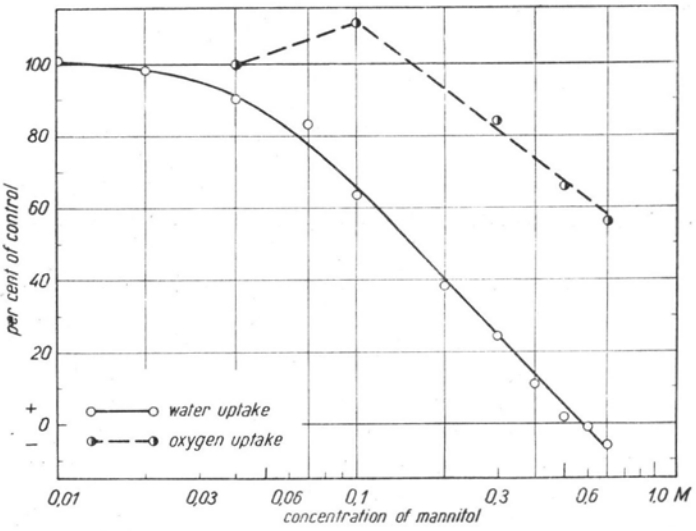

Fig. 1. The effect of external osmotic concentration on water and oxygen uptake. Initial weight of soaked embryo groups was 250 mg; incubation time 24 hrs: the weight of control samples was regarded as 100%

4 experiments run in duplicate). It is evident that embryos stop imbibing water in ca 0.55 M. concentration of mannitol. It is also evident that growth inhibition due to mannitol ranging from 10 to 100 per cent is proportionate to the logarithm of osmotic concentration. A similar correlation was also reported by T h i m a n n et al. (1950) for segments of pea stems.

In concentrations above ca 0.15 M. mannitol prevents respiration — much less evidently however than growth. At 0.15 M. respiration is still on the control level, whereas growth is lowered by 50 per cent.

Table 1

The course of water uptake in mannitol solutions

Mannitol Mol.	Time (hours)										
	0.5	1	1.5	2	3	4	6	9	12	24	48
0	108	130	135	**137**	**137**	**137**	**137**	**137**	146	185	293
0.05	102	122	129	129	**130**	**130**	**130**	**130**	140	170	257
0.1	101	120	127	128	**130**	**130**	**130**	**130**	134	166	179
0.3	96	116	125	126	128	**129**	**129**	**129**	**129**	139	150
0.5	95	111	121	124	125	**127**	**127**	**127**	**127**	128	130

Lots of ten embryos excised from dry seeds (totalling 48 mg) were placed in mannitol solutions. After various timings listed in the table the embryos were taken out, hastily dried with blotting paper and weighed on a torsion balance; the procedure over, they were instantly put back into solutions of the same concentrations. The weight of ten embryos is given in the table in milligrams. The tabulated results are based on means of seven replications. Numbers related to the second phase of water uptake are printed in bold-face type.

In the previous paper (l.c.) three phases were shown to exist in the water uptake of excised, dry embryos: a) imbibition, b) a few-hour suspension in the water absorption and c) further uptake closely related to the aeration of the environment (Table 1) shows the effects of external osmotic concentrations on the course of water uptake in dry embryos during the first 48 hrs.

The given data show that as mannitol concentrations are raised the period of swelling becomes more extended and the phase of water uptake suspension delayed. Interestingly, even concentrations as low as 0.05 M. prevent the embryos to become imbibed in the normal manner (130 mg, in lieu of the usual 137 mg in water). There is no such great difference between the effects of the mentioned concentration and those of higher concentrations.

2. Water uptake in equimolar solutions of mannitol and other substances

Ordin et al. (1956) examining coleoptile segments in oat found that e.g. sucrose, glucose, ethylene glycol and other compounds — while in the same concentrations as mannitol (0.09 M) — bring about a much more evident growth than the latter. Similar findings are reported by Thimann and Marrè (1954). It seemed therefore advisable to investigate the behaviour under these conditions of some totally different plant material, viz. of excised embryos. Swollen embryos were placed in 200 mg lots in equimolar, rising concentrations of mannitol and sucrose. After 24 and 48 hrs of immersion the embryos were taken out, dried and weighed. Average results of four replications are given in Table 2.

These data reveal that, in comparison with mannitol, identical concentrations of saccharose greatly increase the water uptake. This is probably due to the permeation into the cells of the sugar which eo ipso raises the internal osmotic pressure. The process of permeation is relatively rapid. After 48 hrs. 0.05—0.2 M. concentrations of sucrose increase the growth by 1/3, as compared with mannitol solutions.

Table 3 shows the effects of various solutes on water uptake of embryos excised from seeds presoaked for 24 hrs.

Lots of embryos, 200 mg in weight each, were incubated for 24 hrs in various solutions of a uniform, 0.2 M. concentration. Water uptake from the solution of mannitol was settled as 100 per cent. The experiments were triplicated and the mean values given.

Galactose and glucose as well as a few other monosaccharides bear a most visible effect on water uptake of excised embryos; fructose only, is an exception in this respect. Disaccharides like sucrose and maltose

Table 2

Weight increase of embryos in solutions of mannitol and sucrose as percentages of water control — after 24 and 48 hrs.

Concentration M	24-hour growth in solutions of		Diff. %	48-hour growth in solutions of		Diff. %
	Sucrose	Mannitol		Sucrose	Mannitol	
Water	— 100 —		—	100 —		—
0.05	92	89	+3	133	98	+35
0.1	78	73	+5	124	88	+36
0.2	65	58	+7	94	59	+35
0.3	42	35	+7	54	38	+16
0.4	29	18	+11	37	18	+19

are much less effective. Under the experimental conditions raffinose behaves like mannitol. Sorbitol is chemically closely related to mannitol — both these compounds fail to penetrate the embryonic tissues.

Table 3

Effect of various solutes on water uptake

0.2 M solutions of:	Increase of weight
MANNITOL	**100**
Galactose	170
Glucose	167
Arabinose	142
Rhamnose + H_2O	137
Ribose	137
Sucrose	118
Xylose	113
Maltose + H_2O	108
Fructose + H_2O	105
Raffinose + $5H_2O$	99
Sorbitol + 1/2 H_2O	98

Sucrose was supplied by FOCH, Gliwice, Poland. All other chemicals originated from Hoffmann-La Roche, Basle Switzerland.

Ordin et al. (l.c.) examined coleoptiles in *Avena*. The growth rate increase — considered above the growth level in an isoosmotic solution of mannitol — was found to follow a different sequence as regards the solutions in question. E.g. sucrose had the best effect on growth; in the case of lupin embryos it remains far behind several monosaccharides like, in the first place, galactose and glucose. In oat, raffinose caused a considerable increase in length, while in lupin it bears no influence what so ever on growth. All these differences are most certainly due to the great unlikeness in the metabolic and developmental processes of the tested plant material (coleoptiles and excised embryos).

3. The effect of cotyledons on the osmotic behaviour of germ axes

While investigations into the main problem in question were carried out a casual observation was made. It was found that germ axes, isolated from young seedlings in the third or fourth day after the seeds were soaked, and placed in water, absorb it in a much higher rate than embryos excised from seeds presoaked only for 24 hrs. and kept for the remaining three days in a 1% solution of sucrose. In order to gain a better knowledge of these differences in water uptake the following experiment was conducted in two variants (*A* and *B*):

	Time in hours	Experimental procedures
A.	0	Seeds placed in water.
	24	Excised embryos distributed into 3 lots and placed in 1% solution of sucrose in Petri dishes.
	48	From the first lot of embryos, groups weighing 250 mg each, placed for 24 hours in mannitol solutions of increasing concentrations. Thereafter the embryos blotted and weighed.
	72	The second lot of embryos treated as above.
	96	The third lot of embryos treated as above.
B.	0	Seeds placed in water.
	24	The seed coats removed. Coatless seeds distributed into 3 lots and allowed to germinate on moist filter paper in large Petri dishes.
	48	The germ axes, from the first lot of germinating seeds excised and, in groups weighing 250 mg each, placed for 24 hours in mannitol solutions of increasing concentrations. Thereafter the material blotted and weighed.
	72	The second lot of coatless seeds treated as above.
	96	The third lot of seeds treated as above.

Each experiment was triplicated. Average results are plotted on graphs (Fig. 2, curves *A* and *B*).

Excised embryos: 72 hrs. after presoaking of seeds the growth rate rapidly falls (to 1/4 of the rate attained during the 48th hour) and remains for the rest of time on the same level. Embryos placed in mannitol solutions of increasing concentrations reveal a rapidly lowered growth rate during the third and fourth day. The suction force of embryos diminishes at that time from about 0.28 M. to 0.1 M. of the corresponding osmotic value.

The germ axes isolated from the cotyledons are placed in water; the rate at which water is being taken up in the third and fourth day is 3.5—4 times higher than in excised embryos. Mannitol concentra-

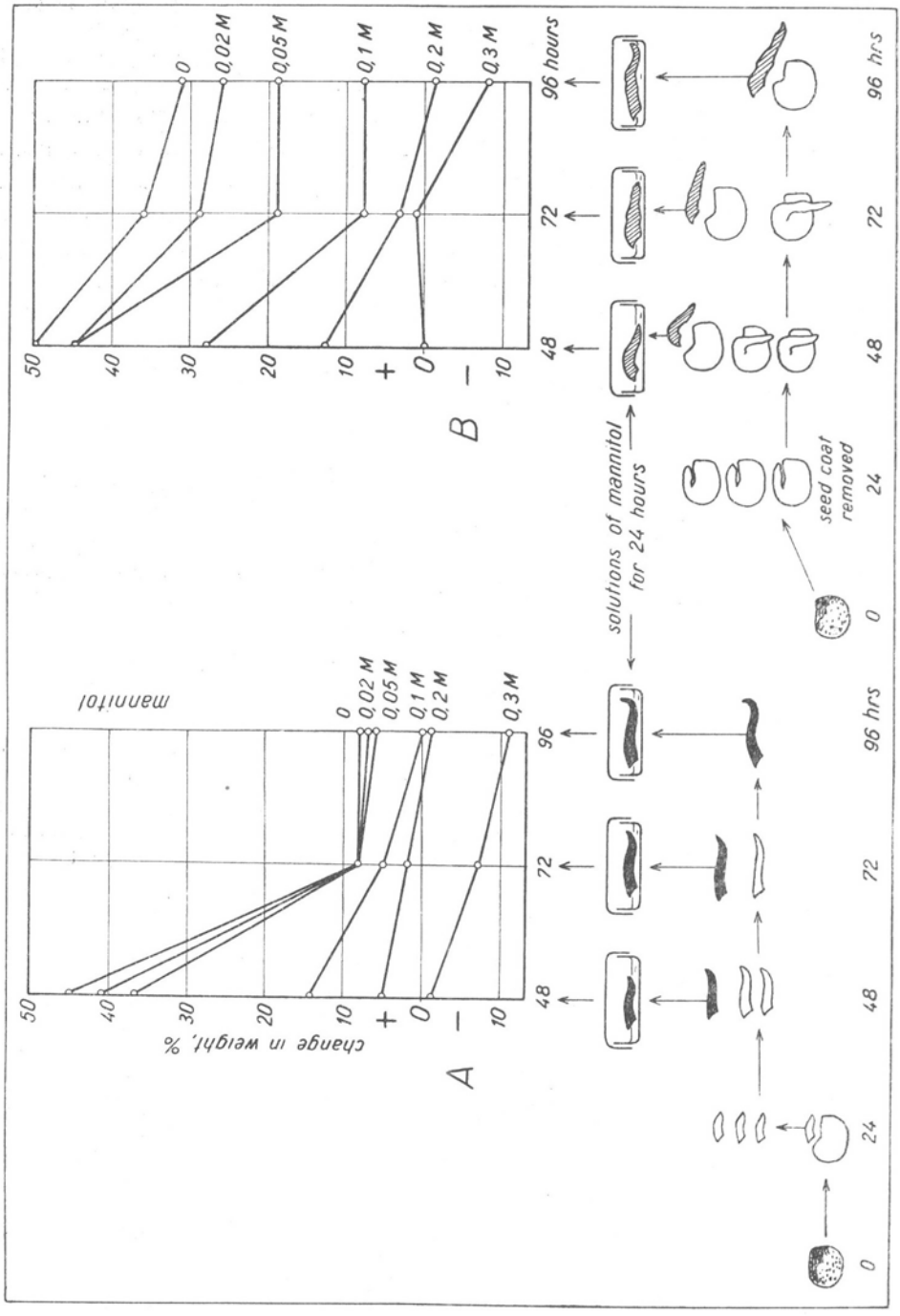

Fig. 2. The course of water uptake of excised embryos (*A*) and seedling xes (*B*) in relation to the external osmotic concentration of mannitol. Detailled explanation in the text

tions of from 0.02 M. to 0.1 M. bring about a much lesser growth restriction than in excised embryos.

The suction force of tissues of the germ axes falls in the third and fourth day from 0.3 M. to about 0.18 M. of the corresponding osmotic value.

Ninety-six hours from the beginning of the trial microcryoscopic measurements of osmotic values of the juice, pressed out of live tissues of excised embyros and germ axes, were carried out in four replications. Similar results were obtained in both cases: Δ_t ranging between 0.31° and 0.33° which corresponds to 0.17—0.18 osmM.

The above given data seem to indicate a much greater plasticity of cell walls, and eo ipso growth rate, in germ axes than in excised embryos, at an identical span of time from the beginning of seed soaking.

DISCUSSION

Many plants respond by a hindrance in growth or in production of fresh mass to increasing concentrations of mannitol (Thimann 1960). The present experiments revealed a similar situation in excised, presoaked lupin embryos. Under our experimental conditions mannitol solutions also prevened oxygen absorption — in a much lesser degree however than water uptake. In general, therefore, the effect of increasing concentrations in the external medium on growth and respiration was much like the action of metabolic inhibitors and of certain growth regulators (marked hindrance of growth and a less effective check in the oxygen uptake — Czosnowski l.c.).

Excised lupin embryos reveal a peculiar response to the action of various kinds of sugars as compared with the isoosmotic concentration of mannitol (0.2 M.). Water uptake is favourably influenced in the first place by galactose, next by glucose, while sucrose occupies a further position in this respect.

Many experiments provide evidence showing that sucrose is one of the most available sugars commonly involved in metabolic process (Schneider 1938; Hoffmannowa 1962 and others). In excised lupin embryos sucrose also bears a favourable effect on the water uptake — particularly after a longer period of action (Tab. 2). What seems most striking, however, is the stronger action of galactose, as compared with sucrose; very often galactose is reported to be a toxic sugar (Farkas 1954; Hoffmannowa 1962) and yet it must permeate the embryonic cells much more easily than sucrose. It is possible that lupin embryos are predisposed, for some length of time after the excision from soaked

seeds, to metabolize galactose or its derivatives. This may be the case, as cell walls in lupin cotyledons include a number of galactanes (W a n n e r 1958) which are made available at time of germination.

For a few days after the excision soaked embryos take up water from mannitol solutions less vigorously than axes of seedlings of the same age. This is a phenomenon closely related to the former problem. Lest the excised embryos should be starved prior to placing them in a mannitol solution they were kept in a 1% solution of saccharose.

It can be assumed that, until the 48th hour of presoaking, excised embryos behave in a usual manner in respect to the osmotic properties of cells and water uptake. At later phases, owing to a scarcity or exhaustion of the substance in question; or else because of disturbances in the dynamic, metabolic equilibrium, the plasticity of cell walls greatly diminishes.

Growth proceeds at a much higher rate in isolated germ axes maintaining for 96 hrs. a contact with the cotyledons than in excised embryos — in spite of the very nearly similar osmotic values of cells in both cases. It can therefore be inferred that the cell wall pressure diminished because of the increase in plasticity (B u r s t r ö m 1953). This situation if followed by an increase of the suction force of the whole cell, corresponding in this case to 0.18 M. as compared with 0.1 M. in excised embryos.

Results of further research after the comparative analysis of metabolism in excised embryos and germ axes will be published in following works of this series.

SUMMARY

1. Water uptake hindrance by swollen excised embryos of yellow lupin from a mannitol solution, varies in the range of ca 10—100 per cent in linear response on log-concentration. Concentrations above ca 0.15 M. check respiration — much less, however, than growth. As regards embryos excised from dry seeds it was found that the higher the mannitol concentrations the longer the swelling phase; also the level of maximum swelling becomes lowered and the phase of restrained water uptake becomes retarded (C z o s n o w s k i 1962).

2. Sugars at concentrations identical with mannitol (0.2 M.) increase water uptake by soaked embryos; galactose and glucose are assimilated most intensely, while raffinose is just as effective as mannitol. Sucrose is in general less effective.

3. Germ axes deprived of cotyledons in the third and fourth day of germination take up water from mannitol solutions much more intensely than embryos

excised from seeds presoaked for 24 hrs. and placed in a 1 per cent solution of sucrose for the following 2 or 3 days. It is assumed that germ axis cells exhibit a much greater plasticity of cell walls than cells of early excised embryos.

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