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ROOT EXUDATES OF PLANTS

V. KINETICS OF EXUDATES FROM BEAN ROOTS AS RELATED TO THE PRESENCE OF RESERVE COMPOUNDS IN COTYLEDONS

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SUMMARY

Changes in the amount and composition of root exudates from young bean plants were investigated. The data were compared with those obtained from plants deprived of cotyledons or of true leaves. With intact plants, it was found that the exudation gradually decreased up to the 15th day of cultivation. Thereafter, the exudation increased. In plants deprived of cotyledons, the decrease in exudation in comparison with intact plants began on the nineteenth day after planting. The decrease in exudation from intact plants and plants without cotyledons is explained by the eventual exhaustion of reserve compounds localized in the cotyledons, which are used in the formation of true leaves. In agreements with the above explanation, it was found that the roots of plants deprived of true leaves released higher quantities of exudates from the eleventh to the twentyfourth day.

The kinetics of exudation of the total amount of compounds exuded from intact plants did not correspond completely to the kinetics of exudation of particular compounds or of groups of compounds. For example, the increase exudation of α-amino and imino nitrogen on the third day of cultivation resulted primarily from an increased exudation of isoleucine and glutamic acid, while the exudation of glycine, serine, threonine, and valine remained constant for fifteen days after planting. The exudation of reducing compounds decreased after the third day of cultivation.

INTRODUCTION

Changes in the exudation of different compounds by roots of cucumbers occurred during the early stages of growth. These changes were related to changes in the nutrition of the plants, primarily to the change from utilization of reserve compounds in the cotyledons to that of organic compounds produced by photo-

synthesis [15]. Changes in the quality of root exudates were found also in bean during the early stages of growth, the germinating seeds and young roots exuded peptides that stimulated the growth of a phytopathogenic bacterium, *Xanthomonas phaseoli* var. *fuscans* [16]. Roots of older plants did not exude these biologically active peptides. Qualitative differences in the composition of seed and root exudates were also demonstrated in a variety of other plants [13].

The purpose of the present communication is to describe quantitative and qualitative changes in root exudates during the early stages of growth of bean and to demonstrate that the observed changes may result from an exhaustion of the reserve compounds of the cotyledons and from an increased amount of nutrients supplied by true leaves to the roots.

MATERIALS AND METHODS

Cultivation of plants and isolation of root exudates

Seeds of bean (*Phaseolus vulgaris* L., cultivar 'Veltruska Saxa') were washed with tap water and surface sterilized for 20 min with a 0.1% solution of mercury dichloride. Excess $HgCl_2$ was removed from the seeds by repeated washings with sterile distilled water. The seeds were germinated on wet filter paper (Whatman No 1). After three days, sterile germinated seeds were planted in washed, sterile, wetted silica sand, and the plants were maintain edat 20°C.

To determine whether exudation patterns in this medium differed from a medium containing inorganic plant nutrients, plants were simultaneously cultivated in sand wetted with a Hellriegel solution. After eleven days of cultivation, one-third of the plants was deprived of cotyledons, leaves and primordia were removed from another one-third, and the remaining plants served as control.

Exudates of germinating seeds were collected by washing the filter papers. Exudates from older plants were obtained by washing the sand at four-day intervals, and the plants were replanted into a new sand medium at the same time. Care was taken to avoid damage to roots: a thick suspension was prepared by adding sterile distilled water to the sand and the whole plant was removed, washed, and transplanted in a new container. Filter papers and sand were washed thoroughly with sterile distilled water, and the washings were centrifuged to remove particles such as epidermal cells and root-hair debris, paper, or sand. After concentration, *in vacuo*, the exudates were lyophilized. Microorganisms were controlled in the media during the experiments, and contaminated containers were discarded. The experiments were terminated 32 days after planting.

Analysis of root exudates

The amount of exudates excreted during each four-day interval was determined gravimetrically. A more detailed analysis was performed on samples taken on the 3rd, 7th, 15th, 24th, and 32nd day. α-amino and imino nitrogen were determined according to Rosen [6] and reducing compounds according to Somogyi [9] and Nelson [3]. Reducing sugars were determined by measuring the size and intensity of spots on chromatograms using a photocolorimeter (Direktschreiber mit Integrator; VEB medizinische Fabrik, Berlin). The results are presented in relative units. Methods used for paper partition chromatography of amino acids and sugars have already been described [11 14]. Amino acids were determined quantitatively, after separation by two-dimensional chromatography, by treatment with ninhydrin and cadmium acetate, followed by exposure of the chromatograms in a dessicator above sulphuric acid for 24 hours. The intensity of eluates from detected spots were measured in a spectrophotometer (Prema; Presná mechanika) at 550 nm. The results were calculated either per 1000 plants or per 1 g exudates.

RESULTS AND DISCUSSION

In a preliminary experiment it was found that plant roots cultivated in sand wetted with distilled water released about twice as much exudates as when wetted with Hellriegel nutrient solution. However, the ratio between the amount of root exudates from plants cultivated in the two media was always constant during the different stages of plant growth, indicating that the level of exudation was influenced to the same extent. On the basis of these results, exudates obtained during cultivation of plants in distilled water were used as the standard to determine the relative ratios of different components excreted during different stages of plant growth, especially as the higher concentrations of salts in the nutrient solution prevented good separation of spots during paper partition chromatography.

Quantitative changes in the exudates from plants cultivated in sand wetted with distilled water are presented in Fig. 1. The amounts exuded from control plants and from plants deprived of their cotyledons decreased up to the 15th day of cultivation and then increased. In plants deprived of true leaves, the amount of exudates increased immediately after their removal (11th day). Beginning on the 24th day, the amounts exuded by roots of plants without cotyledons or leaves decreased, whereas, in the control plants, an increase continued. Generally, the amount of exudates

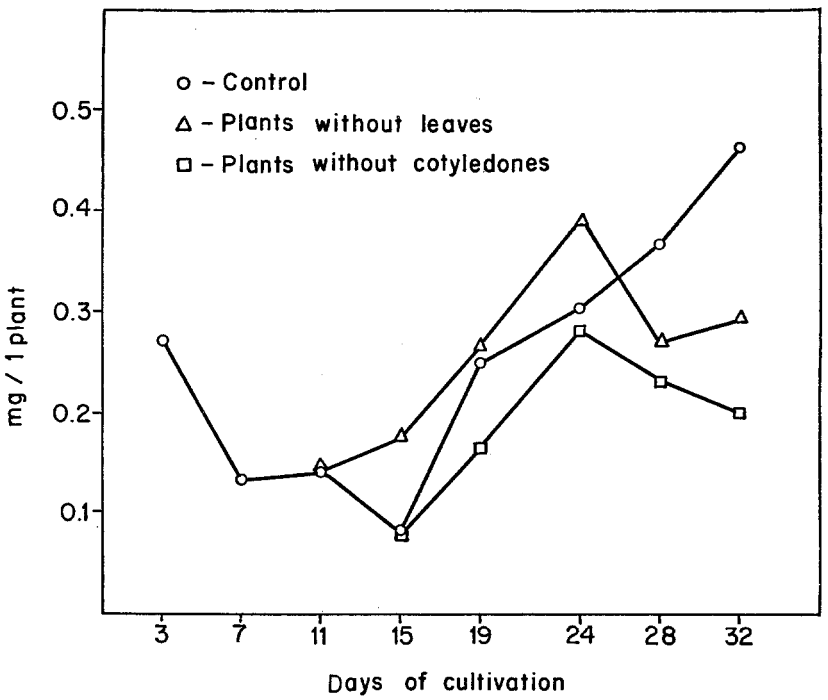

Fig. 1. Kinetics of exudation of root exudates in intact plants, plants deprived of leaves and primordia, and plants deprived of cotyledons.

from plants without cotyledons was always lower and from plants without true leaves higher than from control plants up to the 24th day.

On the basis of these results it was assumed, that plant roots are nourished predominantly by reserve compounds in the cotyledons up to the 24th day of growth and that the decrease of the amount of root exudates between the 3rd and 15th day of growth was associated with the formation of true leaves, which are also initially nourished by these reserve compounds. Due to the removal of the true leaves, the flow of nutrients from the cotyledons to the roots increased, and the amount of root exudates also increased. Conversely, the amount of root exudation decreased below the level of the control plants when the cotyledons and thus the reserve compounds were removed.

The amount of α-amino and imino nitrogen exuded by the

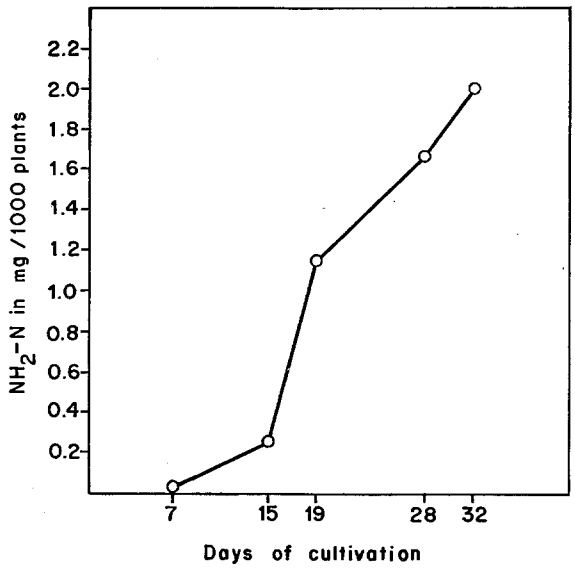

Fig. 2. Kinetics of exudation of α-amino and imino nitrogen.

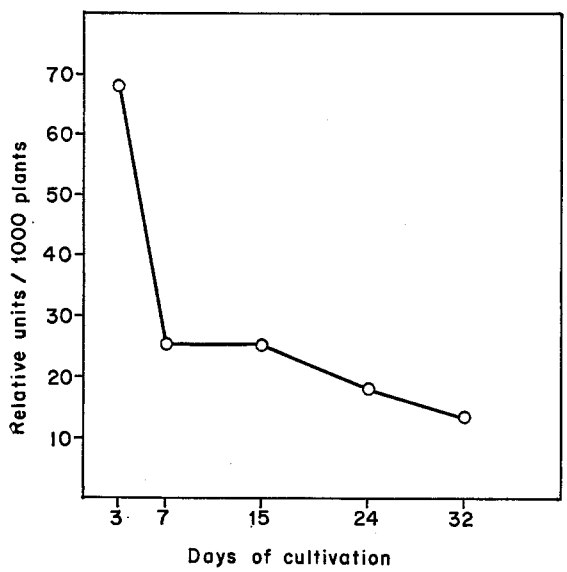

Fig. 3. Kinetics of exudation of reducing sugars.

control plants increased (Fig. 2), whereas the amount of reducing sugars decreased (Fig. 3) during the growth of the control plants.

The amount of reducing compounds exuded by intact plants changed only slightly during 32 days of growth (Table 1). Plants

TABLE 1

Dynamics of exudation of reducing compounds by root of bean (in mg)

Days	Intact plants		Plants without leaves		Plants without cotyledones	
	1000 plants	1 g exudate	1000 plants	1 g exudate	1000 plants	1 g exudate
4– 7	9.14	69.37	–	–	–	–
16–19	12.93	47.50	8.62	33.75	5.29	31.87
25–28	8.83	23.75	4.35	16.25	2.03	8.75
29–32	9.81	21.25	4.38	15.00	3.02	15.00

deprived of true leaves usually exuded lower amounts. A greater decrease occurred in plants deprived of cotyledons. When the data were expressed on the basis of 1 g of root exudates, the amount of reducing compounds exuded steadily decreased and was lower from plants deprived of true leaves or cotyledons.

In agreement with the data presented in Fig. 2, the kinetics of the exudation of individual amino acids were similar (Fig. 4), with the exception of glutamic acid the exudation of which decreased beginning at the 24th day. Isoleucine was exuded most, followed by glutamic acid (the first 24 days), threonine, valine, serine, aspartic acid, and glycine. Fig. 5 shows the amounts of exuded amino acids expressed on the basis of 1 g root exudates. Expressed in this way, the decrease in the exudation of glutamic acid is even more pronounced. The decrease in the exudation of glutamic acid may influence the growth of certain species of bacteria in the rhizosphere of young beans, as it has been shown that this is the only amino acid that can be utilized by the phytopathogenic bacterium, *Xanthomonas phaseoli* var. *fuscans* [12]. Furthermore, this pathogen disappears out of the bean rhizosphere when the exudation of glutamic acid decreases [10]. On the 24th day, serine and aspartic acid decreased slightly, whereas the amounts of other amino acids remained constant or increased slightly with further growth.

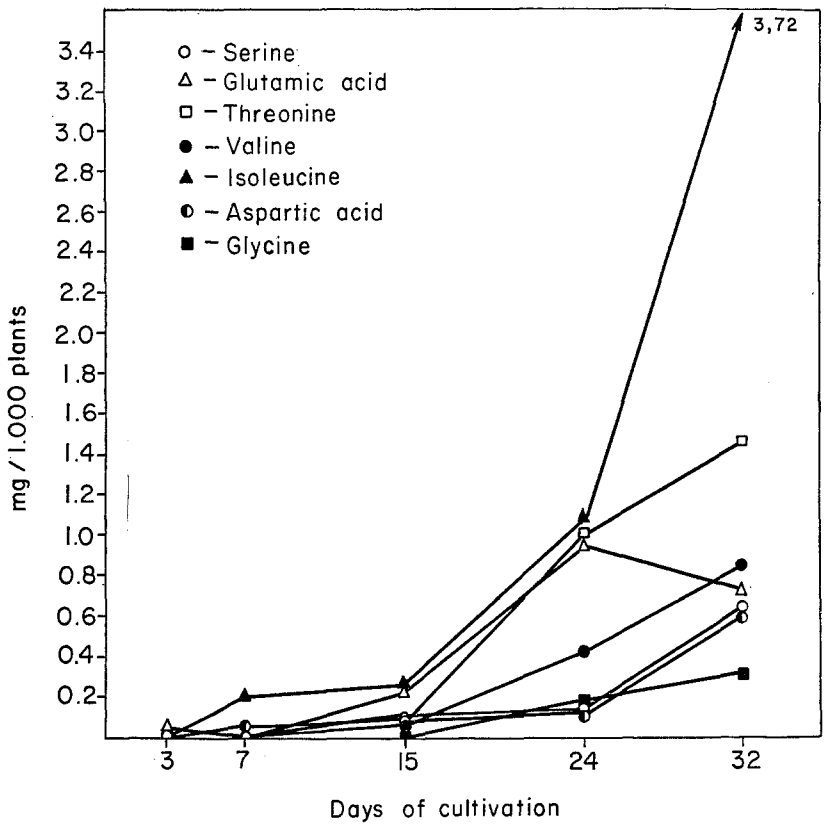

Fig. 4. Kinetics of exudation of certain amino acids.

In plants deprived of cotyledons the exudation of all amino acids decreased (with the exception of isoleucine, where the decrease started later (Fig. 6). With the exception of aspartic acid and glycine, the decrease lasted for more than 24 days.

In plants deprived of true leaves, the exudation of four amino acids increased (isoleucine, threonine, aspartic acid, valine) and slight decrease was observed in three cases, particularly in the case of glutamic acid (Fig. 7). Isoleucine decreased after 24 days, and a less pronounced decrease was observed with aspartic acid.

Qualitative and quantitative changes in the composition of exuded reducing sugars occurred during the first stages of growth (Fig. 8). Fewer spots were detected from 3-day-old plants than from 7-day-old plants. However, one oligosaccharide, excreted by 3-day-

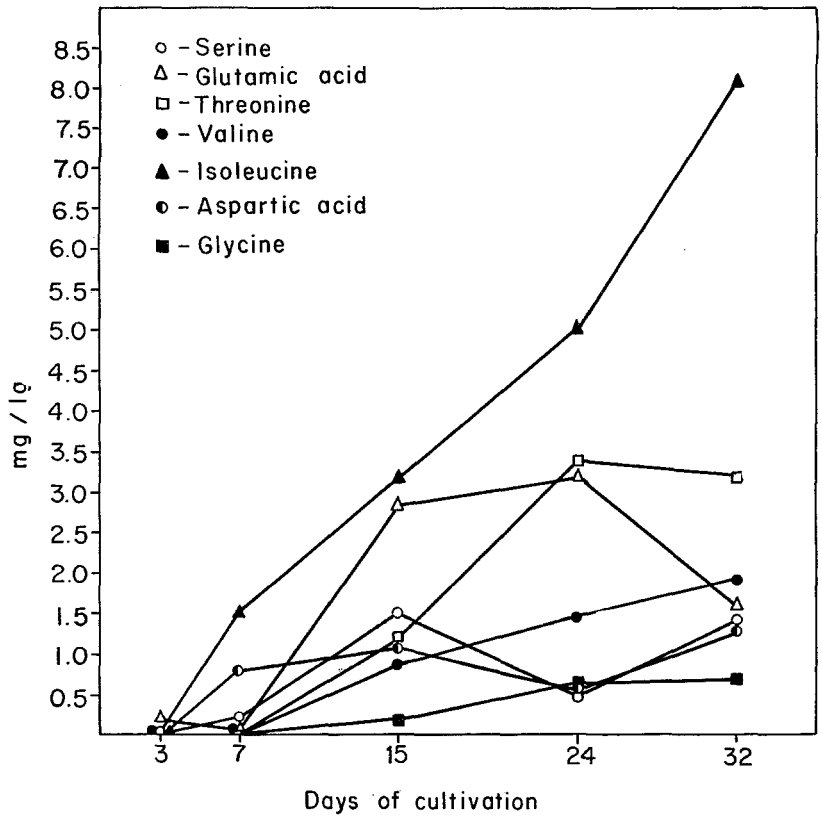

Fig. 5. Kinetics of exudation of certain amino acids (in mg/l g root exudates).

old plants, was not detected in later stages of growth. Seven-day-old plants also exuded xylose and two unidentified sugars, the sugar with the higher R_f value being a keto sugar. The unidentified sugar (spot number 13) was not detected in exudates from 15-day-old plants. Only quantitative differences between control plants and plants deprived of cotyledons or true leaves were found in 15-day-old plants. Fewer spots were found in 24-day-old plants, and a decrease in the exudation of individual sugars also occurred. Plants deprived of cotyledons did not release any reducing sugars, whereas plants deprived of true leaves exuded more sugars and in greater amounts than did control plants. Only a few sugars (three from control plants and two from plants deprived of cotyledons and true leaves) were detected in 32-day-old plants.

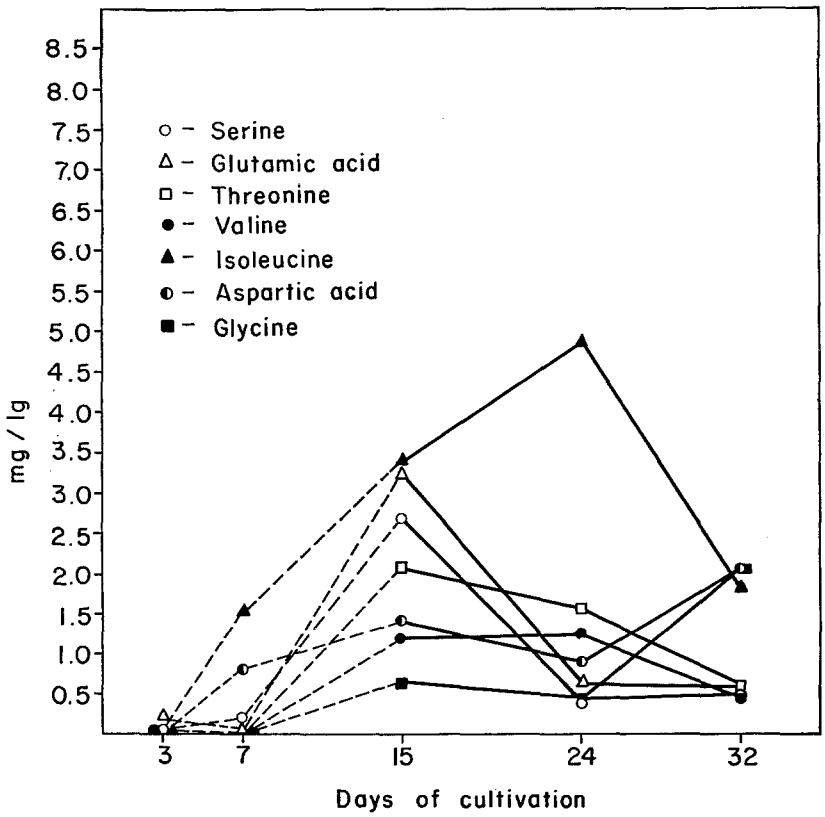

Fig. 6. Kinetics of exudation of certain amino acids in plants deprived of cotyledons (in mg/l g root exudates).

Qualitative and quantitative differences in the exudation of keto sugars could be observed during the early stages of growth (Fig. 9). Three-day old plants exuded two oligosaccharides, with a keto group, and fructose; 7-day old plants exuded only oligosaccharides and a unidentified keto sugar (not shown in Fig. 9; see Fig. 8); whereas, 15-day old plants exuded raffinose, sucrose, fructose, and an unidentified keto sugar. Twenty four- and 32-day-old plants did not exude any keto sugar.

Exudates of germinating seeds and young roots of bean were analyzed earlier [4 8 13]. Sampling of root exudates from plants cultivated for 4-day periods in a fresh medium, throughout the early vegetative period, made it possible to determine, in more

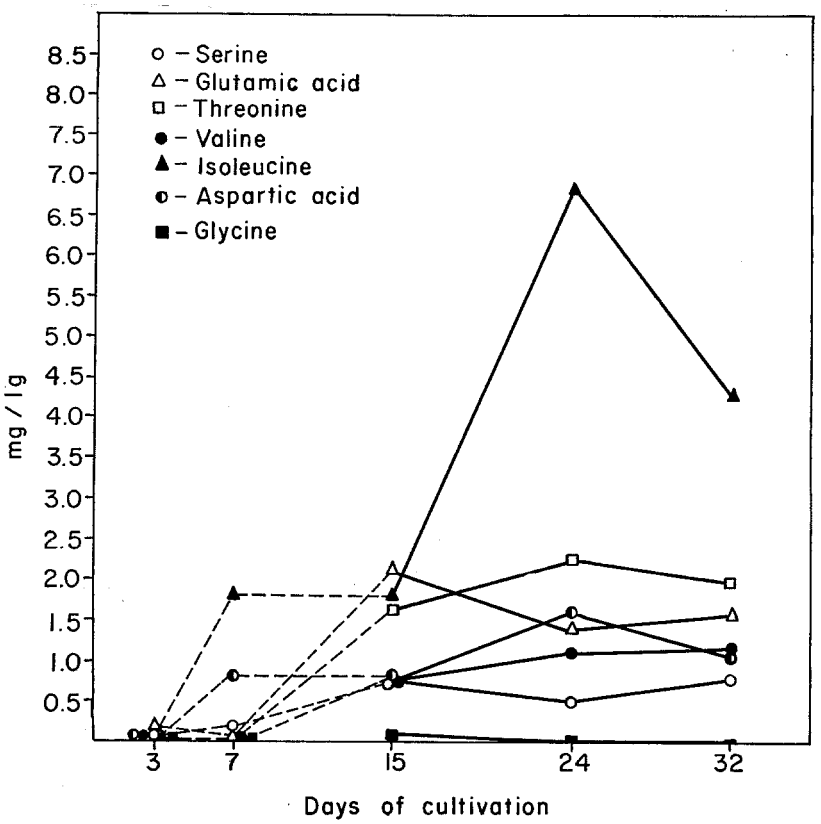

Fig. 7. Kinetics of exudation of certain amino acids in plants deprived of true leaves (in mg/l g root exudates).

detail, changes in the quantitative and qualitative composition of the root exudates. These changes are rather pronounced and occur over relatively short time intervals. These changes probably exert considerable influence on the colonization of the rhizosphere by microorganisms. Certain phytopathogenic fungi (*e.g.* species of *Fusarium* [7], *Pythium* [1]) as well as some bacteria [10], find suitable conditions surrounding germinating seeds and young roots. During the later periods of plant growth, conditions become unsuitable for many of these pathogens and a change in the colonizing fungal biota occurs, as shown by Peterson [5]. The relatively rapid changes in the qualitative and quantitative composition of root exudates during the early periods of plant growth may be caused by

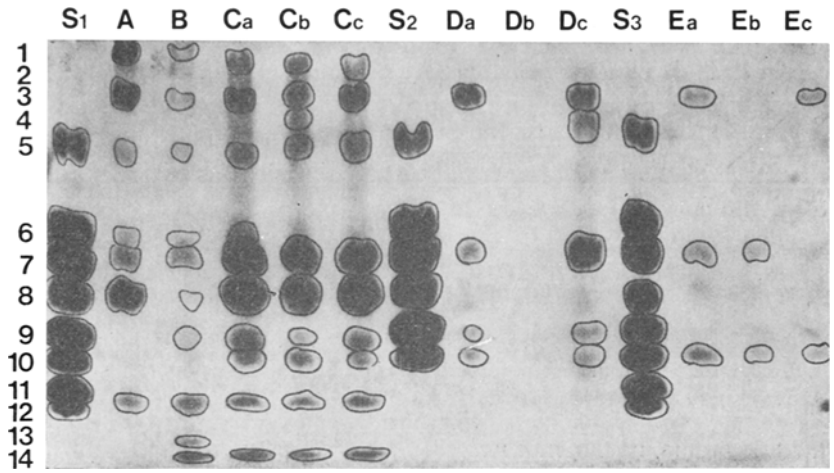

Fig. 8. Reducing sugars during growth of bean. Intact plants (a), plants deprived of cotyledons (b), and plants deprived of leaves and primordia (c); A – three days', B – seven days', C – fifteen days', D – twenty days', E – thirtytwo days' plants; S = standards; 1–4 = oligosaccharides; 5 = maltose; 6 = galactose; 7 = glucose; 8 = fructose with arabinose; 9 = xylose; 10 = ribose; 11 = rhamnose; 12 = deoxyribose; 13 = unidentified; 14 = an unidentified keto sugar.

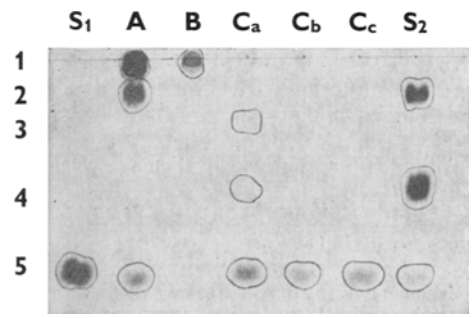

Fig. 9. Keto sugars during growth of control plants (a), in plants deprived of cotyledons (b), and of leaves and primordia (c); A – three days', B – seven days', C – fifteen days' plants; S = standards; 1–2 = oligosaccharides; 3 raffinose; 4 = sucrose; 5 = fructose.

changes in nutrition of the roots by organic compounds from the upper plant parts, as was shown by the qualitative and quantitative changes in root exudates from plants deprived of true leaves or of cotyledons. Furthermore, a bacterium, *Xanthomonas phaseoli* var.

fuscans, that colonizes the surface of germinating seeds and young roots and disappears from the rhizosphere during later stages of plant growth, remained on the surface of roots of plants deprived of true leaves for substantially longer time periods [2]. This phenomenon was explained by a greater supply to the roots of organic compounds from the cotyledons.

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REFERENCES

1 Agnihotri, V. P. and Vaartaja, O., Effect of seed exudates of *Pinus resinosa* on the germination of sporangia and on the population of *Pythium irregulare* in the soil. Plant and Soil **32**, 246–249 (1970).
2 Lasík, J. and Staněk, M., The changes in the population of microorganisms of the bean rhizosphere after the colonization of the root surface by bacteria *Xanthomonas fuscans*. (*In Czech*) Proc. of 11. Ann. Meet. Cz. Microbiological Society 1974.
3 Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**, 375–380, (1944).
4 Ontlová, Anna and Vančura, V., Composition of seeds and root exudates of beans (*Phaseolus vulgaris* L.). Annual meeting of Czechoslovak Association of Microbiology, Prague. Abstract Fol. Microbiol. **12**, 398 (1967).
5 Peterson, E. A., Seed-borne fungi in relation to colonization of roots. Can. J. Microbiol. **5**, 579–582 (1959).
6 Rosen, H., A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. **67**, 10–15 (1957).
7 Schroth, M. N. and Snyder, W. C., Effect of host exudates on chlamydospore germination of the bean root rot fungus *Fusarium solani* f. *phaseoli*. Phytopathology **51**, 389–393 (1961).
8 Schroth, M. N., Tousson, T. A. and Snyder, W. C., Effect of certain constituents of bean exudates on germination of chlamydospores of *Fusarium solani* f. *phasoli* in soil. Phytopathology **53**, 809–812 (1963).
9 Somogyi, M., A new reagent for the determination of sugars. J. Biol. Chem. **160**, 61–68 (1945).
10 Staněk, M. and Lasík, J., The occurence of microorganisms parasitizing on the over-ground parts of plants in the rhizosphere. Plant Microbes Relationships, pp. 300–307, Publ. House Czechoslovak Acad. Sci., Prague (1965).
11 Vančura, V., Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. Plant and Soil **21**, 231–248 (1964).
12 Vančura, V. and Hanzlíková, Anna, Nutritional requirements of *Xanthomonas phaseoli* var. *fuscans*. Fol. microbiol. (Prague) **14**, 27–31 (1969).

13 Vančura, V. and Hanzlíková, Anna, Root exudates of plants IV. Differences in chemical composition of seed and seedlings exudates. Plant and Soil **36**, 271–282 (1972).
14 Vančura, V. and Hovadík, A., Root exudates of plants II. Composition of root exudates of some vegetables. Plant and Soil **22**, 21–32 (1965).
15 Vančura, V. and Hovadík, A., Composition of root exudates in the course of plant development. Plant Microbes Relationships, pp. 21–25, Publ. House Czechoslovak. Acad. Sci., Prague (1965).
16 Vančura, V., Staněk, M. and Hanzlíková, Anna, Effect of seed and root exudates on the growth of *Xanthomonas phaseoli* var. *fuscans*. Fol. Microbiol. (Prague) 14, 23–26 (1969).