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ROOT EXUDATES OF PLANTS

II. COMPOSITION OF ROOT EXUDATES OF SOME VEGETABLES

by V. VANČURA and A. HOVADÍK

Department of Soil Microbiology, Institute of Microbiology,
Czechoslovak Academy of Sciences, Praha 6,
and
Vegetable Research Institute, Olomouc

INTRODUCTION

The composition of the root exudates of barley and wheat in the initial phases of growth has been described in a previous paper [8]. As far as the dynamics of the rhizosphere effect are concerned however, it is necessary to obtain information on possible changes in the nature of the root excretions during the development of the plant. From the technical point of view this problem is a rather difficult one and probably for this reason only two references concerning this aspect can be found in the literature. Katznelson, Rouatt, and Payne [2] analyzed the root exudates of a number of plants for amino acids and sugars at two stages of growth namely seedling and flowering. With the exception of barley, the quantity of amino nitrogen did not show any substantial change. Rovira [5] investigated the amino acids in the root exudate of tomatoes, subterranean clover and phalaris during the first two fortnight's growth and found that a smaller amount of amino acids was exuded in the second than in the first period. The present paper deals with the composition of the root exudates of cucumber, turnip cabbage, tomatoes and red pepper, and for the last two plants two growth phases are compared.

MATERIAL AND METHODS

Root exudates

Seeds of the following plants were used: cucumber (*Cucumis sativus* L.) variety "Bílská", turnip cabbage (*Brassica oleracea* var. *gongylodes* L.)

variety "Dětěnická modrá", tomato (*Solanum lycopersicum* L.) variety "Imun" and red pepper (*Capsicum annuum* L.) variety "Hodonínská". To obtain root exudates in the seedling stage the plants were cultivated in sterile silica sand according to the method described earlier [7]. The isolation of the root exudates in the later stages of growth was carried out as follows:

Tomatoes and red pepper were grown hydroponically in gravel until they reached the fruiting stage when the gravel was removed with tweezers and the root system thoroughly rinsed with distilled sterile water. The plants were then cultivated in a wide necked flask in a sterile nutrient solution until a callus had formed on the injuries (if any). The nutrient solution was then replaced by sterile distilled water which after one week was suctioned off.

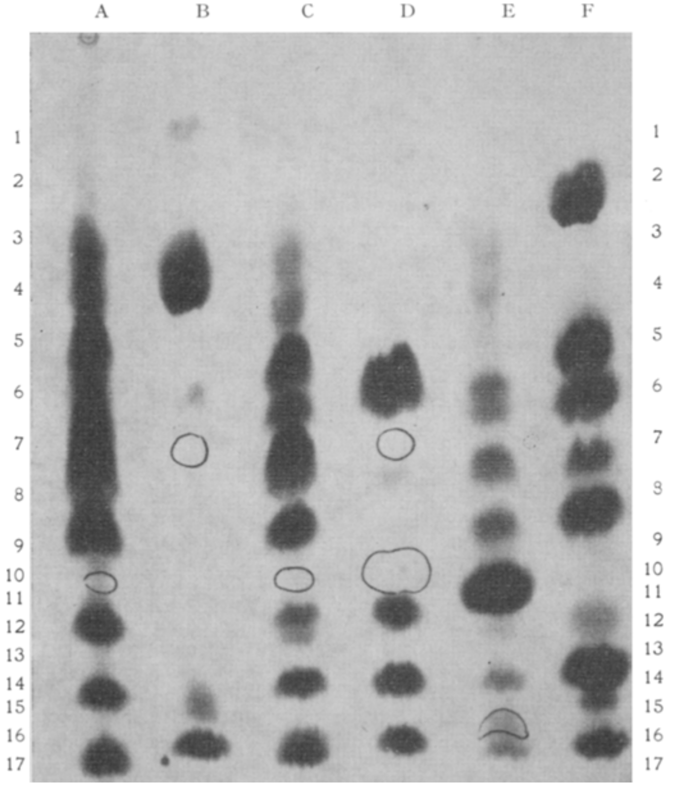

Fig. 1. Amino acids in root exudates of vegetables.

A – tomato, B, D, F – standards, C – red pepper, E – cucumber.
1 – ornithine, 2 – cysteic acid, 3 – asparagine, 4 – aspartic acid, 5 – glutamine, citruline, serine, 6 – glycine, 7 – threonine, γ-hydroxy-α-amino pimelic acid, 8 – glutamic acid, α-amino adipic acid, 9 – α-alanine, 10 – proline, 11 – β-pyrazolylalanine, 12 – γ-amino butyric acid, 13 – tyrosine, 14 – methionine, valine, 15 – tryptophan, 16 – phenylalanine, 17 – leucine, isoleucine.

Analysis of the root exudates

The same procedures and methods were used as described in our previous paper [7] except that the indol and phenol derivatives were not isolated by extraction but analyzed directly in the fractions adsorbed on the ion exchangers. A number of phenolic substances of a non-acid character were sorbed onto the cation exchanger (Zerolit 225) in the H^+-cycle whilst the anion exchanger (Zerolit FF) in the carboxylic cycle also adsorbed acids with aromatic rings. The analysis of gibberellin like substances was carried out by the method described by Vančura [6].

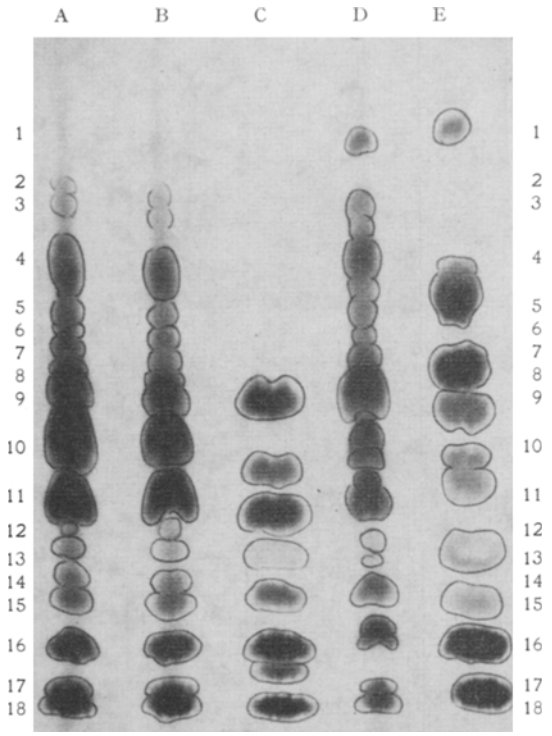

Fig. 2. Amino acids in root exudates of vegetables.

A, B – turnip cabbage, various batches, C, E – standards, D – red pepper in the fruiting stage.

1 – cystine, 2, 3 – un-identified, 4 – ornithine, asparagine, 5 – aspartic acid, 6 – glutamine, 7 – citruline, 8 – serine, 9 – glycine, 10 – threonine, glutamic acid, 11 – α-alanine, 12 – un-identified, 13 – proline, 14 – γ-amino butyric acid, 15 – tyrosine, 16 – methionine, valine, 17 – phenylalanine, 18 – leucine, isoleucine.

RESULTS AND DISCUSSION

In the present work analyses were made of amino, hydroxy-, di and tricarboxylic acids, sugars, phenolic acids, and other phenolic substances as well as of some plant growth promoting factors.

Nineteen amino acids were found in the root exudates of germinating cucumbers (Fig. 1). The main amino acid was β-pyrazolyl alanine which is also present in the free state in seeds [4].

In turnip cabbage 18 amino acids could be identified, the largest spots being given by glycine, α-alanine, leucine (isoleucine), and methionine (valine) (Fig. 2).

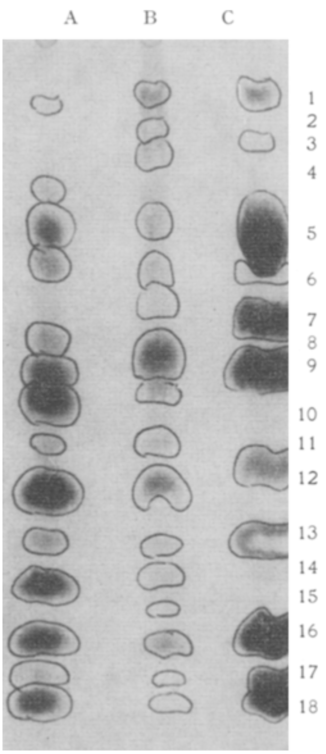

Fig. 3. Amino acids in root exudates of tomato.

A – initial phase of growth, B – fruiting stage, C – standards.
1 – cystine, 2 – ornithine, 3, 4 – un-identified, 5 – asparagine, 6 – aspartic acid, 7 – glutamine, citruline, 8 – serine, 9 – glycine, 10 – threonine, 11 – glutamic acid, 12 – α-alanine, 13 – proline, 14 – γ-amino butyric acid, 15 – tyrosine, 16 – methionine, valine, 17 – phenylalanine, 18 – leucine, isoleucine.

In the root exudate of tomatoes in the initial growth phase 15 amino acids were identified, the most intensively coloured spots being those of glutamic acid, threonine, and α-alanine, while citruline, ornithine, and proline could be detected in traces only. (Figs. 1 and 3). In addition to these tyrosine and three other as yet unidentified nynhydrine-positive substances could be found in the fruiting stage (Fig. 3). The intensity of the colour of the detected spots in this phase was generally weaker than that of the corresponding spots in the initial growth phase although the dry weight of the plants at the fruiting stage was substantially greater. This means that during the vegetation period quantitative changes occur in the distribution of the individual components of the root exudates.

The results for the amino-acid analyses in the initial phases of growth of tomatoes agree with those of Rovira [5]; in addition traces of ornithine, citruline, and proline could be identified.

TABLE 1

Amino acids in the root exudates of cucumber, turnip cabbage, red pepper, and tomato. 1–4 = area of the spots (traces to very large spots)

Amino acid	Cucumber	Turnip cabbage	Red pepper: Initial growth phase	Red pepper: Fruiting stage	Tomato: Initial growth phase	Tomato: Fruiting stage
Cysteic acid	2	0	0	0	0	0
Cystine	1	1	2	2	0	1
Ornithine	3	1	0	0	1	1
Lysine	2	0	0	0	0	0
Asparagine	3	2	1	2	2	1
Glutamine	1	2	2	2	2	1
Citruline	0	1	1	0	1	1
Aspartic acid	3	3	3	2	3	1
Serine	3	2	4	2	2	3
Glycine	3	4	4	2	2	3
α-Amino adipic acid	0	0	1	0	0	0
α-Amino-γ-OH pimelic acid	0	0	2	0	0	0
Glutamic acid	3	2	4	2	2	1
Threonine	3	1	4	2	4	2
α-Alanine	1	4	3	2	4	3
Proline	0	1	3	0	1	1
β-Pyrazolyl alanine	4	0	0	0	0	0
Tyrosine	1	1	0	1	0	1
γ-Amino butyric acid	1	2	2	2	3	1
Methionine (valine)	3	4	3	2	3	2
Tryptophan	1	1	1	1	0	0
Phenylalanine	2	3	3	3	2	1
Leucine (isoleucine)	2	4	3	3	2	1
Not identified	0	2 spots	0	6 spots	1	3 spots

In the red-pepper root exudate 18 amino acids could be identified in the initial growth phase, the most deeply coloured spots beeing those of serine, glycine, threonine, and glutamic acid. The root exudates of this plant also contained traces of asparagine, citruline, α-amino adipic acid, and tryptophane (Fig. 1). Besides tyrosine six other ninhydrine-positive substances could identified and there was a relatively greater quantity of asparagine in the fruiting stage (Tabl. 1).

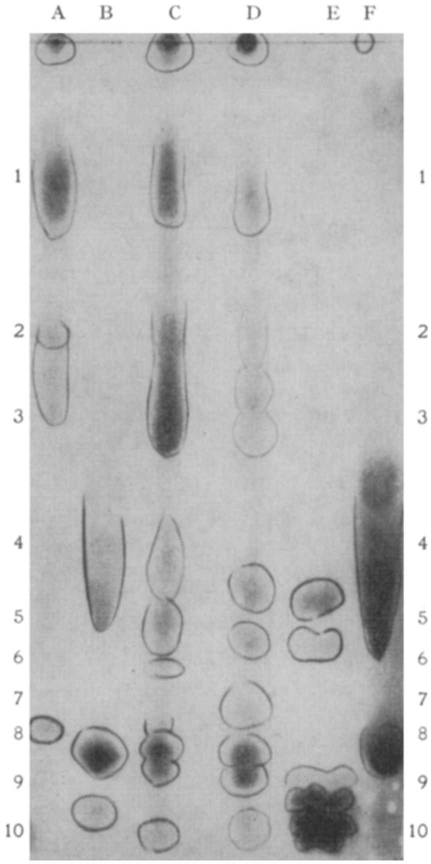

Fig. 4. Organic acids in root exudates of vegetables in initial phases of growth.
A – cucumber, B, E, F – standards, C – tomato, D – red pepper.
1–3 – anorganic acids, 4 – oxalic acid, 5 – citric acid, 6 – malic acid, 7 – glycolic acid, 8 – lactic acid, 9 – succinic acid, 10 – fumaric acid.

Only a small number of organic acids is excreted by cucumber.

In addition to oxalic acid, lactic acid was detected in the root exudates of this plant. (Fig. 4). The root exudates of turnip cabbages contained in addition to these, malic and glycolic acid. (Fig. 5).

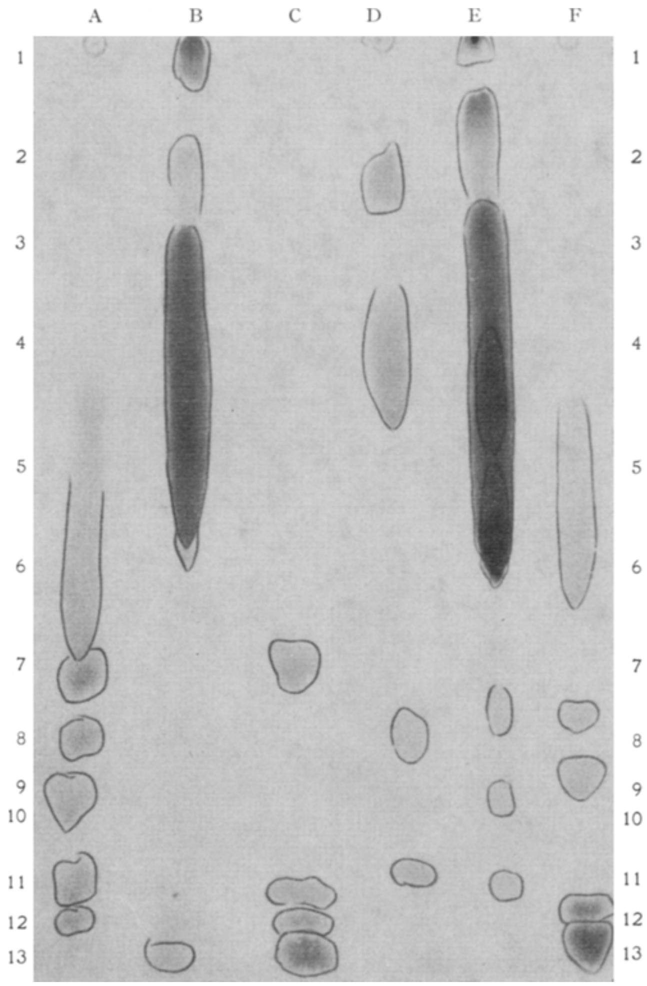

Fig. 5. Organic acids in root exudates of vegetables.
A, C, F – standards, B – red pepper in the fruiting stage, D – tomato in the fruiting stage, E – turnip cabbage in initial phase of growth.
1–5 – inorganic acids. 6 – oxalic acid, 7 – citric acid, 8 – malic acid, 9 – glycolic acid, 10 – lactic acid, 11 – succinic acid, 12 – fumaric acid, 13 –aconitic acid,

Tomatoes and red pepper exude in the initial phases of growth the same organic acids *i.e.* oxalic, citric, malic, glycolic, succinic and fumaric acid (Fig. 4). The same organic acids were identified in tomato root exudates during the fruiting stage but the amount was considerably smaller and some of the acids were present in traces only. (Fig. 5). In red pepper root exudates oxalic, citric, and fumaric acids (Tab. 2) were identified in the fruiting stage while other organic acids found in the initial growth phase could not be detected.

TABLE 2

Organic acids in the root exudates of cucumber, turnip cabbage, red pepper, and tomato (1–4, see Tab. 1)

Acid	Cucumber	Turnip cabbage	Red pepper		Tomato	
			Initial growth phase	Fruiting stage	Initial growth phase	Fruiting stage
Oxalic acid	3	4	3	4	3	3
Citric acid	0	0	2	1	2	1
Malic acid	0	1	2	0	2	2
Glycolic acid	0	1	3	0	3	1
Lactic acid	1	1	2	0	2	2
Succinic acid	0	0	1	1	1	1
Fumaric acid	0	0	2	2	1	1

Fourteen, three, eleven, and nine reducing sugars were detected in the root exudates of germinating cucumber, turnip cabbage, red pepper, and tomatoes respectively. The most intensively coloured spot in cucumber root exudate was that of arabinose, in red-pepper and tomato root exudates that of fructose (Fig. 6). In turnip cabbage root exudate only two oligosaccharides and xylose were detected. The sugars almost disappear from the root exudates in the fruiting stage; only traces of glucose, galactose, and one oligosaccharide could be detected in tomato root exudate while that of red pepper contained only traces of arabinose (Tab. 3).

The distribution of phenolic acids and some other phenolic substances is represented in Tab. 4 and 5. At present the majority of these substances cannot be reliably identified, but it may be concluded from the R_F values and the reactions with various detecting reagents that they are the source of considerable qualitative differences in the composition of the root exudates both between individual growth phases and between plants belonging to the same family.

Among the other substances identified in the root exudates of all samples investigated and in all growth phases was urea. Both

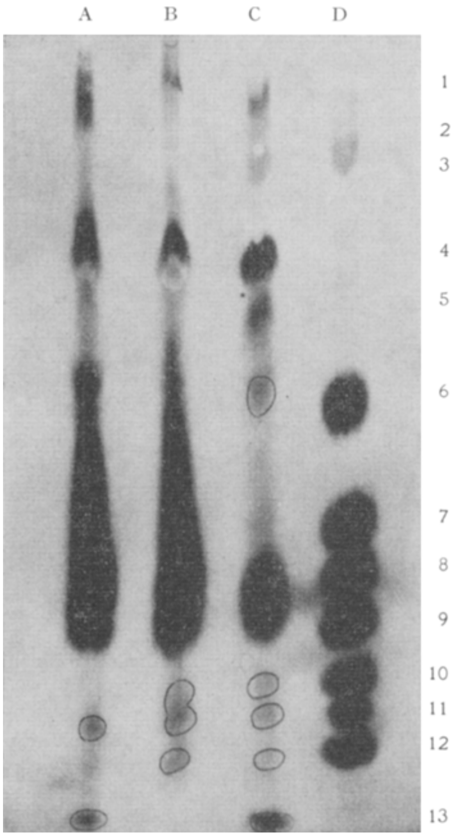

Fig. 6. Sugars in root exudates of vegetables in initial phases of growth.
A – tomato, B – red pepper, C – cucumber, D – standards.
1–5 – oligosaccharides, 6 – maltose, 7 – galactose, 8 – glucose, 9 – fructose, arabinose, 10 – xylose, 11 – ribose, 12 – rhamnose, 13 – desoxyribose.

tomato and red pepper root exudates contain β-indolyl acetic acid as proved by a biological test. A gibberellin-like substance was detected in the root exudate of red pepper in the fruiting stage. It was found that the amount of root exudate in the fruiting stage when calculated in terms of the dry matter content of the plant formed in the same time interval is lower than that in the initial growth phase. It was also confirmed by observation of the dynamics of the rhizosphere microflora of red pepper that in the fruiting stage the population of the root surface decreases to such an extent that an equilibrium with the rhizosphere soil is reached [1]. These

TABLE 3

Sugars in the root exudates of cucumber, turnip cabbage, red pepper, and tomato. Number of spots

Sugar	Cucumber	Turnip cabbage	Red pepper		Tomato	
			Initial growth phase	Fruiting stage	Initial growth phase	Fruiting stage
Oligosaccharides . . .	5	2	5	2	5	2
Maltose	2	0	1	0	1	0
Galactose	1	0	1	0	1	1
Glucose	2	0	2	0	2	1
Arabinose	4	0	1	1	0	0
Fructose	2	0	4	0	4	0
Xylose	1	1	1	0	0	0
Ribose	1	0	1	0	1	0
Rhamnose	1	0	1	0	1	0
Desoxyribose	2	0	1	0	2	0

TABLE 4

Phenolic acids in root exudates of red pepper and tomato

Spot number	R_F*	Red pepper		Tomato	
		Initial growth phase	Fruiting stage	Initial growth phase	Fruiting stage
1	0,05	—	—	orange	—
2	0.09	yellow	brown	—	—
3	0.16	yellow	—	yellow	—
4	0.22	—	—	red	—
5	0.26	red	—	—	—
6	0.27	—	—	yellow	—
7	0.30	—	—	—	red
8	0.33	—	—	red	—
9	0.47	—	—	—	orange
10	0.56	red	—	—	—
11	0.59	—	orange	—	—
12	0.60	—	—	orange	—
13	0.67	—	yellow	—	—
14	0.70	red-violet	—	—	—
15	0.77	—	yellow	—	—
16	0.81	—	—	violet	yellow
17	0.87	—	—	—	orange
18	0.88	—	—	—	yellow

* R_F of spots obtained in the system n-butanol : pyridine : water (14 : 3 : 3). Detection carried out with diazotised *p*-nitroaniline and the colour recorded two hours later.

results agree with those of Krasilnikov [3] who reported that the numbers of microorganisms in the fruiting period was about one third of the maximum reached during the period of intensive plant growth. Hence it appears that a causative relationship exists between the amount and composition of the root exudates and those of the micro-organisms in the rhizosphere.

TABLE 5

Phenolic substances of non-acid character in root exudates of red pepper and tomato

Spot number	R_F	Red pepper		Tomato	
		Initial growth phase	Fruiting stage	Initial growth phase	Fruiting stage
1	0.10	brown	—	—	—
2	0.29	—	yellow	—	—
3	0.34	—	—	—	yellow
4	0.52	red	—	—	—
5	0.56	—	orange	—	—
6	0.80	orange	—	—	—
7	0.91	—	—	—	orange

The qualitative composition of the microflora of the rhizosphere during the development of the plant is in fact not only altered by the nature of the root exudates (differences in nutrition and in the action of physiologically active substance) but also by factors inherent in the micro-organisms themselves *i.e.*, competition for nutrients and antagonism. However, it is assumed here that these changes in the root exudates are basically responsible for changes of the rhizosphere population as a whole.

The analyses made until today allow for the comparison of the root exudates of 6 species of cultural plants from four families. The results suggest that the less phylogenetically related the plants the greater the difference in the composition of the exudate. Plants of the same family have a similar composition: the greatest similarity can be found in the composition and distribution of the organic acids and sugars whereas those of the amino-acids and aromatic substances are not so close.

SUMMARY

Quantitative and qualitative differences are reported in the composition of the root exudates of six plants from four families, barley, wheat [7], cucumber, turnip cabbage, tomato, and red pepper, in the initial stages of growth and for the last two species, also at the fruiting stage. It is concluded that

the less the plants are related phylogenetically, the greater is the difference in the composition of the exudates. These results are discussed in relation to certain aspects of the rhizosphere effect.

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