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Production of carboxylates (C-A)[1] by young sugar-beet plants grown in nutrient solution

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Summary

An experiment was carried out with sugar-beet grown in a nutrient solution to investigate whether decarboxylation takes place in the roots of young sugar-beet plants. Decarboxylation, balanced by uptake of anions in excess of cations from the nutrient solution, were not detected. Only a small amount of carboxylates reached the root system. The reduction of nitrate was almost equal to the carboxylate production. This reduction of nitrate per gram of leaf per day decreased rapidly with time. The supposition is made that the high oxalate content in the leaves might account for the decrease in nitrate reduction.

Introduction

The uptake of nitrate nitrogen by plant roots and its reduction within the plant results in production of an equivalent amount of carboxylates (Dijkshoorn, 1968). In experiments with perennial rye-grass (*Lolium perenne* L.), Dijkshoorn recovered only 1 eq of carboxylates of the 2.5 eq originally produced by the reduction of 2.5 eq of nitrate per kg of dry matter. The missing amount of 1.5 eq was decarboxylated (probably in the root), the bicarbonate released being balanced by uptake of anions in excess of cations from the nutrient solution.
Houba's data (unpublished) on sugar-beet (*Beta vulgaris* L.), sufficiently supplied with nitrate, show that the nitrate equivalent of organic nitrogen equals the amount of carboxylate equivalents. His data refer to sugar-beet plants grown in a field experiment and harvested when the formation of beets had already taken place.
An experiment was set up to investigate whether young sugar beet plants will show the process of decarboxylation supposed to take place in perennial rye-grass. This experiment was carried out with nutrient solution, during the period in which practically no beets are formed.

Experimental

Diploid sugar-beet seeds (No P2167) were sown in a thin layer of gravel on sieves that were placed on top of one-litre polyethylene pots. After germination the roots grew through the gravel on the sieve and entered a well-aerated nutrient solution.

[1] The term (C–A) represents the amount of inorganic cations minus that of inorganic anions both expressed in meq per kg of dry matter.

Table 1 Chemical composition of the nutrient solution

Component	*Concentration (meq/l)*
Na	2
K	4
Ca	2.5
Mg	4
H_2PO_4	1
NO_3	6
Cl	3
SO_4	2.5
Trace elements (except Fe) according to Hoagland	
Fe–EDTA	35 mg/l

The composition of the nutrient solution is given in Table 1. To cope with expected shortages the nutrient solutions were renewed at regular intervals. One plant was grown in each pot. The experiment was carried out in a climate-controlled growth cabinet. The daily light period was 14 hours, the temperature was 25 °C during the light period and 17 °C during the dark period, the relative humidity was 80 to 90 %. Plants were harvested after 30, 36, 39, 43, 46 and 51 days counting from the day of germination, and divided into three sections: leaf blades (including the midribs), petioles and beet + roots. Of each section fresh and dry weights were recorded and the material was finely ground and analysed for Na, K, Ca, Mg, H_2PO_4, NO_3, Cl, SO_4, total N, organic acids (i.e. oxalic acid, citric acid, fumaric acid, succinic acid and malonic acid).
Subsamples were analysed after digestion in concentrated sulphuric acid and hydrogen peroxide in the presence of salicylic acid. In this digest, the Na, K and Ca contents were determined flamephotometrically, the H_2PO_4 content colorimetrically, the Mg content by atomic absorption and the nitrogen content (total N) by the micro-Kjehldahl procedure. Other subsamples were extracted with water (0.5 g of plant material and 50 ml deionised water: shaking time 2 hours). In this extract the nitrate-content was determined with an Orion nitrate electrode, the Cl content was determined coulometrically with a chloro counter and the SO_4 content colorimetrically. The total content of carboxylates (C–A) was calculated by subtracting the sum of inorganic anions from the sum of inorganic cations (de Wit et al., 1963). The carboxylates were converted into organic acids by decationization with a H^+ sulphonic acid resin and these were resolved by partition chromotography and quantitatively determined with an
were resolved by partition chromotography and quantitatively determined with an automatic titrator. All values were calculated on the basis of oven-dried material (70° C).

Results and discussion

Dry-matter production
During the experiment the dry weights showed a lineair increase in dry matter with time (see Fig. 1A). From the fourth harvest onwards the weight of roots (including the beet) expressed in percentage of the total dry weight, increased significantly (Table 2). This indicates the beginning of the stage of beet formation.

The balance of uptake and utilisation of inorganic ions
The most important components which may characterize the balance of uptake and

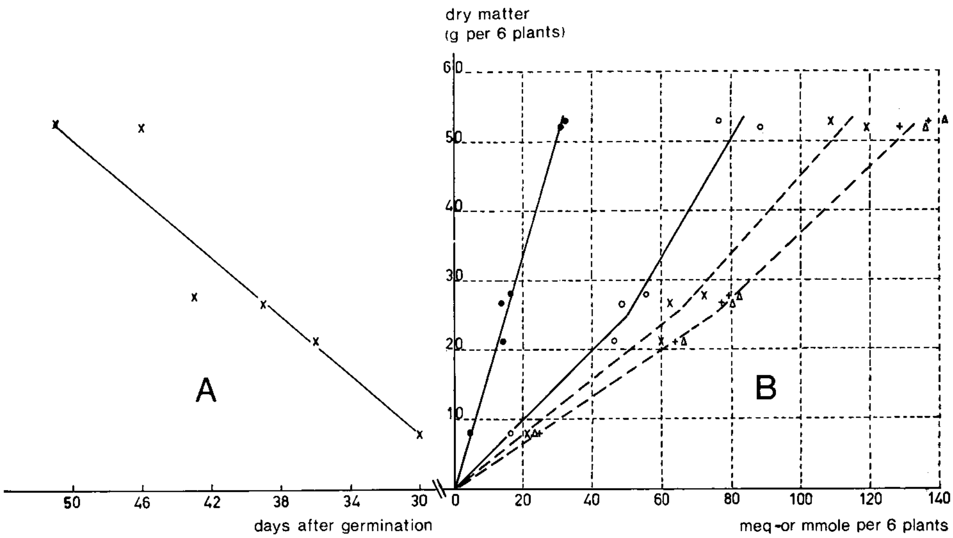

Fig. 1 A. Production of dry matter; B. organic N, (C–A) and carboxylates; ● mobile acids; o oxalic acid; × carboxylates; + organic N; △ (C–A)

utilisation of ions, are given in Table 4 and Fig. 1B. The table shows that the figures for (C–A), and for organic nitrogen are about equal (difference ± 3 %). Generally the amount of organic nitrogen is somewhat lower than the amount of (C–A) (organic N expressed as its ion equivalent of nitrate). Considering the amount of organic sulphur the (C–A) is somewhat lower than the sum of organic N and organic S. (According to Dijkshoorn and van Wijk (1967) organic S is about 6 % of organic N on ion equivalent basis.)

Since the amounts of (C–A) and organic N are about equal, there is no release of carboxylates resulting from NO_3^- and SO_4^{2-} reduction in the leaves in sugar-beet plants and consequently no excess uptake of inorganic anions over inorganic cations. Of the total amount of (C–A) formed by reduction of nitrate and sulphate, only 6–8 % is present in the beet-root system. When only the roots represent the uptake system, a part only of the 6–8 % mentioned above reaches this uptake system. So the process Dijkshoorn et al. (1968) supposed to take place in perennial rye-grass does not take place in sugar-beet plants, or could not be demonstrated in sugar-beet plants because of the very small amount of carboxylates that reaches the uptake system.

Table 2 Dry-weight production (g/6 plants) of sugar-beet plants and the distribution of the produced dry weight over leaf blade, petiole and roots (beet included)

Days after germination	*Leaf blade*	*Petiole*	*Root and beet*	*Total*	*Roots (% of total dry weight)*
30	5.32	1.4	1.24	7.96	16
36	13.6	4.2	3.6	21.4	17
39	16.6	5.4	4.8	26.8	18
43	17.6	5.6	4.8	28.0	17
46	29.0	10.6	12.6	52.2	25
51	28.8	10.65	13.65	53.1	26

Table 3 Content of cations, anions and organic substances in parts of the sugar-beet plants in meq/kg dry matter (total N and organic N in mmole/kg dry matter)

Days after germination	*Dry weight (g/6 plants)*	*Ca*	*Na*	*K*	*Mg*	*C*	H_2PO_4	NO_3	*Cl*	SO_4	*A*	*I*	*II*	*III*	*IV*	*V*	*Total N*	*Organic N*	*Total Z (I–V)*	*(C—A)*
Leaf blade																				
30	5.32	400	570	2637	1194	4801	197	410	177	68	852	296	100	2808	20	16	4000	3590	3240	3949
36	13.6	392	595	2430	1540	4957	152	530	197	72	951	416	72	3016	36	16	4140	3610	3556	4006
39	16.6	410	590	2400	1422	4822	146	480	168	64	858	284	164	2628	28	48	3950	3470	3104	3964
43	17.6	252	557	2466	1500	4775	162	490	186	81	919	220	100	2880	36	32	3860	3370	3268	3856
46	29.0	372	630	2220	1536	4758	118	570	270	76	1034	348	96	2716	12	40	3780	3210	3212	3724
51	28.8	370	567	2430	1460	4827	142	275	169	66	652	328	208	2384	4	24	3380	3105	2948	4175
Petiole																				
30	1.4	176	392	3630	494	4692	162	2040	760	64	3026	352	740	808	44	8	3320	1280	1952	1666
36	4.2	136	430	3480	516	4562	132	1900	883	125	3240	324	684	756	12	64	3330	1430	1840	1322
39	5.4	142	408	3225	634	4409	135	1820	893	69	2917	584	196	604	28	24	3245	1425	1436	1492
43	5.6	156	349	3060	612	4177	154	1760	801	86	2801	1036	148	572	12	56	3250	1490	1824	1376
46	10.6	114	469	3060	606	4219	130	1820	1085	58	3093	380	608	548	4	8	2950	1130	1548	1156
51	10.6	140	327	2283	568	3318	140	1160	1063	60	2423	348	708	364	12	40	3010	1850	1472	895
Roots and beet																				
30	1.24	262	127	1880	378	2647	313	846	262	33	1454	504	28	332	20	8	3560	2714	892	1193
36	3.6	278	112	1670	420	2480	290	720	346	35	1391	568	4	500	20	8	3215	2495	1100	1089
39	4.8	254	116	1690	432	2492	279	810	354	35	1478	260	8	340	4	24	3130	2320	636	1014
43	4.8	194	90	1660	398	2342	282	780	301	51	1414	452	28	364	20	16	3010	2230	880	928
46	12.6	186	117	1520	344	2167	227	570	342	37	1176	420	20	284	36	16	2440	1870	776	991
51	13.6	206	107	1130	334	1777	242	376	222	42	882	260	12	292	20	8	2410	2034	592	895

I = citric acid; II = malic acid; III = oxalic acid; IV = succinic and malonic acid? (traces); V = fumaric acid? (traces).

Table 4 Organic N, (C–A) and carboxylates in the combined plant parts

Days after germination	(C–A) (meq)	Organic N (mmole)	Carboxylates	
			(meq)	(% of (C–A))
30	24.8	24.3	21.0	84.7
36	64.0	64.1	60.1	93.9
39	78.8	76.4	62.3	79.1
43	80.0	78.4	71.9	89.9
46	132.8	128.7	119.4	89.9
51	141.9	136.9	108.7	76.6

A reason for this relatively low amount of carboxylates reaching the root system is perhaps the very low solubility of a large portion of these carboxylates. Illustrative for the situation in sugar-beet plants are the high contents of Ca and Mg (Table 3) together with high contents of oxalates (the solubility of these oxalates is low).
The change in slope of the lines in Fig. 1B at the fourth harvest corresponds with the moment at which the plants start forming beets (Table 2). In the first period of growth when no beets are formed, on the average 3000 mmole of organic N and 2000 meq of oxalate are produced per kg of dry matter. During the second growth period when formation of beets takes place, these values are 2100 mmole and 1200 meq, respectively. The differences in organic nitrogen and oxalate content between the two periods amount to 900 mmole and 800 meq per kg of dry matter, respectively. The rate of production of 'mobile' carboxylates — mostly citrate and malate — does not change and is maintained at a value of about 600 meq per kg of dry matter.

Nitrate reduction

From the data on leaf-blade weights (Table 2), one can calculate an average production of about 1.1 g of leaf blade per 6 plants per day. In the same way one can calculate an average NO_3^- reduction of about 6.0 meq NO_3^- per 6 plants per day (Table 4: data on organic N). On the 30th day after germination, there are 5.3 g leaf blades per 6 plants. With these data the calculations given in Table 5 can be made. Every value recorded in Fig. 2 is based on a calculation as given above. From this figure it is clear that nitrate reduction decreases rapidly with time. This decreasing nitrate reduction in the presence of sufficient amounts of available nitrate in the tissue (Table 3) results in a decreasing production of oxalate (Fig. 1B). Whether a lower nitrate reduction causes a lower oxalate production or whether an accumulation of oxalate causes a lower nitrate reduction was not investigated. It seems, however, quite

Table 5 Calculation of average weight and NO_3^- reduction of leaf blades

Days after germination	Leaf blade (g) per 6 plants	Average weight of leaf blade (g)	NO_3^- reduction per gram leaf blade per day (meq)
30	5.3	5.85 (30–31)	$\frac{6.0}{5.85} = 1.03$
31	5.3 + 1.1	6.95 (31–32)	$\frac{6.0}{6.95} = 0.86$
32	6.4 + 1.1		

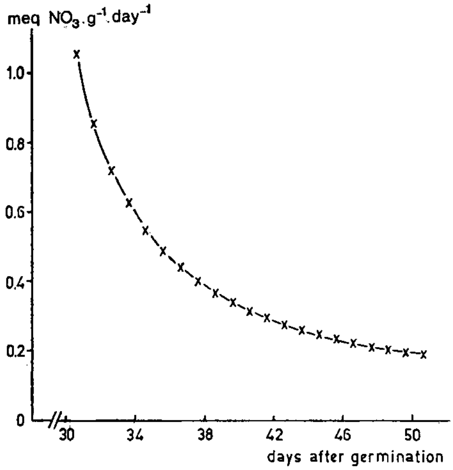

Fig. 2 Reduction of nitrate (meq) per leaf blade per day (calculated from produced organic N and produced dry matter of leaves)

likely that there is a maximum to the quantity of oxalate a leaf can contain.
From the calculated quantity of carboxylates (C–A), 85 % is recovered in the organic-acid analyses (Table 4). The missing 15 % cannot be explained with the water-soluble carboxylates. Van Tuil (1965) assumes the presence of other carboxylates which are difficult to extract.

Conclusions

In contrast with rye-grass, an excess uptake of inorganic anions over inorganic cations accompanied by a decarboxylation of carboxylates, could not be demonstrated in young sugar-beet plants. This could be the result of a relative low amount of carboxylates reaching the roots.
In sugar-beet plants the (C–A) content is not constant during the growth period studied in this experiment.
The nitrate reduction per gram leaf per day decreases rapidly with time.
The decrease in nitrate reduction is accompanied by decrease in oxalate production.

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