14

The Role of C to N Balance in the Regulation of Photosynthetic Function

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1. Introduction

Among the numerous factors affecting plant growth and development one of the most important ones is mineral nutrition. Here, phosphorous and potassium sustain energetics and metabolite transport in the cell. The efficiency of their using can be elevated by repeated circulation of the atoms in conjugated processes. But nitrogen is expended in building plant cell mass and the growth is impossible without continues inflow of new portions of the element. That is why it has a special impact on all physiological processes, including photosynthesis and assimilate transport.

Data accumulated so far about the interaction between carbon and nitrogen metabolisms in plants indicate its key role in regulation of plant vital functions. C to N balance in a whole plant was shown to take part in regulation of photosynthesis, germination, senescence, morphogenesis (Malamy and Ryan, 2001; Martin et al., 2002; Paul and Foyer, 2001; Paul and Pellny, 2003). Nevertheless, mechanisms underlying this regulation are still elusive.

In order to find out the regulatory mechanisms it is necessary to consider all points of contact between carbon and nitrogen metabolisms in plant, and one point that is often overlooked is a significant influence of nitrogen on photo-assimilate transport in plants.

2. The influence of nitrogen nutrition level on photosynthesis and photo-assimilate partitioning

2.1 The influence of nitrogen nutrition on photoassimilate transport

At the first glance, the literature data on the action of nitrogen nutrition on assimilate transport is controversial. For instance, in some publications it is noted that additional nitrogen nutrition delays assimilate export from leaves (Kudryavtsev & Roktanen, 1965; Marty, 1969; Vaklinova et al., 1958; Zav'yalova, 1976), while in others the opposite effect is noted (Anisimov, 1959; Grinenko, 1964; Hartt, 1970; Pristupa & Kursanov, 1957).

Very important here is a period of the plant ontogenetic development, in which plant nitrogen nutrition level changes. In starving for the element juvenile plants, in which the sink-source system consists only of leaves and roots, nitrogen fertilization leads to intensified inflow of photo-assimilates to roots for active metabolization of mineral nitrogen, and these results will probably be interpreted as activation of transport processes by nitrogen. On the other hand, nitrogen supply of older plants with formed sink-source

relationships usually results in relative inhibition of assimilate export from source leaves to sink organs (Table 1).

Treatment	Radioactivity of a plant part, %	
	Leaf	Ear
Control	36.1 ± 4.1	51.8 ± 6.2
Nitrogen	50.6 ± 3.8	28.2 ± 1.7
% to control	140	54

Table 1. The influence of pre-planting nitrogen fertilization of soft wheat cv. Saratovskaya 29 on ^{14}C-photoassimilate export from leaves and their inflow into ears (% radioactivity of above ground plant part) in the milky stage of grain development (Tarchevsky et al., 1973)

The characteristic feature of photosynthetic carbon metabolism in plants grown on increased nitrogen background is a lowered ratio of labeled sucrose to hexoses (Table 2). Using our own method of extraction of the labeled photosynthetic products from the apoplast (Chikov et al., 2001), it was established that at increasing nitrate nutrition level the ratio of labeled sucrose to hexoses decreases in the apoplast rather than in mesophyll cells (Table 2). Thus, the enhanced sucrose hydrolysis is a property of the apoplastic compartment.

Treatment	Upper part		^{14}C-donor part	
	leaves	apoplast	leaves	apoplast
Control (Non-fertilized)	15.0 ± 0.15	149.5 ± 25.0	16.9 ± 7.0	128.0 ± 20.0
NO_3-fertilized	11.6 ± 3.4	36.7 ± 6.7	13.6 ± 0.7	38.1 ± 3.8
control/ NO_3	1.29	4.07	1.24	3.36

Table 2. The influence of nitrate nutrition on the ratio of labeled sucrose to hexoses in the leaves of flax plants (Chikov et al., 2001)

Delayed assimilate export from leaves of plants fertilized with nitrogen is believed to be linked with intensified synthesis of nitrogen containing compounds and diverting to the process carbon fixed in photosynthesis with lesser formation of transport photosynthetic products, i.e. sugars (Champigny & Foyer, 1992). The data on increased sucrose hydrolysis in the apoplast which is an intermediate in the sucrose transfer to the phloem suggest that the reason of lowered export lies not in the shortage of sugars but in the mechanism itself of their transport from leaves.

2.2 The influence of nitrogen nutrition on plant photosynthetic carbon metabolism (PCM)

As a rule, plants grown at various levels of nitrogen differ dramatically in their morphological features such as sizes and densities of leaf blades, photosynthetic pigment contents, the ratios of above-ground part to root weights, etc.; and all this confuses interpretation of data on PCM. That is why to identify differences between the influence of different nitrogen forms it is desirable to assess physiological and biochemical characteristics before pronounced visual changes of plants become obvious. Bearing all this in mind, we compared the influence of nitrate on PCM to that of urea (as a reduced

nitrogen form) the next day after plant fertilization. The experiments were performed with the upper leaf of wheat plants, grown at moderate level of full mineral nutrition till the stage of kariopsides formation. At this time the export function of the upper leaf is most expressed. On the eve of the experiment, plants were watered with solutions of calcium nitrate or urea with concentrations calculated so to be equal to 2 grams of N per pot. Next day from 10 to 12 a.m. the flag leaf was exposed to $^{14}CO_2$ for 2 min and fixed to study PCM. To establish specialities of the influence of nitrogen fertilization on photorespiratory glycolate pathway PCM was investigated under two CO_2 concentrations (0.03 and 0.3%) and two O_2 concentration (21% and 1%). Notably, the gas composition in the leaf photosynthetic chamber was altered only for the period of $^{14}CO_2$ assimilation by the leaf.

The experiment showed significant differences in the action of oxidized and reduced N on PCM (Table 3). The influence of urea and nitrates on PCM had some common features as well as distinct ones. Irrespective of the form of N used, the introduction of ^{14}C into phosphorous esters of sugars decreased and into malate, aspartate and alanine increased, that implied diminished phosphoglyceric acid (PGA) reduction to sugar phosphates and its enhanced non-reductive metabolism. Additionally, in plants fertilized with nitrates, the formation of glycolate pathway products (serine, glycine, glycolate) increased.

Treatment	Photosynthesis intensity ($\mu g\ CO_2 m^{-2} s^{-1}$)	Free sugars	Phosphorus esters of sugars	Serine, glycine, glycolate	Alanine, malate, aspartate
21% O_2; 0.03% CO_2					
Control	680	42.2 ± 1.0	27.6 ± 1.1	11.8 ± 0.1	7.7 ± 0.4
Urea	740	44.5 ± 0.8	24.1 ± 0.9	11.3 ± 1.0	12.2 ± 1.1
Nitrate	260	40.1 ± 1.5	16.3 ± 2.6	18.9 ± 2.2	11.8 ± 0.4
1% O_2; 0.3% CO_2					
Control	990	44.9 ± 1.0	28.9 ± 1.4	4.0 ± 0.6	13.2 ± 0.6
Urea	1430	47.3 ± 1.1	14.1 ± 2.5	2.4 ± 0.4	22.0 ± 0.8
Nitrate	1030	46.8 ± 2.0	14.2 ± 1.6	9.6 ± 0.5	22.5 ± 1.0

Table 3. The influence of different CO_2 and O_2 concentrations and N forms on ^{14}C distribution among some labeled products after 2 min $^{14}CO_2$ assimilation (% radioactivity of water-ethanol soluble fraction) in wheat (Chikov & Bakirova, 1999)

According to common knowledge, glycolate is synthesized from ribulose-1,5-bisphosphate in the RuBP-oxygenase reaction of photosynthesis which requires oxygen, and the oxygenase reaction competes with carboxylase one for RuBP. All this occurs in the joint active centre of the Rubisco enzyme. Thus, O_2 and CO_2 compete for the binding of RuBP in the reaction center, and to lower the activity of oxygenase reaction of Rubisco one needs to decrease the concentration of O_2 and increase the concentration of CO_2. Such a situation was created in the experiment: in the period of $^{14}CO_2$ assimilation a gas mixture of oxygen (1%) and carbon dioxide (0.3%) was delivered into the photosynthetic leaf chamber containing a treated leaf at the same concentration of $^{14}CO_2$.

As a result, ^{14}C incorporation into the products of glycolate metabolism relatively (%) reduced in all plants; however, in nitrate plants it was the least expressed. If one calculates the formation of glycolate pathway products in unit mass of fixed carbon dioxide ($\mu g\ CO_2$

m^{-2} s^{-1}) he will find that in control and urea fed plants the formation of these products lessened twofold while in nitrate plants it augmented twofold.

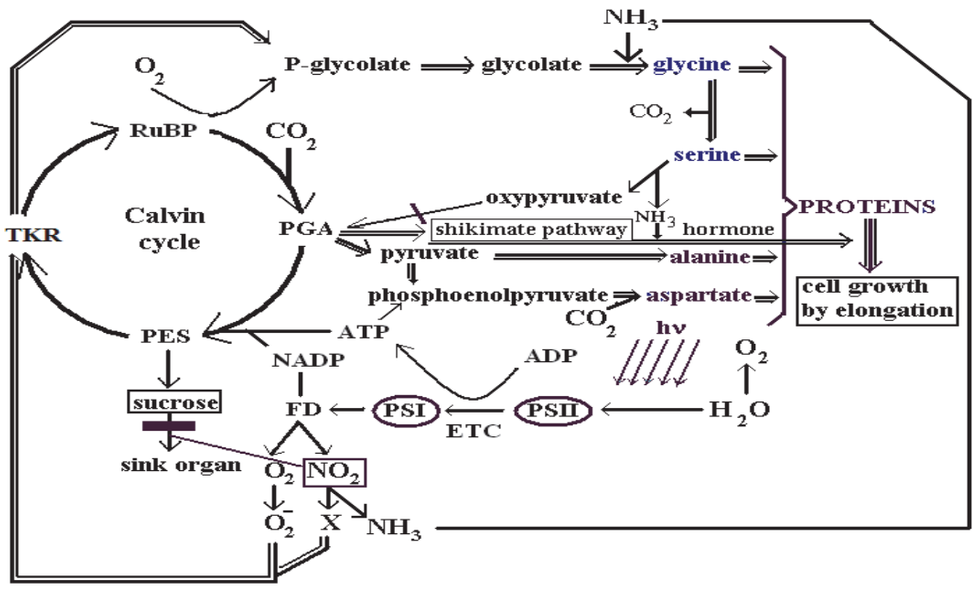


Fig. 1. The scheme of photosynthetic carbon metabolism regulation. ETC – electron transport chain, FD – ferredoxin, PES – phosphorous esters of sugars, PGA – phosphoglyceric acid, PSI – photosystem I, PSII – photosystem II, RuBP – ribulose 1,5-bisphosphate, TKR – transketolase reaction, X – unknown oxidizer, double line – intensification of a process

Based on this data the following conclusions can be made. Firstly, the suppression of RuBP-oxigenase activity by low O_2 and elevated CO_2 occurs only without nitrates. When nitrates are present, the formation of glycolate and its metabolites even enhances. Secondly, as the amount of CO_2 fixation product (PGA) increases at the saturable CO_2 concentration its non-reductive metabolism with appearance of alanine, malate and aspartate rises. Apparently, the latter is partly associated with rising CO_2 fixation in dark type-reaction catalyzed by phosphoenolpyruvate (PEP) carboxylaze (see the scheme in fig. 1).

The active production of glycolate under conditions suppressing RuBP-oxydenase reaction suggests that in the presence of nitrates glycolate is formed from other (not RuBP) phosphorous esters of sugars (PES) in transketolase reactions. Transketolase reaction requires superoxide radical (Asami & Akasava, 1977), produced in the Mehler reaction (Takabe et al., 1980).

The enhanced oxidation of PES may account for their decreased radioactivity and overall elevated non-carbohydrate tendency of photosynthesis in nitrate fed plants. The possibility of such a mechanism was declared long ago (Asami, Akasava, 1977). One of the probable mechanisms of transketolaze reaction (TKR) activation and glycolate formation from sugar di-phosphates upstream of RuBP might be an inhibited activity of phosphatases of fructose- and sedoheptulose-diphosphates (Heldt et al., 1978).

For fully grown leaves, which are typical exporters of assimilates, glycolate metabolism mainly terminates with formation of PGA (see the scheme in Fig. 1), that returns back to the Calvin cycle and is reduced to sugars. In the case of hindered sugar export the glycolate pathway becomes less closed. Glycine and serine, amino acids derived from glycolate, and

also, alanine and aspartate, resulting from non-reducing PGA metabolism, can be used for the protein synthesis when the leaf recommences its growth through expansion. In our experiments with the removal of fruit elements from a cotton plant a leaf which was a source of assimilates could augment its size as many as 1.5-2 times in 10-15 days after the exposure (Chikov, 1987).

The return of the carbon of glycolate into the Calvin cycle is carried along the chain 2 glycolate → 2 glyoxylate + NH_2 → 2 glycine → serine + NH_2 → oxyglycerate + NH_2 → glycerate + ATP → PGA.

If the products of the glycolate pathway do not return to the Calvin cycle but accumulate and used in synthetic processes then the carbon will come to glycerate in smaller quantities. In this case the ratio of radioactivities of glycolate+glycine+serine to that of glycerte must increase.

This conclusion was confirmed in studies of the kinetics of ^{14}C introduction into those compounds. In the fertilized plants labeled carbon entered such compounds as glycolate, glycine, serine and alanine to a greater extent than in the controls, while glycerate, conversely, to a lesser extent (Fig. 2). As a result, the ratio of glycolate+glycine+serine/glycerte increased several-fold. The kinetic curves show that these differences need certain time (not less than 30 s) to reveal oneselves, that supports the metabolism of these substances in the direction of glycolate → glycine → serine → glycerate.

It is interesting that under conditions of hindered assimilate export from leaves after removal of sink organs a similar kinetics of ^{14}C introduction from $^{14}CO_2$ into glycolate and glycine was found at ambient CO_2 concentration (Chikov, 1987). The results were in good agreement with the idea of transketolase mechanism of glycolate formation, because in experimental plants labeled carbon from $^{14}CO_2$ appeared in glycolate earlier than that occurred in control ones. Characteristically, at saturable CO_2 concentration the kinetics curves for control and experimental plants were the same and both resembled the curve for experimental plants under ambient CO_2 (Chikov, 1987). The data indicate again that under the circumstances of slowed assimilate export and saturable CO_2 concentration glycolate and the products of its metabolism are derived from sugar phosphates that are predecessors of RuBP.

Thus, inhibition of assimilate export from the leaf and an increase of the Warburg effect in leaf photosynthetic gas-exchange, observed in some cases (Chikov, 1987), are accompanied by enhanced CO_2 metabolism through the glycolate pathway, but with increasing potion of glycolate formed in reactions not related with RuBP-oxidase one, most likely, in transketolase reaction of the Calvin cycle. This mechanism probably works also at delay of assimilate export from leaves under elevated level of plant nitrate nutrition. In the case of nitrate presence in the leaf, an oxidizer required to perform the transketolase reaction could appear from NO_2^- reduction in the chloroplast ETC (see Fig. 1).

In both cases growth processes become enhanced in the source leaf, for which early photosynthetic products in the form of amino acids alanine, serine, glycine and aspartate are used. It should be mentioned that these four amino acids (of 20 proteinogenic ones) represent over 30% (by number, not by weight) of fraction 1 protein (the main chloroplastic protein).

Furthermore, the suppression of sugar export can enhance the metabolism of phosphoerythroses through the shikimate pathway with the formation of aromatic (see Fig. 1) amino acids (tyrosine, phenylalanine, tryptophan) and then hormonal substances (auxins), creating an additional substrate base for metabolism rearrangement in the leaf (and whole plant). So, a key factor for triggering metabolism readjustment in the leaf (the plant) is inhibition of sugar outflow from leaves.

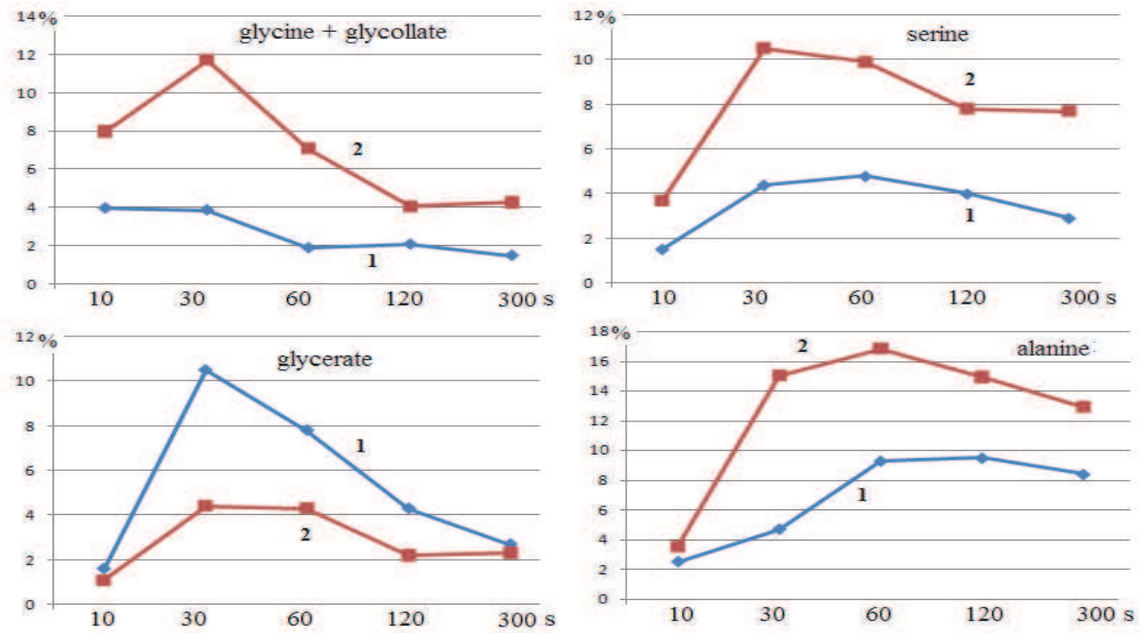


Fig. 2. The kinetics of ^{14}C introduction into some products of photosynthesis (% radioactivity of water-ethanol soluble fraction) in leaves of wheat plants cv. Moskovskaya-35. 1 – control; 2 - fertilized with 2 g of nitrogen as $Ca(NO_3)_2$

2.3 The influence of nitrogen supply on the dynamics of post-photosynthetic conversion of ^{14}C-products of photosynthesis

The actions of oxidized and reduced N vary not only in primary photosynthetic products formation but in their subsequent metabolisation to the end transport compound, sucrose.

After short exposure of leaves to $^{14}CO_2$, ^{14}C content in sucrose was reduced under N treatments, irrespectively of the N form, which seemed to be due to intensive formation of non-carbohydrate substances (organic and amino acids). Then in the next 30 min ^{14}C accumulated in the end photosynthetic product, sucrose, in all plant variants (Fig. 3). However in plants fed with nitrogen this accumulation occurred at higher rates than in control, and as a result, ^{14}C content in sucrose in both N fed variants almost equaled and approached the control level.

From this moment sucrose radioactivity began to decrease in all variants. During 1.5 h it reduced by 75% in control and by 45% in plants fed with urea. In the next 20 h sucrose radioactivity in the plants was running down steadily to the level of 4-5% of the maximal value. Unlike these two variants, nitrate plants exhibited even descent of sucrose radioactivity from 2 h point and during the next 20 h not reaching 30% of the maximal level. All this evidenced significant distinction of the sucrose transport in nitrate plants from those both in control and urea fed ones.

In summary, at water or urea feeding labeled primary photosynthetic products are quite successfully converted into sucrose with its subsequent export in the post-photosynthetic period, while in nitrate fed plants labeled assimilates are piled up as sucrose which remains in the source leaves for a long time period.

To reveal a mechanism of assimilate export delay from leaves special model experiments were performed, where nitrate or urea solutions were injected into isolated plant shoots.

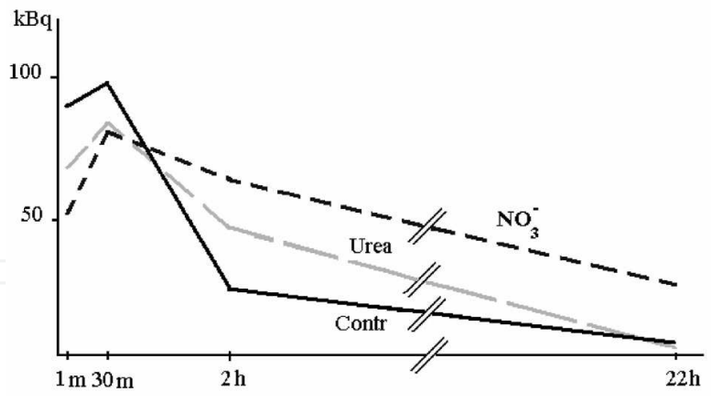
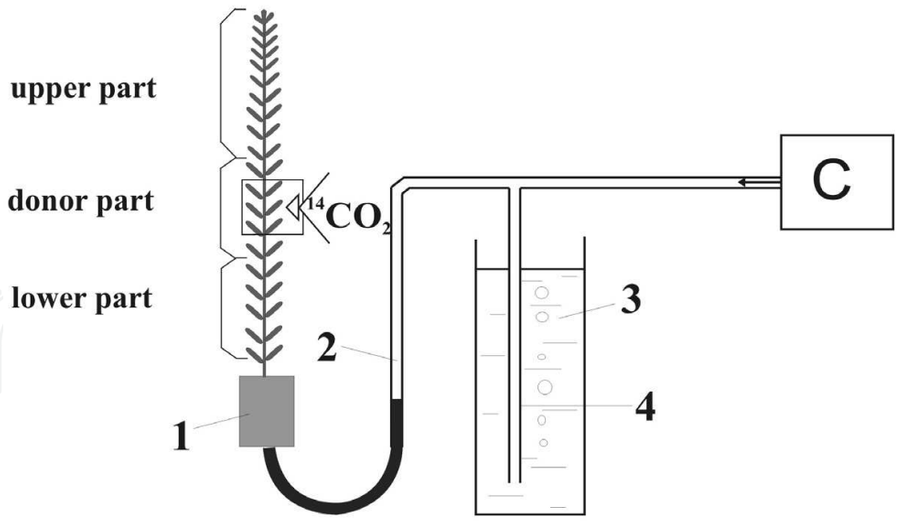


Fig. 3. The influence of nitrate or urea fertilization of wheat plants on the dynamics of ^{14}C content in sucrose after 1 min $^{14}CO_2$ assimilation by leaves

3. The influence of different N forms in the transpiration water stream on photosynthetic carbon metabolism and assimilate transport from leaves

3.1 Methodical peculiarities of the procedure

To study the immediate influence of increased nitrogen nutrition level on photosynthesis and assimilate transport we have designed a special device (Fig. 4) for introduction of solutions or water into a shoot under the pressure of 10^4 Pa, corresponding to a normal root pressure. Preliminary studies had shown that a shoot could survive for several days under conditions of direct sunlight without demonstrating any visible lesions.



1 – a mandrel for shoot fixation; 2 – a silicone tube with a solution fed into the apoplast; 3 – a bath with water and a monostat (4) drowned in it to the depth of 1 m to imitate root pressure; C –a compressor

Fig. 4. The scheme of solution introduction into an isolated flax shoot

For photosynthesis investigations a central shoot part was exposed to $^{14}CO_2$ using a photosynthetic chamber. This allowed not only to determine ^{14}C distribution among the labeled products of 2-3 min photosynthesis, but also to assess the character of allocation of

labeled photosynthetic products along the shoot to different shoot parts (^{14}C-source leaves, the apex, parts above and below the ^{14}C-source part) in the post-photosynthetic period.

There are two main types of plants differing in phloem loading strategy and transportable photosynthetic products (Lohaus & Fischer, 2002). These are plants with the apoplastic phloem loading strategy in which photoassimilates move outside the plasma membrane of parenchyma cells and are loaded from the apoplast into the companion cells or sieve elements using energy-dependent transporters. In other plants photoassimilates move from assimilating to conducting cells along the system of plasmodesmata without membrane crossing. For our experiments we have chosen flax (*Linum usitatissimum* L.) as a plant with apoplastic phloem loading and a willow-herb (*Chamerion angustifolium* (L.) Holub.) as a symplastic plant.

3.2 The introduction of nitrate solution into an apoplastic plant

The study of $^{14}CO_2$ distribution among the labeled products of photosynthesis in flax leaves has shown that nitrate injection into the apoplast had the same effect on metabolism as fertilization of plants with nitrogen via soil. The introduction of nitrates resulted in decreased ^{14}C incorporation into sucrose and the ratio of labeled sucrose to hexoses, and increased ^{14}C distribution into the glycolate pathway products (Table 4).

Compounds	Water	KNO_3 (0.5%)	KNO_3 (1.5%)	$Ca(NO_3)_2$ (0.5%)	Urea (2.5%)
Sucrose	59.2 ± 1.6	52.6 ± 2.5	48.1 ± 2.8	47.0 ± 1.8	56.4 ± 0.9
Phosphorous esters of sugars	3.2 ± 0.3	2.1 ± 0.8	3.5 ± 0.3	0.3 ± 0.0	2.9 ± 0.8
Hexoses	4.3 ± 0.6	4.0 ± 0.5	6.0 ± 0.5	5.0 ± 0.8	3.5 ± 0.5
Sucrose/hexoses	13.8	13.2	8.0	9.4	16.1
Amino acids	21.1 ± 1.1	29.2 ± 3.1	32.1 ± 3.2	29.4 ± 3.3	24.5 ± 0.8
including: glycine	1.6 ± 0.2	2.1 ± 0.5	2.6 ± 0.7	3.7 ± 0.4	1.8 ± 0.1
serine	5.7 ± 0.3	14.9 ± 2.5	15.3 ± 1.6	11.8 ± 2.3	5.3 ± 0.3
alanine	11.7 ± 0.7	9.4 ± 0.3	10.8 ± 1.0	10.1 ± 1.0	13.5± 0.7
Organic acids	3.6 ± 0.3	6.5 ± 0.9	4.3 ± 0.4	5.9 ± 0.7	6.4 ± 0.2
including: glycerate	0.8 ± 0.1	4.5 ± 0.9	1.6 ± 0.1	1.7 ± 0.2	0.7 ± 0.1
malate	1.8 ± 0.1	0.9 ± 0.1	1.5 ± 0.3	3.1 ± 0.5	4.8 ± 0.1
Pigments	2.7 ± 0.2	1.2 ± 0.1	2.0 ± 0.2	3.0 ± 0.4	1.5 ± 0.1
Others	5.9	4.4	4	9.4	4.8

Table 4. The influence of nitrate and urea feeding into the apoplast on ^{14}C distribution among labeled products in flax source leaves immediately after exposure to $^{14}CO_2$ (% radioactivity of water-soluble fraction)

As it was indicated above, increased label incorporation into the glycolate pathway products is also characteristic of enhanced soil nitrogen nutrition, however at soil fertilization ^{14}C was mostly incorporated into glycine and glycolate while at direct nitrate feeding into the shoot

the radioactivity of serine was higher (Table 4). Watering of plants grown in the soil with $Ca(NO_3)_2$ solution on the eve of the day of experiment apparently leaves the plants enough time for activation of protein synthesizing systems, utilizing serine. The protein synthesizing systems of plants supplied with nitrates through the transpiration water stream were probably not ready for utilization of ample amounts of newly-formed amino acids and this resulted in ^{14}C accumulation in amino acids (first of all, serine). The speed of nitrate reduction exceeds the flow through the GOGAT-pathway approximately by 25%, leading to reduced nitrogen piling up in the immediate products such as ammonium and glutamine as well as photorespiration metabolites, glycine and serine (Stitt et al., 2002).

Why does ^{14}C distribution into sucrose immediately after $^{14}CO_2$ assimilation decrease under nitrate nutrition of plants? The common explanation of reduction in sucrose synthesis after nitrogen nutrition of plants is the following: at enhanced inflow of nitrogen into the plant photosynthetic products and energy are diverted from sucrose synthesis to the formation of nitrogen-containing compounds (Champigny & Foyer, 1992). However, this explanation does not accord with different actions of oxidized and reduced nitrogen on sucrose production (Batasheva et al., 2007). Undoubtedly, the reduction of nitrates requires more energy spending, but the electron transport in chloroplast is known to have significant plasticity and different types of mitochondrial electron transport are probably take part in the maintenance of necessary ATP/NAD(P)H ratio in the cell (Noctor & Foyer, 1998; 2000).

Another reason of decreased sucrose synthesis under nitrate nutrition may be a feedback regulation of photosynthesis resulted from slowdown of sucrose transport from leaves.

Injection of nitrate solution into a flax shoot resulted in pronounced response of photosynthetic rate, photosynthetic carbon metabolism and assimilate transport measured in 3 h after $^{14}CO_2$ assimilation (Batasheva et al., 2007). That is why we decided to study those changes in more detail.

Studies of the dynamics of ^{14}C distribution along the shoot have revealed that feeding nitrates to the apoplast resulted in inhibition of assimilate export. In 30 min after $^{14}CO_2$ assimilation in shoots fed with KNO_3, relative content and distribution of ^{14}C outside the ^{14}C-donor part did not virtually differ from those in control (Table 5). In additional 2.5 h of post-photosynthesis, in control plants, ^{14}C relative contents outside the donor part increased with significant rising of ^{14}C content in the lower part (Table 5). In shoots fed with nitrates, ^{14}C content outside the donor part in 2.5 h also increased but in lesser degree than in control, and the overall ^{14}C relative content in the lower shoot part became smaller whereas in the upper part – greater compared to control.

In our previous experiments it was found that feeding urea solution (0.15% (w/v)) containing the same amount of nitrogen as potassium nitrate solution (0.5% (w/v)), led to almost the same distribution of ^{14}C through the shoot as feeding water (Batasheva et al., 2007). NH_4NO_3 solution feeding led to lesser inhibition of assimilate export and slightly changed the pattern of ^{14}C partitioning along the shoot compared to those under KNO_3 solution feeding (Batasheva et al., 2007).

In flax, most ^{14}C-sucrose after being loaded into the phloem terminals in source leaves moves downwards within stem phloem vessels, and ^{14}C content in the plant lower parts gradually raises (Chikov & Bakirova, 2004). During their movement along the stem assimilates can partly escape into the stem apoplast, and long distant transport is controlled by retention and retrieval mechanisms in the phloem (Ayre et al., 2003). The assimilates lost to the apoplast can either be re-loaded back into the phloem (Kühn et al., 1997) or be

transported upwards with the transpiration water stream. If assimilates leaked in the apoplast cannot be quickly loaded back into the phloem due to some reason then the portion of the assimilates inflowing into the upper plant part with the transpiration water stream would increase. It seems likely that this is the reason of increased ^{14}C content in the upper parts of the shoots fed with nitrates. Enhancement of sucrose hydrolysis in the apoplast in the presence of nitrates must result in appearing large amounts of labeled hexoses, which can not be loaded into the phloem (Turkina et al., 1999) and are easily carried away upwards with the transpiration stream.

Shoot part	H_2O		KNO_3	
	30 min	3 h	30 min	3 h
Above ^{14}C-donor part	3.5 ± 0.6	5.2 ± 0.9	3.1 ± 0.4	9.8 ± 2.0
including: top	0.2 ± 0.0	1.0 ± 0.3	0.2 ± 0.0	3.3 ± 1.2
leaves	2.0 ± 0.	1.7 ± 0.4	1.3 ± 0,5	1.4 ± 0.3
cortex	0.9 ± 0.0	1.5 ± 0.4	1.1 ± 0,1	3.1 ± 0.6
wood	0.4 ± 0.05	1.0 ± 0.30	0.5 ± 0,1	2.0 ± 0.5
^{14}C-donor part	82.2 ± 2.8	67.5 ± 0.9	82.0 ± 1.3	76.3 ± 2.5
Below ^{14}C-donor part	14.3 ± 0.9	27.3 ± 1.1	14.9 ± 1.3	13.9 ± 3.8
including: leaves	9.9 ± 1.1	13.5 ± 1.5	7.3 ± 2.1	9.0 ± 1.4
cortex	3.0 ± 0.3	6.7 ± 0.8	5.8 ± 1.7	2.2 ± 0.6
wood	1.4 ± 0.2	7.1 ± 1.6	1.8 ± 0.7	2.7 ± 0.8
Above/below	4.1	5.25	4.8	1.4

Table 5. The influence of nitrate feeding through the transpiration water stream on ^{14}C distribution among the organs of flax in 30 min and 3 h after $^{14}CO_2$ assimilation by the middle shoot part (% of whole shoot radioactivity)

As we have shown previously (Batasheva et al., 2007), increased ^{14}C content in the lower part of plants fed with water is not connected with label accumulation due to synthesis of any high-molecular weight substances in this period.

In 30 min after $^{14}CO_2$ assimilation relative content of ^{14}C-sucrose in source leaves increased both in control and in nitrate fed plants (Table 6). In the following 2.5 h in water fed shoots ^{14}C-sucrose content decreased to lower values compared to those observed immediately after $^{14}CO_2$ fixation whereas in nitrate fed shoots it continued growing. Thus, in 3 h after $^{14}CO_2$ assimilation relative ^{14}C content in sucrose in source leaves of the shoots fed with KNO_3 rose significantly up to 75%. Relative radioactivity of hexoses practically did not change which resulted in increase of the labeled sucrose/hexoses. The similar picture of ^{14}C-sucrose dynamics was described above for wheat plants fertilized with nitrates (Fig. 3).

Localization of labeled sucrose in leaves was determined by autoradioagraphy of whole leaves taken from ^{14}C-donor part in 30 min and 3 h after $^{14}CO_2$ assimilation. It turned out that in 30 min after $^{14}CO_2$ assimilation in water fed shoots the label was concentrated mainly in leaf large veins whereas in nitrate fed shoots – outside them (Fig. 6). It indicates that upon nitrate feeding the accumulation of the labeled assimilates occurred either in mesophyll cells or in minor vein cells from which they were not transported to large veins.

Labelled compounds	H_2O		KNO_3	
	30 min	3 h	30 min	3 h
Sucrose	71.6 ± 1.7	50.7 ± 1.6	67.7 ± 0.5	75.0 ± 1.6
Glucose	9.3 ± 1.7	17.2 ± 1.7	7.6 ± 0.3	4.8 ± 0.6
Fructose	4.9 ± 0.5	15.0 ± 1.9	3.3 ± 0.6	4.2 ± 0.4
Glycine	0.9 ± 0.5	1.9 ± 0.6	2.4 ± 0.3	1.0 ± 0.3
Serine	1.7 ± 1.3	2.2 ± 0.2	5.3 ± 1.3	1.4 ± 0.5
Aspartate	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Glutamate	0.9 ± 0.1	0.5 ± 0.1	0.9 ± 0.2	0.5 ± 0.0
Malate	1.3 ± 0.1	2.5 ± 0.5	1.4 ± 0.3	1.0 ± 0.2
Alanine	1.3 ± 0.1	1.0 ± 0.3	1.2 ± 0.1	1.4 ± 0.1
Pigments	1.9 ± 0.3	2.2 ± 0.5	1.5 ± 0.1	1.6 ± 0.1
Others	6.0	6.6	8.5	8.9

Table 6. The incorporation of ^{14}C into the products of photosynthesis in 30 min and in 3 h after 2.5 min $^{14}CO_2$ assimilation

In 3 h of post-photosynthesis in control leaves ^{14}C mostly disappeared from large bundles. In nitrate fed leaves the differences between ^{14}C contents inside and outsides the large bundles became even more contrasty. It seemed likely that in control shoots ^{14}C-sucrose export exceeded its synthesis while in nitrate fed shoots ^{14}C-sucrose synthesis was not compensated by its removing from the leaf. Earlier it was shown that feeding urea (0.15% (w/v)) into the apoplast led to the same picture of ^{14}C-sucrose dynamics as feeding water (Batasheva et al., 2007).

Thus, nitrate feeding resulted in graduate accumulation of sucrose in source leaves, which was observed on the background of assimilate export suppression. It is interesting that similar dynamics of sucrose radioactivity changes in mature leaves was observed by Möller and Beck (1992), who studied metabolism of labeled sucrose when unlabelled sucrose was constantly inflowing into the apoplast. In the work high sucrose content in the apoplast led to labeled sucrose accumulation within cells. A question arises why labelled sucrose was not hydrolyzed especially under the conditions of inhibited export appearing when sucrose is fed into the apoplast or observed in our experiments upon nitrate feeding.

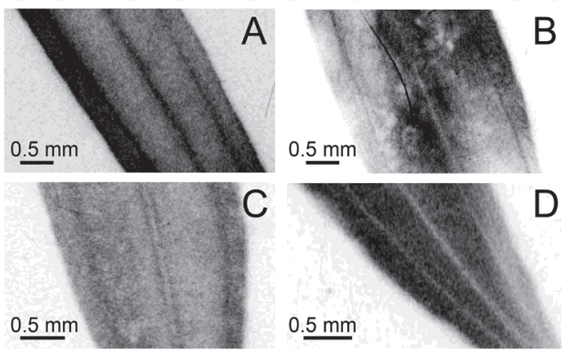


Fig. 6. The influence of water or KNO_3 solution (0.5%) feeding on ^{14}C distribution within the donor leaf in 30 min (A – water; B – nitrate) and 3 h (C – water; D – nitrate) after exposure to $^{14}CO_2$. Darker places correspond to greater ^{14}C contents

It is probable that labeled sucrose was in the leaf conducting system where sucrose hydrolyzing activity is negligible (Dubinina et al., 1984). On the other side, we can not rule out the possibility that at least a part of labeled sucrose was accumulated in mesophyll cells. For instance, in barley shoots, suppression of assimilate export resulted in sucrose accumulation in bundle sheath parenchyma cells and in mesophyll cells and probably in the bundles themselves. In the mesophyll and bundle sheath cells an increase in glucose contents was also observed (Koroleva et al., 1998; Pollock et al., 2003).

Thus, it turned out that nitrates fed into the cut shoots with the transpiration water stream exerted the same action on assimilate outflow and ^{14}C distribution among the products of photosynthesis as nitrate fertilization.

The similar action of nitrate fertilization (Chikov et al., 2001) and artificial nitrate infusion into the apoplast on ^{14}C distribution among the products of photosynthesis, primarily, the ratio of labeled sucrose to hexoses, allowed us to conclude that enhanced sucrose hydrolysis under increased nitrate fertilization of plants was in some way connected with the presence of nitrate anion in the apoplast. Urea did not exert such an effect (Batasheva et al., 2007).

Thus, a possible reason of changes in metabolism at soil fertilization of plants with nitrates may be an enhancement of sucrose hydrolysis in the apoplast at nitrate income therein. Because monoses formed in the process of sucrose hydrolysis cannot be loaded into the phloem terminals and, consequently, take part in assimilate transport, assimilate outflow from leaves becomes lowered. The monoses have to return back to mesophyll cells and then, decreased photosynthesis rate and its non-carbohydrate tendency can be the consequences of feedback inhibition of photosynthesis and sucrose formation.

The possible influence of nitrates through activation of sucrose hydrolysis by cell wall invertase is supported by numerous data on the similar action of nitrates and sugars on certain gene expressions, primarily those connected with nitrogen metabolism (Stitt et al., 2002). Furthermore, it was shown that one of nitrate carrier genes is induced by NO_3^- and decrease of its transcript abundance in the dark could be prevented by addition of sucrose. The gene could also be induced in the absence of external NO_3^- by a sharp decline of medium pH from 6.5 to 5.5 (Forde, 2000), which could cause an activation of the apoplastic invertase, because its pH optimum lies in the acidic pH range (Brovchenko, 1970).

In plants with the apoplastic type of phloem loading inhibition of photoassimilate transport by putting a cold collar on the petiole was associated with appearance of large central vacuoles in phloem companion cells (Gamalei & Pakhomova, 2000). So, it could be possible that in our experiments in the post-photosynthetic period sucrose accumulated not in the transport path itself but in vacuoles formed in companion cells.

Significant changes in minor vein cell structure as a response to increase in nitrate concentration in the apoplast were found (Fig. 7). In control leaves, companion cells were characterized by well developed system of cell wall invaginations and very slight vacuolization of their protoplasts (Fig. 7A). In 30 min after beginning of KNO_3 (0.5%) solution feeding sieve elements filled up with electron-transparent vesicles, and a large central vacuole was formed in companion cells (Fig. 7B). Some indication of endocytosis allows to guess that the vacuolation was a result of seizure of extracellular milieu containing sugar in high concentration. This could be the way by which companion cells protected themselves from osmotic stress. The vacuole was growing in size for the next 2 h (Abdrakhimov et al., 2008).

When the dynamics of changes in ultrastructure of leaves in the presence of nitrate in the apoplast was studied it turned out that in mesophyll, bundle sheath and phloem parenchyma cells the mitochondria matrices clarified and dictyosomes curled in the first 30 min, but repaired their structure by 1 h after beginning of nitrate feeding (Abdrakhimov et al., 2008). We supposed that such a two-phase response could consist of a quick direct action of nitric oxide, formed from nitrate, on leaf ultrastructure and slower changes related with inhibited photoassimilate export (Abdrakhimov et al., 2008)

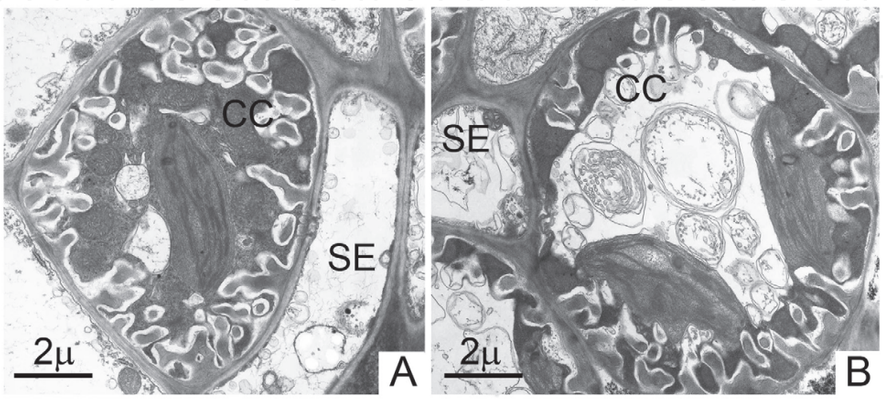


Fig. 7. Ultrastructure of terminal vein cells in the leaves of common flax 1 h after supplying water (a) or 0.5% KNO_3 (b) to the apoplast. CC - companion cell; SE - sieve element (Batasheva et al., 2007)

Thus, analysis of flax leaf terminal vein ultrastructure revealed vacuole formation in companion cells in response to nitrate feeding into the apoplast. One can guess that, similarly to putting a cold collar on the petiole, nitrate feeding into the apoplast initially creates hindrances to assimilate transport within the sieve elements or assimilate transport from companion cells to sieve elements. Augmentation of callose synthesis is known to happen very quickly in response to low temperature, within several minutes (Kursanov, 1976). It is tempting to speculate that when nitrates are excessive NO is generated, which, as a stress signal, can trigger synthesis of callose.

3.3 The influence of nitrates fed into the apoplast of a symplastic plant

The introduction of nitrate solution into the plant apoplast of willow-herb shoots (control – water) caused an inhibition of $^{14}CO_2$ fixation by leaves (Khamidullina et al., 2011). Analysis of distribution of labeled photosynthetic products among the plant organs in 3 h after $^{14}CO_2$ assimilation has shown that the intensity of assimilate export from willow-herb leaves (both in control and treated plants) was lower than that from flax leaves. In willow-herb plants it was 8.1 % in control and 4.5% in nitrate plants, while under the same conditions in flax plants more than 40% of ^{14}C-assimilates formed in leaves were exported (Khamidullina et al., 2011). Such a difference in the export rate in plants with various types of phloem loading could be linked with synthesis of transport products represented by tri- and tetra-saccharides in symplastic plants. Probably it is due to variable mechanisms of transport, longer time required for oligosaccaride synthesis, and different diurnal dynamics of assimilate export (Gamalei, 2004) the transfer of labeled photosynthetic products was slower in willow-herb than in flax.

As in the flax plants, in the willow-herb plants nitrates stimulated the transfer of labeled assimilates to the upper shoot part. The difference will become larger if the content of labeled assimilates in the upper shoot part is assessed relatively to that exported from the ^{14}C-donor leaf. As a result the ratio of exported labeled assimilates in the upper part to that in the lower part was 4.8 in control and 2.4 in nitrate plants. Because the labeled assimilates are likely to get into the upper shoot part with the transpiration water stream after their leakage from the phloem into the stem apoplast the data evidences the enhancement by nitrates of the permeability of phloem tubes to transport photosynthetic products. The latter could be due to the slowdown of phloem transport and increased concentration of products in the stem phloem or reduced return of sugars from the apoplast back into the phloem (Kühn, et al., 1999).

The analysis of ^{14}C distribution among the labeled products of low molecular weight fraction revealed both common traits and differences in the action of nitrates on photosynthetic metabolism in flax and willow herb. In both cases ^{14}C introduction into sucrose decreased under nitrates, that indicates the similarity of nitrate influence on photosynthetic carbon metabolism in symplastic and apoplastic plants. The influence on photosynthetic carbon metabolism is not connected with the presence of potassium cation, because the same changers were also observed under calcium nitrate nutrition of plants (Chikov et al., 1998).

After comparing the dynamics of ^{14}C acquirement by sucrose in flax and willow herb the similarity was also found. After short expositions to $^{14}CO_2$ mirroring the intensity of sucrose synthesis a decrease in sucrose radioactivity was observed. Later in treated plants the label piled up in sucrose as a result of sucrose export arrest. Thus, the characters of post-photosynthetic changes of sucrose radioactivity in both plant types were resemblant.

As it is known in symplastic plants a significant fraction of exported sugars is represented by oligosaccharides (Pristupa, 1959). Probably that is why the dynamics of changes in ^{14}C content in this group of compounds is similar to that of sucrose. After analysis of the data on oligosaccharide and sucrose content in the willow-herb it was found that in nitrate plants ^{14}C was gradually accumulated with time in sucrose and oligosaccharides. In control, this parameter changed insignificantly (within the error).

Thus, nitrate anion hinders assimilate export irregardless of the way of sugar transfer from leaf mesophyll cells to the phloem, suggesting that the mechanisms of nitrate action on assimilate transport in both plant types have much in common.

The radioactivity of phosphorous esters of sugars (PES) in nitrate fed willow-herb plants immediately after 3 min exposition to $^{14}CO_2$ was increased apparently indicating their hampered conversion to export sugars (Khamidullina et al., 2011). The general elevation of radioactivity in the glycolate pathway products (serine, glycine and glycolate) suggests that photooxidation processes become activated under nitrate feeding. As it was shown by Chikov and Bakirova (1999) the enhanced production of glycolate pathway products under increased nitrate nutrition may be connected not with atmospheric oxygen but with reactive oxygen species, generated in the course of nitrate reduction in the chloroplast electron transfer chain, because their formation only slightly declined after a decrease in oxygen concentration down to 1%. A possible existence of other sources of glycolate and its metabolites under delayed assimilate export from the leaf was demonstrated previously in the kinetic experiments (Chikov et al., 1985).

In 3 h after beginning of nitrate feeding into the apoplast the most evident changes of ultrastructure were observed in the mitochondrion and vacuolar system of cells of conducting system (Khamidullina et al., 2011). The electron density of mitochondrial matrix increased and christa lumens swelled indicating increased osmotic pressure in the cytosol of cells in conducting system. This was paralleled by accumulation of fibrillar inclusions represented by polymer substance that generated a homogenous network evenly distributed throughout the organelle interior on the cross-sections (Fig. 8).

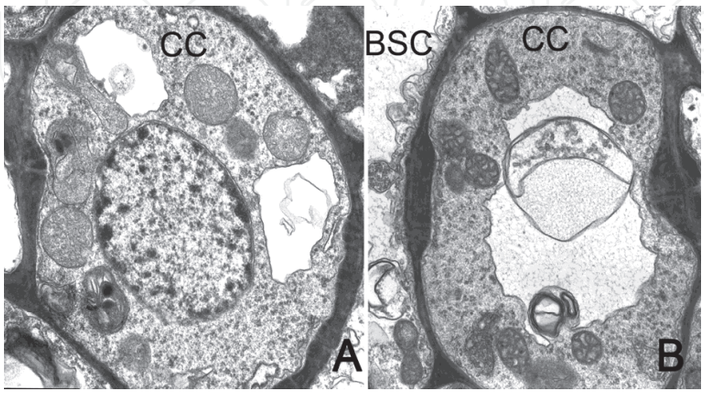


Fig. 8. Companion cells in the leaves of fireweed plants in 3 h after water or potassium nitrate (50 mM) feeding into the apoplast. BSC – bundle sheath cells, CC – companion cells (Khamidullina et al., 2011)

The nature of the inclusions is unknown, but, bearing in mind the abundance of the polymer and the fact that analogous formations were revealed also in the sieve elements we have guessed that the observed structures is either photosynthetic transport product itself or the result of its polymerization. But irregardless of the way of formation its accumulation must also express a blockage of transfer of carbohydrate transport product in willow-herb plants.
The organelles covered with semi-permeable membranes, mitochondria and plastids are known to be ideal intracellular osmometers (Gamalei, 2004). Obviously, hindered transport of low molecular weight compounds from leaf blades must result in their piling up in the places of phloem loading and osmotic contraction of the organelles. That is just what was observed in our experiments (Fig. 8). Similar changes we have earlier seen in an apoplastic plant, flax, in 3 h after beginning of nitrate feeding through the transpiration stream (see above). However, oligosaccarides rather than sucrose are accumulated in companion cell vacuoles, diagnosable by fibrillar inclusions.

4. The influence of NO donor, sodium nitroprusside on photosynthetic carbon metabolism

As it was shown above in model experiments, under direct feeding of salts, containing nitrate anion, in the plant apoplast, decreased $^{14}CO_2$ assimilation rate and photoassimilate export from leaves with changes in photosynthetic carbon metabolism and ultrastructure of phloem companion cells were observed. Elevation of nitrate content in a plant is a condition advantageous for nitric oxide generation from nitrite by both enzymatic and non-enzymatic ways (Neill et al., 2003). From whence a participation of NO signal system in triggering a mechanism, arresting assimilate export from leaves under increased nitrate nutrition level

may be proposed. To test this proposition we investigated the influence of NO donor, sodium nitroprusside, on photosynthetic metabolism and ultrastructure of cells of flax leaf blades.

As in experiments with injection of potassium nitrate solution into the shoot, sodium nitroprusside solutions (SNP) with concentrations of 50 μM, 100 μM and 1mM were introduced into cut flax shoots. In 30 min a middle shoot part containing 8-10 leaves was placed into a photosynthetic chamber, where $^{14}CO_2$ (0.03%) was delivered at natural sunlight (^{14}C-donor part of a shoot).

Feeding SNP solution into the apoplast resulted in reduced $^{14}CO_2$ assimilation, with the inhibition of $^{14}CO_2$ fixation by SNP (50 μM, 100 μM) being almost equal to that by potassium nitrate (50 mM). Further elevation of nitroprusside concentration to 1 mM enhanced the inhibition of photosynthesis up to 75% (Table 7, Batasheva et al., 2010). Such a sharp drop of carbon dioxide uptake after increasing SNP concentration up to 1 mM can partially be explained by NO participating in stomatal closure (Mata & Lamattina, 2001). NO involved in stomatal movements can be generated from nitrate anion by nitrate reductase (Desikan et al., 2002).

SNP introduction into the shoot affected ^{14}C-assimilate distribution throughout the plant similarly to nitrate feeding. In the treated plants assimilate outflow from the donor part slowed down and their transfer into the upper part relatively increased (Table 7). Whereas in control the main part of ^{14}C-products of photosynthesis exported from the donor-part appeared in the lower shoot part, after feeding SNP the relatively higher content of exported ^{14}C-assimilates was found in the upper shoot part (Batasheva et al., 2010).

Shoot parts	Control (water)	SNP (50 μM)	SNP (100 μM)	SNP (1mM)
Top	2.5 ± 0.5	3.0 ± 1.4	6.0 ± 0.8	1.8 ± 0.2
Upper shoot part	5.3 ± 1.3	6.3 ± 2.3	9.1 ± 0.5	11.5 ± 6.2
Above ^{14}C-source part	7.8	9.8	15.1	13.3
^{14}C-source leaves	42.3 ± 4,5	49.2 ± 4,4	46.0 ± 1,8	21.4 ± 2.8
^{14}C-stem	24.2 ± 3.4	31.5 ± 8.1	28.5 ± 4.2	57.1 ± 13.2
^{14}C-source part summarized	66.5	80.6	74.5	78.5
Below ^{14}C-source part	25.7 ± 4.8	9.5 ± 3.1	10.4 ± 2.0	8.0 ± 4.4
Above/Below part	3.29	0.97	0.69	0.60
Uptake of $^{14}CO_2$ (% control)	100	88	86	25

Table 7. The influence of sodium nitroprusside solution introduced into the flax shoot with the transpiration water stream on ^{14}C distribution among different plant parts in 3 h after $^{14}CO_2$ assimilation by the middle shoot part (% total shoot radioactivity) (Batasheva et al., 2010)

SNP treatment resulted in altered photosynthetic carbon metabolism. Analysis of ^{14}C distribution among the labeled products of photosynthesis has shown that the largest relative changes occurred in the glycolate pathway compounds (serine, glycine, glycolate) and sugars (Table 8). As in the case of nitrate feeding into the apoplast (Batasheva et al., 2007) the portion of ^{14}C in sucrose decreased, leading to the lowered ratio of labelled sucrose to hexoses. Elevated monosaccharide availability relatively enhanced production of other storage sugars (oligosaccharides). As a result the ratio of sucrose to oligosaccharides reduced almost three times.

Compounds	Control (H_2O)	Sodium nitroprusside (1 mM)
Sucrose	48.0 ± 3.9	33.6 ± 1.1
Hexoses	5.3 ± 0.9	10.1 ± 1.9
Serine+glycine+glycolate	0.7 ± 0.2	4.1 ± 0.4
Amino acids	22.0 ±1.7	23.3 ± 1.8
Oligosaccharides	3.3 ± 0.9	6.2 ± 1.2
Pigments	2.6 ± 0.5	3.1 ± 0.7
Others	20.7	22.7

Table 8. The influence of sodium nitroprusside solution (1 mM) infused into the flax shoot with the transpiration water stream on ^{14}C distribution among the labeled compounds of 2.5 min $^{14}CO_2$ assimilation by leaves (% water-ethanol soluble fraction) (Batasheva et al., 2010)

On the background of lower ^{14}C content in sucrose the formation of the glycolate pathway products substantially increased, which is also a characteristic of metabolism under increased nitrate concentration in the plant. However, in distinction from nitrate treatment under nitroprusside injection ^{14}C income into amino acids did not increase compared to control. This was probably related with the absent of additional nitrogen as a substrate for amino acid synthesis.

Infiltration of plants with NO donor lead to appreciable alterations in the organization of both assimilating and conducting system cells (Fig. 9). In 30 min after beginning of feeding sodium nitroprusside into the apoplast the structural changes became obvious (Fig. 9 B, C). They were expressed in vacuolization of companion cells with appearing of a large central vacuole (Fig. 9B). The peaks of the crests of cell wall labyrinth became osmophilic and often reached the vacuole interior. Structural changes of the assimilating cell-bundle sheath cell-phloem parenchyma domain expressed in clarification of mitochondrion matrices (a place of glycine decarboxylation) and dictyosome curling into ring-shaped structures (Batasheva et al., 2010).

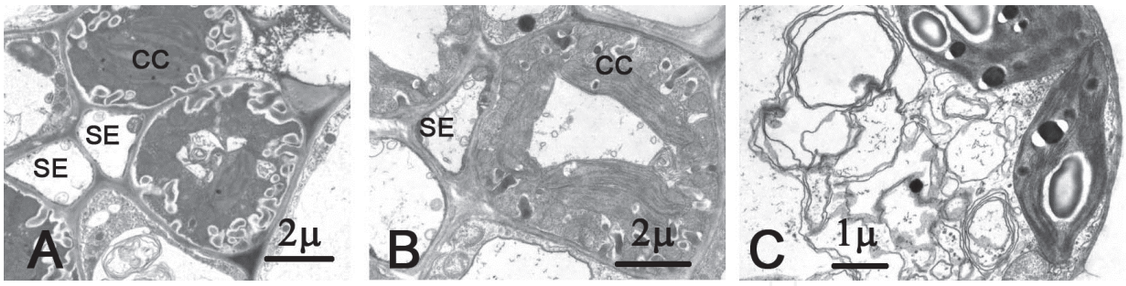


Fig. 9. The influence of sodium nitroprusside solution (50 μM) on ultrastructure of flax leaf companion cells. A – control, B, C –after 30 min of introduction of sodium nitroprusside with the transpiration water stream; CC – companion cell, SE – sieve element (Batasheva et al., 2010)

NO induced formation of numerous multimembrane and multivesicular structures in assimilating cells (Fig. 9C) in many ways analogous to those observed in 30 min after beginning of nitrate salts feeding into the apoplast (Abdrakhimov et al., 2008).

Because the effects of NO and nitrates on cell ultrastructure were similar, one can suppose that a product of incomplete reduction of nitrate, NO, is capable of inhibiting assimilate

transport directly in phloem cells. This suggestion is supported by the observation that NO is able to elevate callose content in the leaf, β-1,3-glucane, taking part, inter alia, in plugging sieve plate pores (Paris et al., 2007). Exhibitive of this mechanism is also data on increased NO synthesis (Zottini et al., 2007) and inhibited callose destruction (Serova et al., 2006) in the presence of salicylic acid, implying that the suppression of callose breakdown may be mediated by nitric oxide.

Appearing of vesicles inside the vacuole owing to endocytosis can be indicative of rising osmolarity of cell environment, probably as a consequence of sugar accumulation in the leaf apoplast due to slowdown of sugar export along the phloem.

The discovered facts allow us to propose that a likely reason of assimilate transport suppression by nitrate is generation of nitric oxide under increased nitrate concentration in cells and point to involvement of NO signal system in regulation of assimilate movement in the whole plant system.

5. A possible regulatory interaction of nitrates and sugars at alterations of other external factors

In the plant there is a regulatory link between the two main mass flows – sugars of photosynthetic origin and nitrates. The flows are heading towards each other and their interaction reacts to a change in sink-source relationships between assimilating and photosynthate consuming organs.

Because an influx of nitrates into a plant is determined by root system activity, this assimilate consumer holds a special place compared to other sink organs, competing with the latters for photosynthates. Different sink organs are uneven in their functions. Unlike other acceptors, such a sink as the root system, could provide feed-back to photosynthetic apparatus not only through sugar consumption but also through export of mineral nutrients (primarily nitrates) and triggering NO signal system (Fig. 10).

The results of the works (Batasheva et al., 2007; Abdrakhimov et al., 2008) on ultrastructure and radioautography of leaves after NO_3^- intrusion into the apoplast evidence a block to the sugar flow at the level of long-distance transport, most likely, either on the stage of transition of primary sieve tubes to the vascular bundles or when sucrose is moving along the phloem. NO donor, sodium nitroprusside, is known to induce callose accumulation (Paris et al., 2007), involved in clogging the pores in the sieve elements of phloem.

The degree of nitrate reduction is directly related to the sugar availability (Stitt & Krapp, 1999). The process of nitrate uptake has long been known to require photosynthetic energy. At low doses of nitrates and intense photosynthesis they are almost completely reduced in the roots with nitrogen coming to aboveground organs in the form of amides and amino acids. As nitrate concentration in the roots rises, in substances exported from roots to leaves a fraction of amino acids increases at first, and then also NO_3^-.

A disproportion of the two main mass flows (nitrate and sugars) is likely to be of a great importance. Here, two modes of events are possible:

1) a sudden elevation of nitrate income into the plant relatively to the existing (established) small amount of synthesized photosynthetic products (sugars);

2) sugar availability increases at unchanged (or decreased) nitrate influx into the plant.

The first may occur after additional input of mineral (nitrate) fertilizers, shortage of irradiance or partial loss of plant leaf blades, while the second, conversely, under raise of lighting, damage or cutting of root system by machinery in the course of planting treatment.

Depending on the character of such a disequilibrium the meeting of the two disturbed mass flows may occur either in the aboveground plant part or in the root system. According to the place of the meeting, generation of NO and triggering of NO signal system may take place in the root area or in the shoot. Both are also influenced by the intensity of the transpiration, that carries mineral nutrients from roots to leaves with the water stream.

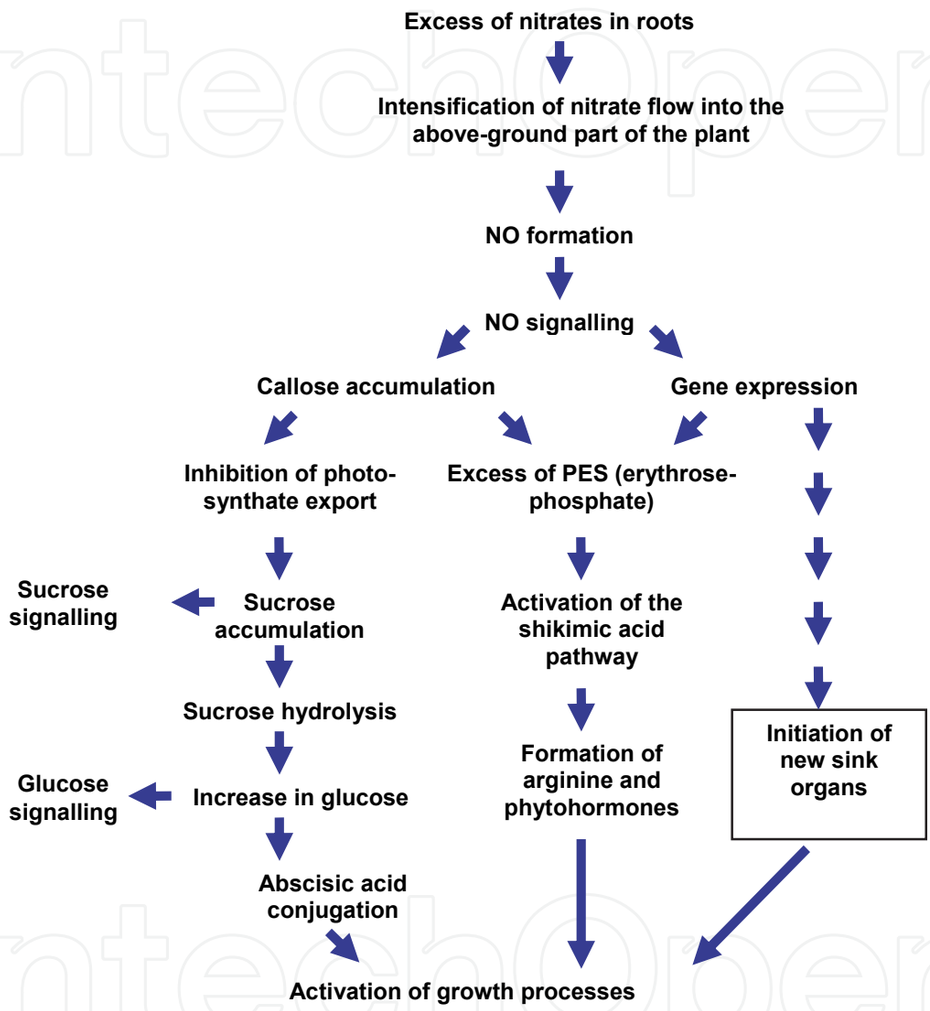


Fig. 10. Scheme of NO-signal system participation in the rearrangement of metabolism in the whole plant under increased nitrate

When excessive nitrates appear and the available nitrate reducing enzymes and their activity are not enough to utilize this massive flow of nitrates, the latters will surge into the upper plant part without having been reduced, where they will interact with the sugar flow, and NO signal system will become triggered. Oppositely, when sugars are in excess, they will be transported to roots and interact with nitrates therein.

In the first case, growth processes will be initiated in the above-ground plant part, and gradual nitrate utilization in leaves (shoots) will lead to a step-down in root nitrates, and augmented photosynthetic apparatus will send extensively increased sugar flow to the

roots. In the second case, interaction of nitrates with sugars and NO signal system triggering will occur in roots and the process of new secondary root formation will become activated.
This thesis is well illustrated by experiments of L. B. Vysotskaya (Vysotskaya, 2001). Removal of the largest part of roots from seven-days old wheat seedlings suppressed shoot growth and activated biomass growth of remaining roots in as soon as 2 h. In the remaining roots auxins and cytokinins were accumulated while in the growing part of the shoot a rapid decline of auxin content compared to intact plants was found. This suggests that excessive sugar flow to the reduced root system creates prerequisites for interaction of the changed nitrate to sugar ratio and NO signal system triggering (alike an analogue of apical dominance alleviation) and synthesis of cytokinines that activate new root formation. Initially, the prerequisites are most probably the immediate fueling of the nitrate uptake process by better sugar supply of roots. Additional nitrates, in their turn, will trigger NO signal system.
The proposed concept on the role of NO signaling in the regulation of plant metabolism is supported by split-root experiments where plant roots were exposed to culture mediums of different concentrations (Trapeznikov et al., 1999). By placing one part of roots of an individual potato plant into a medium of high concentration and the other part into low-salt one, the authors have found that in the concentrated medium a massive formation of small (absorbing) roots occurred while in the low-salt one numerous tubers appeared (Fig. 11).

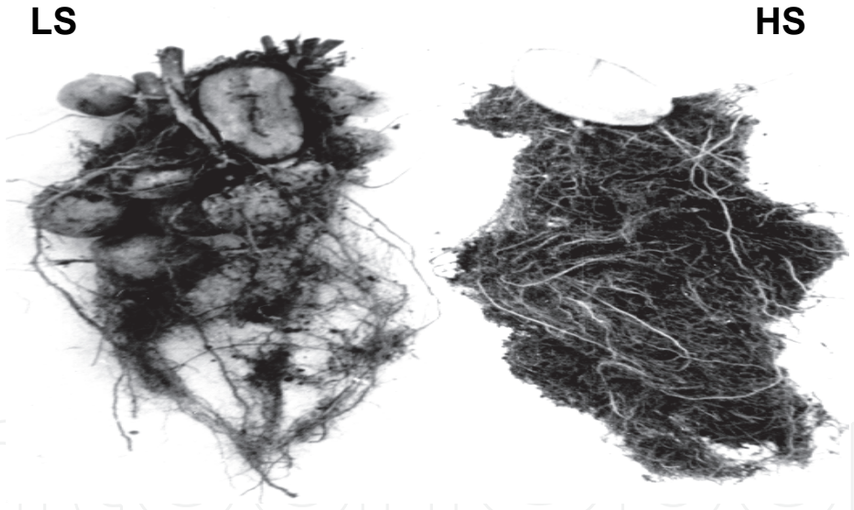


Fig. 11. Root system of an individual potato plant at local nutrition. HS – high-salt culture medium, LS – low-salt culture medium (Trapeznikov et al., 1999)

6. Conclusion

Nitrate has been shown to act as a signal molecule, inducing expression of genes, primarily related with nitrogen metabolism and organic acid synthesis. However, low sugar level in the plant inhibits nitrate assimilation, overriding signals from nitrogen metabolism (Stitt et al., 2002). In this regard a concept has appeared that for regulation of various processes in the plant not sugar and nitrate concentrations are important but a certain ratio between them which was called a C/N-balance (Coruzzi & Bush, 2001).

We believe that the link between nitrates and sugars is to be sought not at the molecular level, i.e. at the level of their metabolism in the cell or their influence on gene expression, but at a higher level - at the level of transport of these substances within the plant. This view is supported by observation that information on the nitrogen and carbon status of the plant is transmitted over long distances, revealed by the well known effect of root nitrate on the metabolism of the above-ground plant part and on shoot to root weight ratio (Scheible et al., 1997). In this connection, there is now a large group of studies devoted to the search of a "signal" coordinating shoot and root responses to nitrogen availability (Walch-Liu et al., 2005).

Activation of the hydrolysis of sucrose in the apoplast in the presence of nitrates is in good agreement with a similar effect of nitrate and sugars on the expression of several genes (Stitt et al., 2002), as well as with discerned differences in systemic and local effect of nitrate on the morphogenesis of the roots (Zhang et al., 2007). And the systemic action of nitrate is associated with its negative influence on the flow of assimilates to roots (Scheible et al., 1997).

Currently, signaling functions are ascribed not only to nitrate but also to products of nitrate reduction. Depending on the ratio of available carbon and nitrogen in the plant the ratio of oxidized and reduced nitrogen will vary. The influence of the products of nitrate reduction was noted to be opposite to nitrate influence, though the mechanism of their action is also as yet unknown, but supposed to involve glutamine content or glutamine/2-oxoglutarate ratio (Foyer and Noctor, 2002; Stitt et al., 2002).

Since an increase in amount of nitrates in the plant creates conditions favorable for the generation of nitric oxide from nitrite in both enzymatic and non-enzymatic ways (Neill et al., 2003), we can assume that the signaling effects of nitrate are partially realized through the formation of nitric oxide and triggering of NO-signaling system. This is confirmed by found similarities in actions of nitrate and nitric oxide generator, sodium nitroprusside, on assimilate transport and metabolism. However, in contrast to nitrate, nitric oxide preferably activates genes involved in plant defense (Grün et al., 2006).

Actually, the difference of nitrate and nitric oxide actions may be due to differences in the activity of amino acid synthesis, that, as was mentioned above, can also perform signaling roles. Study of the dynamics of gene expression activation under the influence of nitrate showed that many genes induced by nitrate in the first 0-5 and 5-10 minutes are subjected to negative regulation by as early as 20 minutes (Castaings et al., 2011).

Thus, there remains a lot to be elucidated in the signaling mechanism of nitrate and the study of mechanisms of nitrate influence on the transport of sugars can be very promising, not only for this area of research, but also to discovering how the different processes in the plant are interrelated.

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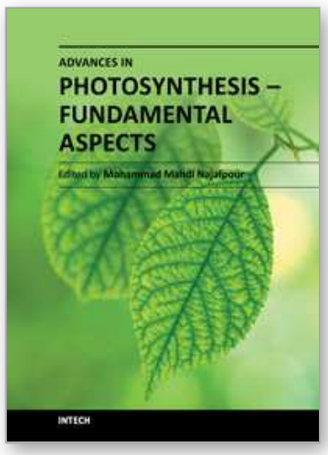

Advances in Photosynthesis - Fundamental Aspects
Edited by Dr Mohammad Najafpour

ISBN 978-953-307-928-8
Hard cover, 588 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

Photosynthesis is one of the most important reactions on Earth. It is a scientific field that is the topic of many research groups. This book is aimed at providing the fundamental aspects of photosynthesis, and the results collected from different research groups. There are three sections in this book: light and photosynthesis, the path of carbon in photosynthesis, and special topics in photosynthesis. In each section important topics in the subject are discussed and (or) reviewed by experts in each book chapter.

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