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BRIEF COMMUNICATION

The influence of ammoniates on $^{14}CO_2$ assimilation in flax

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Abstract

A 1 μM solution of ammoniates $[ZnCu(NH_3)_n]^{2+}(CO_3)^{2-}$ was inserted into a cut shoot of flax with the transpiration stream of water. Analysis of the ^{14}C content after $^{14}CO_2$ assimilation by the shoot showed that ammoniates increased radioactive label contents in the tissues (especially in the young leaves and stem). In the leaves the higher sucrose to hexoses ratio, an increased radioactivity of glycerate and malate and decreased incorporation of ^{14}C into oligosaccharides and pigments were observed. These effects were more pronounced in the young leaves. Spraying of plants with 20 mM solution resulted in an increase of plant height and leaf number.

Additional key words: apoplast, assimilate transport, *Linum usitatissimum*, photosynthesis, physiologically active substances, plant productivity.

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It is often found that high nitrogen nutrition leads to increased shoot to root mass ratio, which can be negative in the case of root crops such as cassava (Cruz *et al.* 2004). In the previous investigations it was shown that the reason of a relative decrease of assimilate export from leaves in plants fertilized with nitrogen was not a decrease of sucrose synthesis as a result of diverting photosynthesis primary products to the formation of nitrogen-containing compounds in the leaf, but sucrose hydrolysis in the apoplast (Chikov *et al.* 2001). Since in the apoplast sucrose hydrolysis occurs mainly by acid invertase it should be expected that apoplastic fluid acidity must influence this process. The validity of this conclusion was proved in the experiments with artificial changing the extracellular space acidity. Measurements of $^{14}CO_2$ fixation rates and ^{14}C content in the apoplast in plants transiently placed under a bell-jar with the atmosphere containing either HCl vapour or NH_3 vapour showed (Chikov 1987) the opposite actions of the investigated factors on photosynthetic rate and the ^{14}C content in the leaf apoplast. The acidification suppressed $^{14}CO_2$ fixation by the leaf, with the labelled assimilates being accumulated in the apoplast. Conversely, the ammonia vapours had some stimulating effect on photosynthesis and decreased the ^{14}C-labelled substances content in the apoplast. Thus, to weaken sucrose hydrolysis in the apoplast it is necessary to decrease the extracellular aqueous medium acidity. To make such a situation in the apoplast we decided to use complex compounds with the general formula of $[M(NH_3)n]^{+m} A^{-m}]$. It was supposed that the metal of the complex in the form of a cation surrounded with ammonia would be adsorbed on the cell walls in the apoplast (Blinda *et al.* 1997) and thereby would extrude H^+ out of the aqueous medium increasing the pH value.

Furthermore, the alkalization of the apoplastic fluid may also occur when a part of NH_3 molecules goes out of the complex compounds to their environment, because the complex persists even if a part of NH_3 molecules has released. An increase in apoplastic pH after fumigation of a leaf with NH_3 was observed by Felle and Hanstein (2002). Although in some cases an acidification of medium due to NH_4^+ was found (Bennett *et al.* 2003/4), the influence of NH_3 and NH_4^+ on pH could be different because NH_3 takes H^+ from the medium to form NH_4^+ and in the case of NH_4^+ salts a great role may belong to anion resting after NH_4^+ uptake. In the given paper the results of testing this hypothesis are presented.

Flax plants (*Linum usitatissimum* L. cv. Novotorzhsky) were grown under field conditions in pots containing 7 kg of air-dry gray wood soil, each pot supplied with 1 g N, P and K (as $Ca(NO_3)_2$ and KH_2PO_4). The humidity of the soil was maintained at 70 % of the full moisture capacity. A solution of ammoniates (1 μM) was inserted into a cut shoot of flax (50 - 60 cm high) under the pressure of 10^4 Pa (equal to root pressure). We used plants with

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inserted distilled water as controls. After 40 min of the solution inserting into the shoot a photosynthetic chamber was put on the upper (21 cm) part of the shoot into which $^{14}CO_2$ from a gasholder was delivered using a compressor. After 3 min the shoot part exposed to $^{14}CO_2$ was cut, divided into parts (the young leaves, the mature leaves, the bast and the wood) and fixed with boiling ethanol.

The experiments were carried out in sunny weather from 11:00 to 13:00. The concentration of CO_2 in the chamber was 350 μg g^{-1}, the specific activity 12 MBq dm^{-3}.

The fixed samples were ground in 50 % ethanol and after placing the aliquots of the homogenates on chromatographic paper discs their radioactivities were determined. The ethahol-water-soluble labelled compounds were analyzed using paper chromatography and radioautography. The radioactivity was determined using a liquid scintillation spectrophotometer *Delta-300* (*Tracor Analytic*, Elk Grove Village, IL, USA) taking the counting efficiency into consideration.

To study the influence of ammoniates on growth flax plants (13 - 15 cm high) were sprayed with 20 mM solution. Upon achievement of yellow ripeness stage the plants were taken out of soil and their heights (from the hypocotyls) were measured. Then the stem was divided into three parts: the lower part (up to 15 cm above the epicotyl), the middle part (15 - 60 cm), and the upper part (above 60 cm). In each part of the stem the number of existed leaves was estimated (by the traces on the stem).

The number of biological replications was five. The arithmetic means with standard errors are given in the tables and figures.

Analysis of ^{14}C content in different parts of the shoot showed that ammoniates increased (Fig. 1) the label content in the plant (most of all in the young leaves and stem). An increased incoming of radioactive label to the stem tissues could occur in two different ways: either photosynthesis of the stem chlorenchyma itself increased or labelled carbon appeared in the phloem after its export from the leaves. The latter conclusion was based upon the following reasons. Since flax leaves are small (about 8 mm) and have very short petioles (about 1 mm) it is likely that the labelled assimilates synthesized in leaves can rapidly be transferred to the sieve tubes.

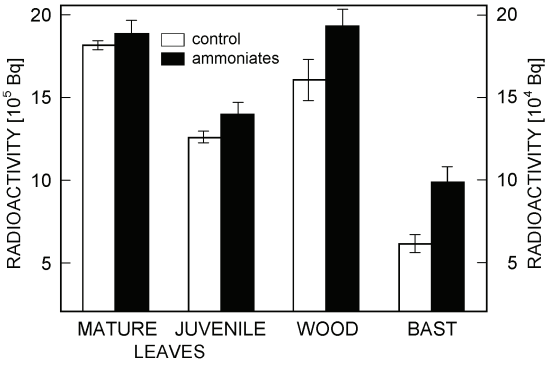

Fig. 1. The influence of ammoniate (1 μM) inserting into the apoplast on the ^{14}C incorporation into different tissues (leaves - *left axis*, stem - *right axis*) in flax after 3 min $^{14}CO_2$ assimilation by the shoot. Means ± SE, $n = 5$.

The study of ^{14}C distribution among the labelled products of the ethanol-water-soluble fraction (Table 1) showed more prominent differences between the control and the experiment in young leaves. In the leaves of the experimental plants the higher labelled sucrose to hexoses ratio, less ^{14}C incorporation into oligosaccharides and pigments as well as an increased radioactivity of glycerate and malate than in control plants were observed.

The increased sucrose to hexoses ratio supports our hypothesis that sucrose hydrolysis decreases under the action of ammoniates and therefore there are prerequisites for its successful transport from the leaf. In this context it is an increased export function of the leaves with which the greater labelled carbon content in the stem may be connected. The same reasons can account for the

Table 1. The influence of ammoniate (1 μM) inserting into the apoplast on ^{14}C distribution among the labelled products of photosynthesis in leaves of different ages after 3 min $^{14}CO_2$ assimilation by a whole flax shoot [% of the water-ethanol-soluble fraction]. Means ± SE, $n = 5$.

Compounds	Young leaves control	experiment	Mature leaves control	experiment
Sucrose	40.1 ± 1.5	45.4 ± 1.7	59.4 ± 1.6	59.3 ± 1.1
Hexoses	8.3 ± 0.6	6.1 ± 0.7	4.3 ± 0.5	3.3 ± 0.2
Sucrose to hexoses ratio	4.8 ± 0.5	7.4 ± 0.6	13.8 ± 1.3	18.0 ± 1.1
Phosphorus esters of sugars	4.5 ± 0.5	3.2 ± 1.1	3.2 ± 0.3	5.2 ± 0.4
Amino acids	30.0 ± 1.7	27.3 ± 1.6	21.1 ± 1.3	19.3 ± 1.2
Serine	9.5 ± 0.4	7.2 ± 0.5	5.7 ± 0.3	4.8 ± 0.3
Glycerate	1.7 ± 0.3	3.6 ± 0.3	0.8 ± 0.1	2.0 ± 0.2
Malate	4.5 ± 0.2	5.6 ± 0.3	1.8 ± 0.1	3.9 ± 0.3
Pigments	4.2 ± 0.3	2.6 ± 0.1	2.7 ± 0.2	2.2 ± 0.2
Oligosaccharides	4.0 ± 0.3	0.6 ± 0.1	2.9 ± 0.2	2.3 ± 0.1
Other compounds	2.7 ± 0.3	5.6 ± 0.5	3.8 ± 0.4	2.5 ± 0.3

increased ^{14}C content in the young leaves, where the labelled carbon (after going out of the stem phloem to the apoplast) can be transported with water in the transpiration stream (Chikov *et al*. 2001).

The less label content in hexoses also explains why there is a lower label content in oligosaccharides in the experimental plants, because hexoses are substrates in oligosaccharide synthesis. The suppression of sucrose hydrolysis by ammoniates prevents carbon of sucrose transported from the lower part of the shoot from being used in synthetic processes in young leaves (including pigment synthesis).

The increased radioactivity of malate and glycerate may be associated with the following processes. Absorption of the ammoniate metal ions on the mesophyll cell walls seems to oppose transport into the apoplast of not only protons but also potassium ions. At the same time K^+ is known (Lips *et al*. 1997) to be transported from roots to leaves with nitrate ion and to return back to the roots for the next portion of nitrate with malate anion. Difficulties (in the presence of ammoniates in the apoplast) in potassium ion going out of the mesophyll cells are likely to result in under-utilization of malate, formed in photosynthesis, for the potassium export. Under these conditions malate accumulates in the cells and its excess after being decarboxylated is converted into glycerate (Table 1).

The lower radioactivity of serine in the experimental plants can be connected with decreased carbon flow into glycollate pathway under the circumstances of enhanced assimilate export from the chloroplasts.

Thus, affecting the leaf apoplast with ammoniates one appears to be able to prevent an excessive (under enhanced nitrogen feeding conditions) sucrose hydrolysis and through this to increase the export function of leaves and, consequently, plant sink organs productivity. The treatment of plants in the fast growth period resulted in an increase in shoot length (86.2 ± 1.6 compared with 81.6 ± 1.2 in control) and leaf number in the middle shoot part that had grown after the treatment with the preparation (23.4 ± 1.0 compared with 18.4 ± 0.7 in control). Leaf numbers in the upper and lower parts were equal to those in control plants. Flax stem grows in length in its upper part. Treated with ammoniates leaves exported photosynthesis products more intensively for both the fiber synthesis process and new leaves formation. The lower part was fully-grown before the treatment, and the upper part grew when the treated leaves began to wither and fall off. Such effectiveness of ammoniates in crop yield increasing makes them useful for practical application.

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