ROOT EXUDATES OF REVERSIBLY WILTED MILLET PLANTS *(PANICUM MILIACEUM L.)*

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SUMMARY

Exudation of various substances by millet plant roots (*Panicum miliaceum* L.), was compared in plants reversibly wilted and plants that were not affected by a moisture lack. The plants reversibly wilted exuded by 53 per cent more exudates, from which the content of α-aminonitrogen was by 190 per cent higher, of reducing sugars by 113 per cent higher, and of reducing substances by 50 per cent higher. The content of fructose, glutamic acid, α-alanine, valine and leucines increased essentially in root exudates of reversibly wilted plants as compared to the same weight unit.

RÉSUMÉ

Exsudats racinaires du millet après flétrissement réversible

Nous avons comparé l'exsudation de diverses substances par les racines de plantules de millet (*Panicum miliaceum* L.), dans le cas de plantes flétries de façon réversible et de plantes non affectées par un manque d'eau. Les plantules flétries ont exsudé 53 % fois plus d'exsudats que les témoins, leur teneur en azote α-aminé étant supérieure de 190 %, celle des sucres réducteurs de 113 % et celle des substances réductrices de 50 %. Pour une même quantité d'exsudats, les teneurs en fructose, acide glutamique, α-alanine, valine et leucines ont nettement augmenté dans les exsudats racinaires des plantes, après flétrissement réversible.

ZUSAMMENFASSUNG

Die Wurzelausscheidungen der vorübergehend welkenden Hirsepflanzen

Die Ausscheidung verschiedener Stoffe durch Wurzeln einerseits von vorübergehend welkenden Hirsepflanzen und anderseits von Pflanzen mit genügender Wasserversorgung, wurde verglichen. Die vorübergehend welkenden Pflanzen schieden um 53 % mehr Ausscheidungen (davon α-Amino-Stickstoff um 190 %, reduzierende Zucker um 113 % und reduzierende Stoffe um 50 %) aus. Bei der Umrechnung auf die gleiche Gewichtseinheit erwies sich der Gehalt von Fruktose, Glutaminsäure, α-Alanin, Valin, und der Leucine in den Wurzelausscheidungen der vorübergehend welkenden Pflanzen wesentlich höher.

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KATZNELSON, ROUATT and PAYNE (1954) reported an increased exudation of aminoacids in barley, oat, tomato, and soybean plants after desiccation and remoistening. We made use of this phenomenon in some of our experiments, in order to obtain a higher amount of root exudates, that were applied as nutrition and energy source in nutrient media for growing of microorganisms (VANCURA and MACURA, 1961; VANCURA, STANEK and HANZLIKOVA, 1969). This paper is giving data on quantitative and qualitative differences in exudates from plants thus treated.

MATERIALS AND METHODS

PLANT CULTIVATION

Seeds of millet (*Panicum miliaceum* L., varietas " Hanácké ") were sterilized on their surface with mercuric chloride (VANCURA, 1964) and seeded in 3 cm layer of washed sterile silica sand in glas boxes (diameter 10 cm, height 7 cm). The boxes were placed in SHERER's plant growth chamber Model CEL 25-7-HL at 20-22 °C for a fortnight. Half a number of these boxes were opened thereafter and the plants were cultivated for 32 hours at 24 °C till they wilted slightly. The other plants in closed boxes kept a normal turgor. The wilted plants were remoistened (to original weight), and their cultivation continued for another 16 hours (in light for 4 hours, and in oscurity for the rest).

ISOLATION OF ROOT EXUDATES

Over-ground parts of plants together with seed remainders were detached and a sand layer, forming together with roots a block was placed on Büchner funnel and washed with 100 ml distilled water. The eluate obtained was used for washing of another sand block and thus it has been continually enriched by root exudates from other boxes used in experiment. The eluates from both variants (control and reversibly wilted plants) were centrifugated (4 000 revolutions/min.) and then these pellucid eluates were freezed and lyophilized. The lyophilizate was homogenized in a rubbing bowl to a powder and stored in exicator to be used for various analysis later.

ANALYSIS OF ROOT EXUDATES

Most of the methods used were described in previous papers (VANCURA, 1964; VANCURA and HOVADIK, 1965). α-Aminonitrogen was determined as to ROSEN (1957), reducing sugars by SOMOGYI and NELSON's method (NELSON, 1944; SOMOGYI, 1945). The amount of substances found in the exudates was calculated both per 1 000 plants and per 1 g of exudates obtained.

RESULTS AND DISCUSSION

1) Analytical data on the exudation of plants reversibly wilted and control plants are given in the Table 1. Control plants exuded 50.8 μg, and reversibly wilted plants exuded 77.7 μg of various substances per one plant during 16 days of cultivation. However, it is necessary to take into consideration that the substances

TABLE 1

Some analytical data on root exudates of control and reversibly wilted plants

	Control plants	Wilted plants	Difference in %
Exudation per 1 000 plants (in mg)	50.8	77.7	+ 52.9
Number of plants needed for 1 g of exudates	19 680	12 860	− 34.7
Reducing substances in mg/1 000 plants	2.19	3.30	+ 50.6
Reducing substances in mg/g of root exudates	43.09	42.47	− 1.5
Reducing sugars (relative unit/1 000 plants)	200.0	426.0	+ 113.2
Reducing sugars (relative unit/weight unit)	236.0	349.0	+ 47.9
α-NH_2-nitrogen in mg/ 1 000 plants	0.130	0.377	+ 190.0
α-NH_2-nitrogen in mg/1 g of root exudates	2.55	4.85	+ 90.1

are not only exuded but also resorbed by plant roots. Data are known (MESHKOV, 1950; VANCURA, 1967), that the more frequently the exudates are determined during a certain period, the higher their total amounts is. Therefore, each amount of daily exudation per one plant that was calculated from records obtained in a longer period, can serve only for orientation. The Table 1 shows that plants reversibly wilted exuded almost by 53 per cent more exudates than control plants.

2) Another our experiments showed that a difference in a quantity of exudates liberated decreased with a time of cultivation after wilting and remoistening. When the reversibly wilted plants were remoistened and cultivated for 3 days, their exudation was higher only by 6 per cent. It seems that in addition to others phenomena, an increased resorption of substances liberated before occurred, what was observed already in experiments on so called " cold shock ", reported in a previous paper (VANCURA, 1967). Reversibly wilted plants liberated reducing substances by 50 per cent more, and amino-nitrogen by 190 per cent more than control plants.

3) A calculation of these compounds per 1 g of exudates showed that especially what aminoacids were concerned, a change in their exudation was inadequate to the total increase in exudates. Analysing separately reducing sugars that formed only a part of reducing substances, the striking increase in exudation of reversibly wilted plants was observed; however, a change in exudation of sugars with respect to the total exudation was observed, too. A relative occurrence of particular sugars

TABLE 2

Relative incidence of reducing sugars in 15 mg of root exudates from millet plants (*)

	Control plants		Wilted plants	
	Relative unit	%	Relative unit	%
$Oligosaccharide_1$	14	5.93	21	6.50
$Oligosaccharide_2$ $Oligosaccharide_3$	73	30.93	64	19.82
$Oligosaccharide_4$	17	7.20	30	9.29
Glucose	71	30.09	90	27.86
Fructose + Arabinose	25	10.60	66	20.43
Ribose	17	7.20	28	8.67
Rhamnose	19	8.05	24	7.43
Total	236	100.00	323	100.00

(*) Area and density of the spots were recorded in relative units by " Direktschreiber mit Integrator " (VEB Medizinische Gerätefabrik, Berlin).

in the total amount of sugars changed too (Tab. 2). Glucose and some of oligosaccharides were liberated in reversibly wilted plants in relatively lesser amount, and in contrast, fructose was exuded in larger amount than in control plants. Relative representation of other sugars almost didn't change. An increased exudation of fructose, as compared to plants growing under normal conditions, was observed in plants subjected to a " cold shock ", too (VANCURA, 1967). In addition to sugars mentioned in the Tab. 2 also saccharose was found in exudates in both variants.

The changes in the composition of aminoacids in the same weight units of exudates from control and reversibly wilted plants are given in the Tab. 3. It is of a great interest that the quantity of some aminoacids, such as basic amino acids, amides, cysteic acid, homocysteic acid, aspartic acid, serine, glycine, etc... didn't change; some of them (threonine, proline, tyrosine, γ-aminobutyric acid, phenyl-

TABLE 3

Relative incidence of aminoacids in 3 mg of root exudates from millet plants

Aminoacids	Control plants	Wilted plants
Cysteic acid	2	2
Homocysteic acid	2	2
Ornithine	2	2
Lysine	2	2
Asparagine	2	2
Glutamine	2	2
Aspartic acid	4	4
Serine	2	2
Glycine	2	2
α-Aminoadipic acid	1	1
Glutamic acid	2	4
Threonine	1	2
α-Alanine	2	5
Proline	1	2
Tyrosine	1	2
γ-Aminobutyric acid	4	5
Valine (Methionine)	2	5
Methionine sulphoxide	1	1
Phenylalanine	1	2
Leucine, isoleucine	2	4
unidentified	1	2

1 - 5 = area of spots (from traces to very large spots).

alanine) changed a little, whereas the exuded amount of some aminoacids, such as glutamic acid, α-alanine, valine, methionine, leucines increased essentially. The most striking increase was observed in α-alanine, valine and glutamic acid.

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