

Changes in Content of Sugars and their Exosmose from Maize Kernels in Relation to Cold Resistance

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Received October 8, 1969

Abstract. Changes in soluble sugar content in individual parts (embryo, endosperm and pericarp) of maize caryopses exposed to the influence of 24°, 10° and 6° were studied. At the same time the eluates from sand in which the seeds were planted and exposed for various periods were analysed by paper chromatography.

It was found that the lower was the temperature of exposure the greater were the amounts of sucrose, glucose, raffinose and other sugars diffused from the caryopses into the medium before the start of germination. The exosmose course and the changes in sugar content in kernel tissues proved that the hydrolysis of storage polysaccharides is not inhibited by low temperatures but that under such conditions the use of products of hydrolysis for the growth of the embryo is inhibited.

It is concluded that not only surface parts of endosperm but also the embryo participates in exosmose. The relatively high sugar exosmose found from seeds germinated at low temperatures explains the well-known interaction of pathogenic and parasitic microorganism in cold resistance during emergence.

Studies on cold resistance of germinating maize proved that the emergence of kernels at low temperatures is reduced by parasitic and pathogenic microorganisms. Cold hardiness in maize was therefore sometimes considered as the ability to resist attacks by different pathogenic organisms or as the problem of the effect of low temperature on maize susceptibility to microorganism invasion (ISELY 1951).

Changes in the count and composition of microorganism population in cold soil in the seed surroundings and on its surface (HOPPE and MIDDLETON 1950, SEGEŤA and STANĚK 1960) are attributed by some authors to influence of cool temperatures affecting less the activity of microorganisms than that of imbibed but as yet unspouted seeds. To explain the fact that optimum temperatures for vegetative growth of fungi differ from those inducing the most intensive invasion of maize kernels and that noxious effects of microorganisms on higher plants are especially pronounced in cold soil DICKSON (1923) proposed that some microorganisms in spite of their wide temperature ranges are pathogenic only at temperatures just

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low enough to prevent maize germination and seedling growth, whereas McLAUGHLIN (1946) supposed that in warm soil microorganisms remain inactive.

Although mutual interactions among green plants and bacteria and fungi in the rhizosphere have been explained earlier (VIRTANEN 1936) and also the occurrence of microorganisms in the sphaerosphere proved to be influenced by organic compounds exosmosed from the seed (VÁGNEROVÁ *et al.* 1960), no attempt has been made to attribute the changes in the activity and composition of microorganisms found at cool temperatures to the effect of seeds or seedlings themselves.

Our preliminary experiments (SEGEŤA 1963) enabled us to propose a hypothesis which explains the interaction of microorganisms in maize germinating at low temperatures by the exosmose of organic compounds from the kernels.

The objective of this study was to investigate the exosmose of soluble saccharides from the kernels exposed to different low temperatures and to evaluate the relationship between the exosmose of sugars and their content and transformations in different parts of the seed until germination began. It was noticed before that both direct effects of cold and the action of microorganisms on maize caryopses result in their mortality just before the emergence of the radicle from the seed coat (SEGEŤA 1964).

Material and Methods

Experimental Procedure

Maize (*Zea mays* L.) cv. Český bílý koňský zub (ssp. *indentata*) and Kočovská raná (ssp. *indurata*) were used for the experiments. Careful examination of each grain insured that only seeds with intact pericarp and of equal size and shape were selected. The difference in 100 seed weight of each replication did not exceed 5%. The germination capacity was not lower than 90% under optimum temperature of standard germination tests. Caryopses were soaked for 5 minutes in a solution of mercuric chloride (1:1 000), rinsed in 90% ethanol and washed in sterile distilled water before planting. A total of 50 seeds were planted out into a 2 cm layer of sterilized quartz sand in Petri dishes (25 cm in diameter), moistened with distilled water to 70% maximum capillary capacity. Although no particular precautions were devised the seeds were practically free from contamination during the whole period of experiments. Each temperature variant included 6 Petri dishes containing kernels of a single cultivar. The experimental temperatures of 6°, 10° and 24° were maintained with accuracy of $\pm 0.1^\circ$.

Estimation of Sugars in the Eluate

At certain time intervals all kernels were removed from the sand in the Petri dish and were transplanted in the sand in another Petri dish prepared in the same manner as before. The eluate obtained by repeated washing of the sand from each dish was concentrated by distillation *in vacuo* at 50°. After dissolution in 50% ethanol it was used for one-dimensional descending

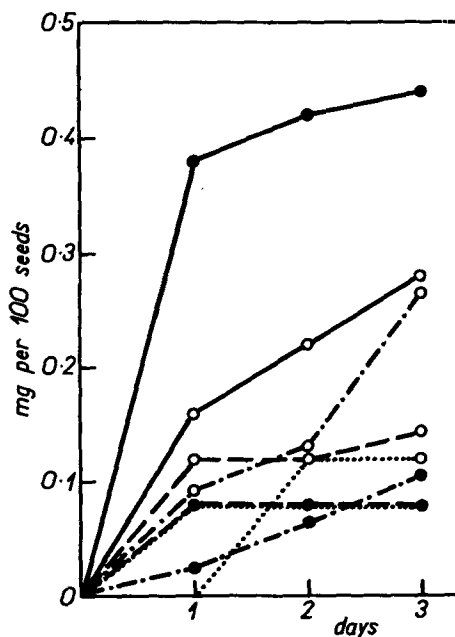
paper chromatography on Whatman No 1. In four replications of each eluate sugars were separated by developing in the system: n-butanol: acetic acid: water (4:1:5). Two of them were used for separate detection of aldoses (with anilin-acetic acid solution) and other two replications for estimation of ketoses (using 1% ethanol solution of alfa-naphthol) according to DE WHALEY *et al.* (1951) (after Čincarová-Hejná 1957).

Estimation of Sugar Content in Seed Parts

The content of soluble sugars in different parts of kernels during their exposure to experimental temperatures has been studied in parallel experiments. At corresponding intervals caryopses were taken out of the sand and were carefully dissected into their various anatomical regions, embryo, endosperm and pericarp. In addition to water content determination they were used for the extraction of sugars according to the method described by JIRÁČEK *et al.* (1966). The same procedure was applied in estimation of sugar content in dry seed kept for a longer period under laboratory conditions.

When estimating quantitatively different sugars in eluates and extracts the intensity of colour and the size of individual spots were compared with values obtained in standard solutions applied in gradually (in 5 mg) increasing concentrations. The results were expressed in mg per 100 seeds and were evaluated by the usual statistical methods. Most of the results taken into consideration were found statistically significant.

Fig. 1. Time course of glucose (—), fructose (---), sucrose (....) and raffinose (....) exosmose from caryopses of Český bílý koňský zub (○) and Kočovská raná (●) exposed at 24°. Abcissa: time in days; ordinate: mg per 100 seeds



Results

Besides sugars (glucose, fructose, sucrose and raffinose) the course of whose exosmose from kernels and whose changes in content in different parts of caryopses are demonstrated in Figs. 1 to 7, we found mannose, galactose, arabinose, xylose, ribose and 17 other compounds not yet identified. Since their appearance was irregular, no special attention has been paid to them for the present.

In Figures 2 and 3, representing the exosmose of individual sugars at all experimental temperatures, the differences in trend and level of sugar exosmose at 24° are not distinct enough; the values found in this variant

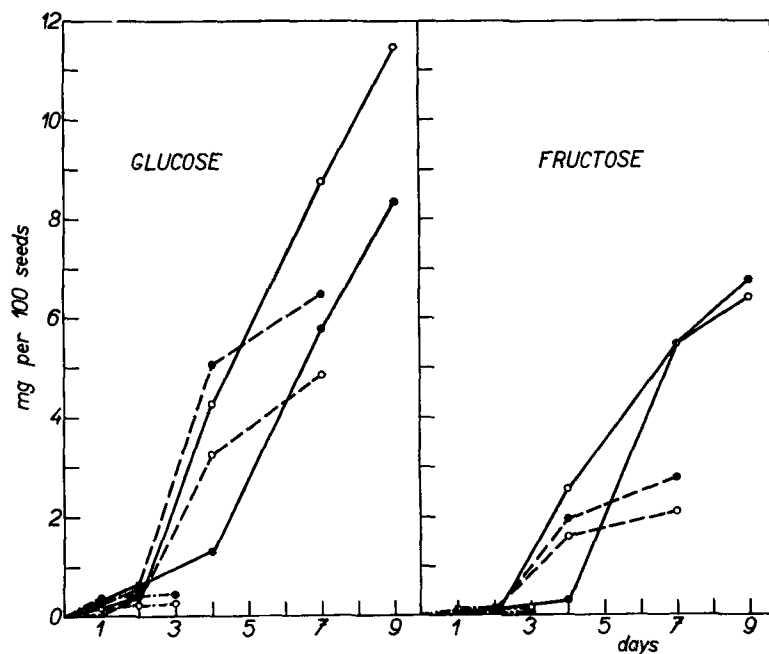


Fig. 2. Time course of glucose and fructose exosmose from caryopses of Český bílý koňský zub (○) and Kočovská raná (●) exposed at 24° (.....), 10° (- - -) and 6° (——).

Abseissa: time in days; ordinate: mg per 100 seeds

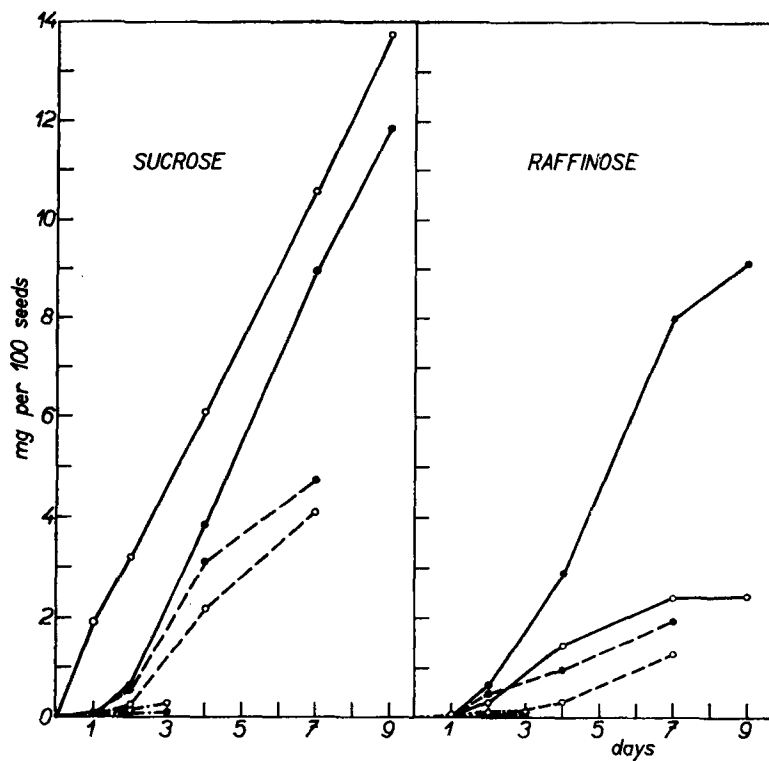


Fig. 3. Time course of sucrose and raffinose exosmose from caryopses of Český bílý koňský zub (○) and Kočovská raná (●) exposed at 24° (.....), 10° (- - -) and 6° (——).

Abseissa: time in days; ordinate: mg per 100 seeds

are presented in Fig. 1. The curves show that under conditions favourable for germination the rate of sugar exosmose fell rapidly as individual kernels began to germinate (25—48 hours). Glucose dominates among the sugars to be exosmosed under such conditions.

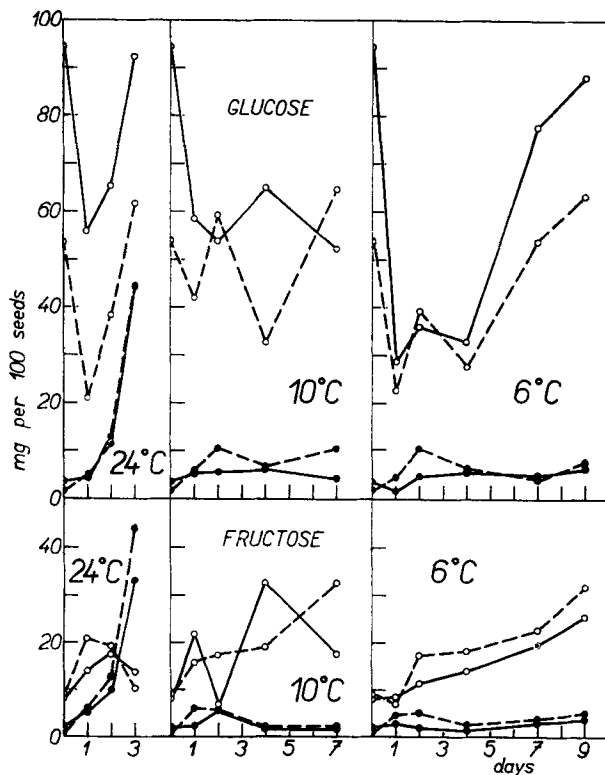


Fig. 4. Glucose and fructose content of embryo (●) and endosperm (○) tissues cv. Český bílý koňský zub (—) and cv. Kočovská raná (- -) from seeds exposed at indicated temperatures. Abscissa: time in days; ordinate: mg per 100 seeds

In striking contrast to this, at low temperatures the rate of exudation and likewise the total amount of sugars exosmosed until the onset of germination are many times greater. Especially in the period between 2 to 4 days there is a steep rise in saccharide exudation. The velocity is different in individual sugars, sucrose reaching the maximum. The total exosmose of all sugars from seeds exposed at 6° is greater than that at 10°, exceeding 100 fold the exudation at 24°.

Differences in exosmose rate between seeds of both cultivars can be found at low temperatures only and especially in the period of intensive exudation.

A comparison of changes in the content of saccharides in embryo and endosperm (Figs. 4 to 6) and pericarp tissue (Fig. 7) of seeds exposed to various temperatures not only confirms the known differences in the role played by different parts of caryopses in germination processes but also contributes to the explanation of the exosmose.

The relatively low level of sugars in the pericarp remains nearly unaltered in time and under various temperatures. In the endosperm there are considerable changes in the content of mono- and oligosaccharides, glucose being present in greatest amounts. The stepwise declining level of sucrose, as that of raffinose, depends directly on temperature.

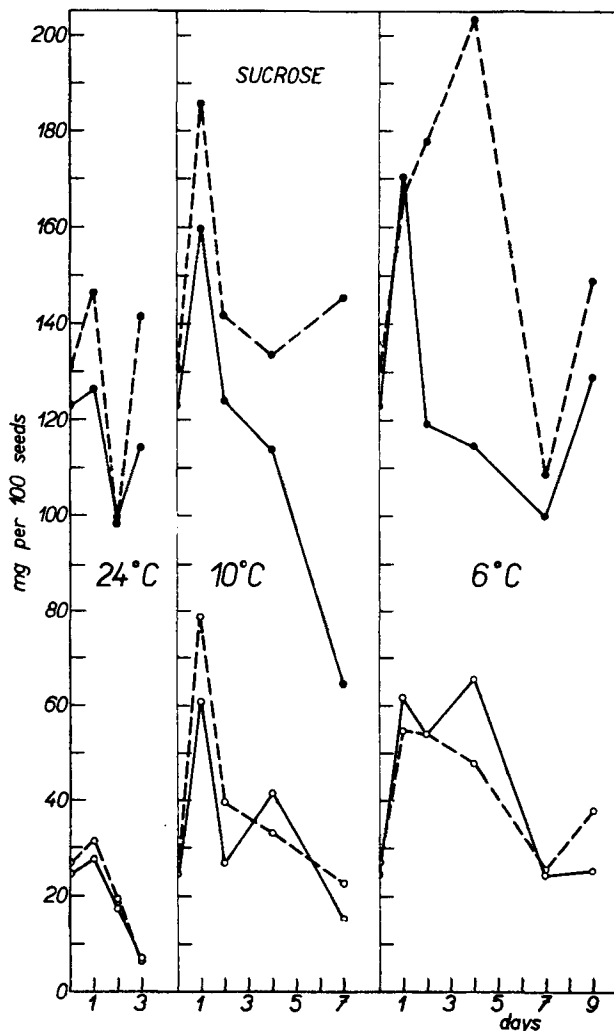


Fig. 5. Sucrose content of embryo (●) and endosperm (○) tissue cv. Český bílý koňský zub (—) and cb. Kočovská raná (- - -) from seeds exposed at indicated temperatures. Abscissa: time in days; ordinate: mg per 100 seeds

At low temperature the content of monosaccharides in the embryo is small and relatively constant. The amount of sucrose contained in the embryo of dry seed and during the exposure to low temperature was much higher than that in the endosperm. The increase of sucrose content found in the

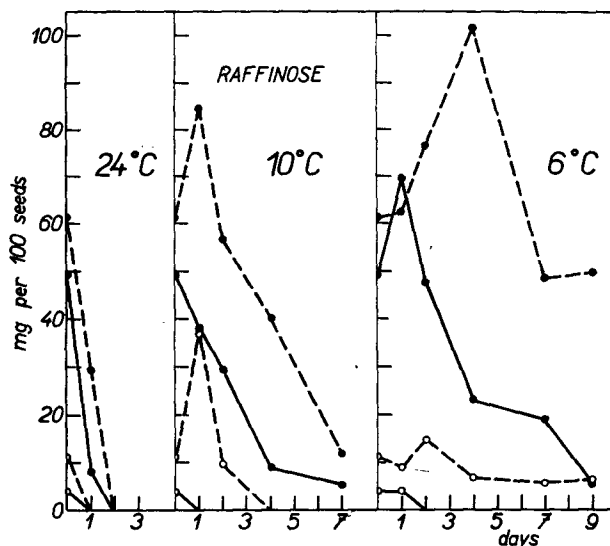


Fig. 6. Raffinose content of embryo (●) and endosperm (○) tissues cv. Český bílý koňský zub (—) and cv. Kočovská raná (- - -) from seeds exposed at indicated temperatures. Abscissa: time in days; ordinate: mg per 100 seeds

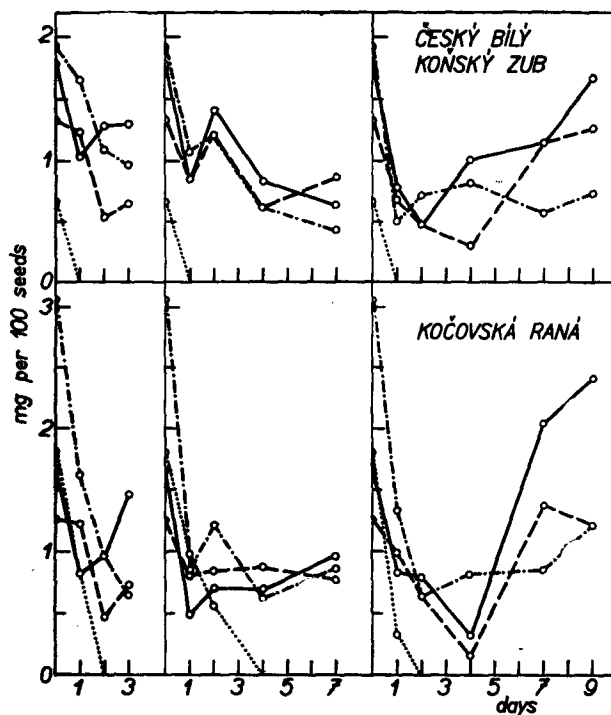


Fig. 7. Glucose (—), fructose (- - -), sucrose (.....) and raffinose (- . - .) content of pericarp tissue cv. Český bílý koňský zub and cv. Kočovská raná from seeds exposed at indicated temperatures.

Abscissa: time in days; ordinate: mg per 100 seeds

first 24 hours at all experimental conditions is followed by a decline which is the slower the lower is the temperature of exposure. The same dependence can be seen in the level of raffinose.

Discussion

Although low temperatures inhibit germination (SEGEŤA 1964) and reduce water absorption rate in maize caryopses (SEGEŤA 1963), they do not inhibit the enzymatic hydrolysis of reserve carbohydrates. This has been proved by the fact that changes in the content of soluble sugars from polysaccharides, their translocation and use by the embryo either for growth of meristematic tissues or as substrates for respiration, proceeded without interruption. But under these conditions early appearing exosmose of sugars ceased in a short time. These results were confirmed by data found simultaneously by SCHWÄR and JÄHNE (1964).

On the other hand, at low temperatures as a consequence of slower hydration of kernel tissues the activation of hydrolytic enzymes is also delayed. The enzymes taking part in the interconversions of reserve compounds in cereal grains (amylases, maltase, phosphorylases *etc.* TSAI and NELSON 1968) are localized in the aleurone layer, scutellum or in the endosperm, and according to recent proofs (VARNER 1964) the activation or even *de novo* synthesis of some of them is induced by gibberellic acid.

The reason for substantially greater sugar exosmose from kernels influenced by low temperatures can be looked for in the fact that under such conditions soluble sugars formed in hydrolytic processes are not used by the embryo for the growth of its tissues. This assumption is supported not only by the pronounced inhibition of the embryo development but also by the fact that dry matter weight of the embryo, despite sufficient hydration of its tissues, remains unaltered even at long exposures (SEGEŤA 1970).

Consequently considerable amounts of soluble sugars produced by enzymatic hydrolysis pass through the pericarp out into the surrounding medium. The inhibitive effect of increasing amounts of the breakdown products on enzyme activity is thus eliminated by their stepwise diffusion. The more the germination processes are inhibited the greater is the sugar exudation. This phenomenon is not identical with the leaching out of saccharides from soaked unviable seeds as has been proposed by TAKAYANAGI and MURAKAMI (1968) for testing seed vitality.

With regard to mechanical structure and chemical composition of maize kernels it is not possible to assume the participation of more deeply located tissues in the sugar diffusion outside. The most important process therefore is the mobilisation of reserve saccharides in the outermost parts of the endosperm and their translocation in the embryo, respectively. Values of actual content of saccharides in pericarp show the relative stability of exudation rate and also the passive role of this histologically rather complex kernel covering.

Since in addition to sucrose, glucose and fructose occur in the exudate in large quantities in the cold and since at the same time the greatest changes in the content of these latter compounds could be detected in the endosperm,

it may be concluded that surface parts of this storage organ play a great role in the exosmose. However, especially at low temperatures, the much more pronounced changes in raffinose content of embryo than of the endosperm prove that the embryo participates in relatively high exosmose of this sugar.

In both the eluate from sand medium and the extracts from embryo and endosperm of seeds exposed to cold, sucrose seems to be the major component. The exudation rate of this sugar is high from the beginning of cold exposure, being accompanied by a rise in its content in the embryo and endosperm. Therefore sucrose synthesis, proved in fragments of maize endosperm by WANNER (1958) is not inhibited in the endosperm by low temperatures. The increase in sucrose content of embryo cannot as yet be unequivocally explained by the function of the scutellum, where the site of sucrose synthesis from glucose and its translocation to the embryo was found (EDELMAUN *et al.* 1959). During the period of experiments at low temperatures the glucose and fructose content in the embryo remained very low.

The lower the temperatures to which the caryopses were exposed, the higher was the initial rise in raffinose content in the embryo and the slower was the decline later on. It seems probable that analogically to the appearance of raffinose in wheat shoot apex when the growth of plants was inhibited (TELTSCHEROVÁ 1962) these changes in raffinose exosmose are connected with the inhibition of sugar utilization for the growth of embryo at low temperatures.

Even without direct evidence it seems probable to suppose that large amounts of sugars exosmosed from maize caryopses distinctly inhibited by low temperatures in germination, represent easily assimilable energetic material for the growth and activity of pathogenic and parasitic microorganisms in the spermosphere. In this way our hypothesis makes possible a causal explanation of complex interactions between higher plants and microorganisms.

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ELIŠKA VEDRALOVÁ, V. SEGEŤA, Výzkumný ústav rostlinné výroby, Praha-Ruzyně: **Změny v obsahu cukrů a jejich exosmosa z obilek kukuřice vzhledem k chladuvzdornosti** — Biol. Plant. 12 : 265—274, 1970.

Byly sledovány změny obsahu rozpustných cukrů v jednotlivých orgánech (embryo, endosperm a perikarp) obilek kukuřice, vystavených vlivu teplot 24°, 10° a 6° C. Současně byly chromatograficky analyzovány eluáty z písku, v kterém byly vysazeny a po různou dobu za uvedených teplot ponechány karyopse kukuřice.

Bylo zjištěno, že čím nižší teplotou jsou zrna ovlivněna, tím větší množství sacharosy, glukosy, fruktosy a rafinosy a jiných cukrů z obilek do doby jejich klíčení difunduje do prostředí. Průběh exosmosy i změn obsahu cukrů v jednotlivých částech karyopse ukazuje, že nízké teploty neinhibují hydrolýzu rezervních polysacharidů, ale brzdí především využití produktů hydrolýzy pro růst embrya. Je vyvozeno, že na exosmose se podílí nejen povrchové části endospermu, ale též embryo. Za chladu značně vysoký výdej cukrů z obilek vysvětluje kausálně v takových podmínkách výrazný rozvoj a aktivitu patogenních a parazitických mikroorganismů, jež způsobují značnou mortalitu semen a klíčenců při vzcházení.