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Dependence of Nitrate Reduction in Green Plants on Reducing Substances

ARTTURI I. VIRTANEN and SYNNÖVE SAUBERT-v. HAUSEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

The decisive role of reducing substances (ascorbic acid, reductone, and SH-compounds) in the reduction of nitrate and nitrite in green plants has been ascertained during some years in this laboratory by growth experiments with pea seedlings and wheat embryos isolated from cotyledons and endosperm [1]. Similarly, it has been noted that a cotyledonless pea, growing in a nutrient solution without combined nitrogen but inoculated with an effective strain of pea *Rhizobium*, grows only if reducing substances are added to the nutrient solution although white nodules are formed without any addition [2]. On the other hand, in a nutrient solution containing ammonium nitrogen, the growth is good even without reducing substances. Used in greater amounts they will even injure the growth [1].

A quantity of 100—120 mg ascorbic acid per plant given in three doses at intervals of about a week produced in best cases with cotyledonless pea in nitrate nutrient solution a growth almost comparable with that of normal pea. *Cotyledons were thus successfully replaced by ascorbic acid.* On the basis of the results it seems that the most important function of the cotyledons and the endosperm is to produce reducing substances to the plant at the early stage of its growth. The action of the reducing substances affects probably primarily the iron metabolism [2].

Nitrate reduction in the cotyledonless pea plants is presented by data in Table 1.

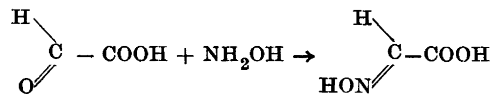
It appears from the results that when a cotyledonless pea grows in nitrate nutrient solution without ascorbic acid, nitrate-N accumulates in the plant in very great amounts, in extreme cases up to 20 % of total N. The growth of plants is then nil or very poor. In parallel experiments, the addition of ascorbic acid, which produces a vigorous growth, lowers the nitrate content of the plants very much, to less than 1 % of total N. The percentage of nitrite nitrogen also drops sharply by the addition of ascorbic acid, thus nitrite, too, is accumulated in abundance though less by far than nitrate. It is interesting to note, that when a cotyledonless pea seedling grows in the dark in a nutrient solution containing cane sugar and nitrate (a modified method of

Table 1. Cotyledonless pea (Torsdag) in sterile culture system. Cotyledons removed 5 days after moistening the seeds. One seedling in 1 litre suction flask. Mineral nutrient solution with 243 mg $NaNO_3$ (40 mg N) and 1 mg Fe as $FeCl_3$ per litre. Sterile seedling transferred to suction flask 21. XI. 1949. Experiment interrupted 15. XII. 1949, 13. I. 1950 and 3. II. 1950. Ascorbic acid (Aa) added in three doses, 50 mg each, to each flask 21. XI, 10. XII, and 20. XII. Artificial light.

No.	N-source and additions	Date of harvest	Fresh weight g	Total N, mg	N in plant extract, mg	NO_3—N γ	NO_3—N % of total N	NO_2—N γ	NO_2—N % of total N	NO_2—N % of NO_3—N
1	40 mg NO_3—N	15. XII	0.8	3.2	—	510	15.9	3.7	0.12	0.7
2	—„—	15. XII	0.3	1.7	—	200	11.8	3.5	0.20	1.8
3	—„—	13. I	0.4	1.8	0.9	340	18.8	2.6	0.14	0.8
4	—„—	13. I	1.7	5.1	0.8	250	5.0	2.8	0.06	1.1
5	—„—	13. I	2.1	6.8	1.4	225	3.3	4.6	0.07	2.0
6	40 mg NO_3—N + 150 mg Aa	15. XII	3.0	8.8	—	70	0.8	10.0	0.12	14.3
7	—„—	13. I	10.7	44.1	6.7	130	0.3	4.2	0.01	3.2
8	—„—	13. I	13.8	42.1	8.4	160	0.4	4.2	0.01	2.6
9	—„—	13. I	4.0	15.6	4.7	70	0.4	4.8	0.03	6.9
10	—„—	3. II	9.2	41.5	10.5	225	0.5	9.0	0.02	4.0
11	—„—	3. II	6.1	26.8	5.3	170	0.6	5.6	0.02	3.3

Bonner [3]), nitrate and nitrite are not accumulated to any noteworthy extent, although even then an addition of ascorbic acid promotes the growth. This implies that the mode of nitrate reduction may be different in the light (in green parts of the plant) and in the dark (in roots), as Burström [4] has suggested*. However, it must also be considered that the seedlings utilize nitrate comparatively little in this system, inspite of ascorbic acid addition.

* The hypothesis is attractive that the reduction of nitrate in the green parts of plants proceeds partly only as far as, *e.g.* to hydroxylamine, and this then reacts with compounds containing the CO-group, *e.g.*, glyoxylic acid, produced by the CO_2-assimilation. The reaction



is very intense according to the determinations made in this laboratory, and besides, the enzyme system which oxidizes glycolic acid to glyoxylic acid is present according to Tolbert and Burris (*J. Biol. Chem.* **186** (1950) 791) in the green parts of plants but not in roots. It is activated by light in intact etiolated plants. The said authors suggest that glycolic acid and the enzymatic system for its oxidation may be concerned with photosynthesis. According to Benson and Calvin (*J. Exp. Bot.* **1** (1950) 63) glycolic acid is one of the earliest products to contain C^{14} after a short exposure of plants to $C^{14}O_2$.

It is seen from Table 2 that the cotyledonless peas did not grow at all with nitrite-N without ascorbic acid while in most cases and addition of ascorbic acid produced a fairly good growth.

Table 2. Parallel experiments with those in Table 1, but $NaNO_2$ as the source of nitrogen.

No.	N-source and additions	Date of harvest	Fresh weight g	Total N, mg	N in plant extract, mg	NO_3—N γ	NO_3—N % of total N	NO_2—N γ	NO_2—N % of total N	NO_2—N % of NO_3—N
1	40 mg NO_2—N	15. XII	0.40	3.1	2.1	170	5.49	7.6	0.25	4.5
2	—,,—	3. II	0.44	5.2	2.9	70	1.35	4.2	0.08	6.0
3	—,,—	3. II	0.08	1.9	0.6	45	2.37	7.2	0.37	16.0
4	—,,—	3. II	0.08	3.6	1.9	50	1.38	4.6	0.13	9.2
5	—,,—	3. II	0.07	2.8	—	48	1.71	5.6	0.20	11.7
6	40 mg NO_2—N + 150 mg Aa	3. II	11.36	38.9	10.7	130	0.33	7.7	0.02	5.9
7	—,,—	3. II	13.61	41.9	13.2	155	0.37	10.0	0.02	6.5
8	—,,—	3. II	1.85	10.2	2.5	95	0.93	8.5	0.08	9.0
9	—,,—	3. II	5.30	22.8	5.2	160	0.70	4.9	0.02	3.1

The recent finding of Hewitt *et al.*[5] of the notable decrease in the ascorbic acid content of plants caused by the lack of molybden is very interesting and is apt to throw more light on the action of molybden. In our experiments the nutrient solution has contained molybden in so great amounts that a further addition has caused no increase in the growth. To make certain, it has been a rule during the last two years to add to the nutrient solution a mixture of trace elements containing also molybden.

The effect of the addition of reducing substances has been examined not only with plants whose cotyledons or endosperm were removed at an early stage of germination but also with normal plants. When pea plants, whose cotyledons were not removed, were grown in sterile nitrate nutrient solution with and without ascorbic acid, it was noted that the plants administered with ascorbic acid contained noticeably less nitrate nitrogen than those grown without ascorbic acid. In a previous paper[6] we have recorded results of experiments, in which the quantity of NO_3-N in the nutrient solution has been comparatively low, *viz.*, 15, 30 and 60 mg per plant.

When the nutrient solution contains plenty of nitrate nitrogen (150—300 mg N per plant per litre nutrient solution) an addition of ascorbic acid also causes an increase in the crop. When growing without ascorbic acid the peas then regularly develop chlorosis. The plants supplied with ascorbic acid soon overcame the slight chlorosis that first developed and the growth continued normally. Results of these experiments are given in Table 3.

Table 3. Normal pea plant (Torsdag) in nitrate nutrient solution. Sterile culture system, 1 litre suction flasks. Experiment started 5. V. 1950. 1 mg Fe as $FeCl_3$ per litre. Ascorbic acid (Aa) given in 50 mg doses May 5, 12, 28, and June 9. Date of harvest appears from the table.

No.	N-source and additions	Date of harvest	Fresh weight g	Total N, mg	N in plant extract, mg	NO_3-N γ	NO_3-N % of total N	NO_2-N γ	NO_2-N % of total N	NO_2-N % of NO_3-N
3	150 mg NO_3-N		10.7	31.0	7.6	1700	5.5	15.0	0.05	0.9
4	150 mg NO_3-N + 200 mg Aa	June	12.7	38.8	10.7	1400	3.6	9.8	0.03	0.7
5	300 mg NO_3-N	1—3	8.3	36.3	13.5	1900	5.2	23.0	0.06	1.2
6	300 mg NO_3-N + 200 mg Aa		13.4	44.0	13.3	1500	3.4	9.5	0.02	0.6
9	150 mg NO_3-N		20.9	76.5	14.8	5190	6.8	57.7	0.08	1.1
10	150 mg NO_3-N + 200 mg Aa	June	35.7	127.6	32.8	4880	3.8	20.7	0.02	0.4
11	300 mg NO_3-N	12—16	8.8	36.6	6.4	5290	14.4	12.9	0.04	0.2
12	300 mg NO_3-N + 200 mg Aa		26.8	102.7	26.5	9000	8.8	—	—	—
15	150 mg NO_3-N		19.6	87.3	18.4	5990	6.9	38.8	0.04	0.6
16	150 mg NO_3-N + 200 mg Aa	June	32.9	142.1	33.5	1395	1.0	27.0	0.02	1.9
17	300 mg NO_3-N	26—27	15.9	67.3	14.5	7620	11.3	21.5	0.03	0.3
18	300 mg NO_3-N + 200 mg Aa		26.5	98.4	27.8	4900	5.0	20.0	0.02	0.4

A similar effect on the nitrate content of plants as produced with an addition of ascorbic acid is also brought about with an addition of sodium sulphide to the nutrient solution. In open pot cultures with either quartz sand or soil as a root support, the nitrate content of plants is also often lowered by the effect of sulphide.

Our idea of the function of the reducing substances in the plants is that they lower the redox potential and effect in this way on the uptake and assimilation of iron. As a result of the disturbance in the iron metabolism the reduction of nitrate is checked or weakened. Chlorosis, which appears in pea plants in our nutrient solution with plenty of nitrate but no reducing substances, is an indication of the disturbance in the iron metabolism.

SUMMARY

The addition of reducing substances to the root support promotes nitrate reduction in normal pea plants. In sterile cultures the effect is strong and distinct at least in the experimental conditions used. In open pot cultures the effect is also often to be found. The primary influence, however, affects the uptake and metabolism of iron.

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