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Apoplastic transport of ^{14}C-photosynthates measured under drought and nitrogen supply

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Abstract

Using water infiltration of the plant and individual shoots with the subsequent intercellular liquid extraction by the pressure chamber, dynamics of the movement ^{14}C-photosynthates from cell to apoplast, and ^{14}C distribution among photosynthetic products in mesophyll cells and apoplast were studied. The relative quantity of ^{14}C-photosynthetes in leaf apoplast depended on growing conditions; drought increased, and nitrate supply decreased it. When the middle leaves absorbed $^{14}CO_2$, photosynthates moving down in stem phloem appeared in intercellular space, where they were transported up by transpiration stream. ^{14}C-photosynthates entering to the apex and young leaves were utilized and accumulated, and photosynthates transported to the mature leaves were reloaded into the phloem and reexported. Thus, photosynthates circulated through the plant and were redistributed to the plant organs according to their transpiration. In leaf apoplast photosynthetic sucrose was partly hydrolyzed to glucose and fructose. This increased under high nitrogen supply. The result indicate that apoplast sucrose hydrolysis is the basic cause of the reduction of photosynthate flux from leaves when the nitrate concentration in soil increases.

Additional key words: assimilate transport, bean, flax, hexoses, *Linum usitatissium, Phaseolus vulgaris,* photosynthate utilization, sucrose.

Introduction

In recent increase of interest to processes occurring in apoplast has been observed (Sonnewald *et al.* 1991, Lu *et al.* 1995, Van Rensburg *et al.* 1996). It was connected with the development of new methods for studying the apoplast content, the principle of which is the extraction of extracellular liquid from tissues and plant organ previously infiltrated by water or buffer (Chikov 1987, Li and McClure 1990).

Since apoplast of the majority of plants is an intermediate compartment in export of photosynthetic sucrose from a leaf (Kursanov 1984), the processes occurring in it seems to influence both photosynthesis and transport photosynthates. To verify this assumption, we carried out the experiments with use of our own technique. The labelled photosynthates from leaf (shoot) apoplast were extracted and the dependence of their quantity and structure on photosynthetic rate was estimated.

Materials and methods

Plants of a French bean (*Phaseolus vulgaris* L.) cultivar Triumf in a phase of first true leaf and flax (*Linum usitatissimum* L.) cultivar Novotorzhsky during fast growth period were used. Plants were grown in field conditions in 7 kg pots with air-dry grey wood soil supplied with 1 g of N, P, and K each per pot). For elucidating the role of the increased N nutrition 1 g N as $Ca(NO_3)_2$ was added. In drought treatment, watering of the bean plants was stopped till the visible wilting occurred (water potential decreased from about -0.55 to -1.35 MPa).

For the extraction of labelled photosynthates from

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apoplast the pressure chamber was used (Chikov 1987). A leaf or a shoot segment was exposed (15 s or 2 min) in $^{14}CO_2$. Then a leaf was left on a plant to assimilate $^{12}CO_2$ and within a defined time (1, 2, 3, 5, 10 and 20 min) it was cut off and, after infiltration by water, it was placed in the pressure chamber (Fig. 1). Due to pressure of the

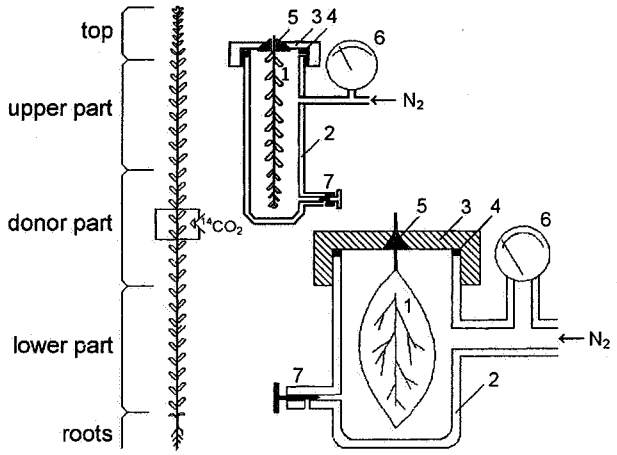

Fig. 1. Scheme of the flax plant dismemberment and a pressure chamber for the extraction of apoplast content. 1 - experimental leaf (shoot); 2 - chamber body; 3 - lid; 4 - rubber; 5 - rubber plug; 6 - manometer; 7 - drain cock.

gaseous nitrogen, the movement of a water in a direction opposite to transpiration stream was caused. The pressure was chosen previously and corresponded to water potential of experimental plants. A liquid, secreting from a leaf petiole (stem of shoot) was collected by filter paper. The secretion of a liquid was finished within 12 - 15 min, and water potential of a leaf was restored up to the initial (up to infiltration by water) level. The collected apoplast liquid was analyzed for the content of labelled ^{14}C-substances.

In experiments with extraction of labelled substances from the apoplast of flax, a central part of shoot (Fig. 1) absorbed $^{14}CO_2$. The plants were of 40 cm high. The size of the leaf photosynthetic chamber was 2.0 × 2.5 cm. After exposure in $^{14}CO_2$ a shoot was taken from the ground and divided into three parts, equal in length. The ^{14}C-absorbed site remained in the middle section of shoot. Each part of shoot was infiltrated by water and placed in the own pressure chamber. In all three pressure chambers the pressure was created simultaneously by gaseous nitrogen for extraction of a liquid from intercellular spaces.

After the collection of apoplast liquid the remained leaf (shoot) and apoplast content was fixed by 80 % boiling ethanol. The shoot of flax was previously divided into three parts. Then the samples were analyzed to estimate the radioactive ^{14}C-carbon. Water-ethanol solution of labelled substances from leaves and apoplast was analyzed using paper chromatography and radioautography as described earlier (Chikov *et al.* 1985). Radioactivity was determined using liquid scintillation spectrophotometer *Delta-300* (*Tracor Analytic*, USA). The content of labelled photosynthates in apoplast was expressed in percentage of radioactivity of all shoot. The experiments were carried out in 6 - 7 biological replications. The arithmetic means with standard errors are given in tables and figures.

Results

The content of ^{14}C-photosynthates in apoplast reached no more 5 - 8 % from a common radioactivity of a leaf (Fig. 2). In 3 - 5 min the content of ^{14}C in this compartment reached a maximum, which after some decrease was kept at a constant level during all time of supervision. It means, that photosynthates quickly enter the extracellular space, and then for a long time the stationary flow of ^{14}C-photosynthates from mesophyll cells through apoplast to phloem is kept.

Under water stress, the accumulation of ^{14}C-photosynthates in apoplast was observed. It allows to conclude, that under drought a "bottle neck" in photosynthesis is not a low assimilation of CO_2 and formation of primary products of photosynthesis, but an export of the already formed products of photosynthesis from mesophyll cells through apoplast to phloem.

To confirm the assumption on an efflux of photosynthates from stem cells in apoplast, in the next experiment ^{14}C-photosynthates were taken from apoplast of different section of flax shoot. For this purpose $^{14}CO_2$ within 2 min was introduced into an middle part of shoot.

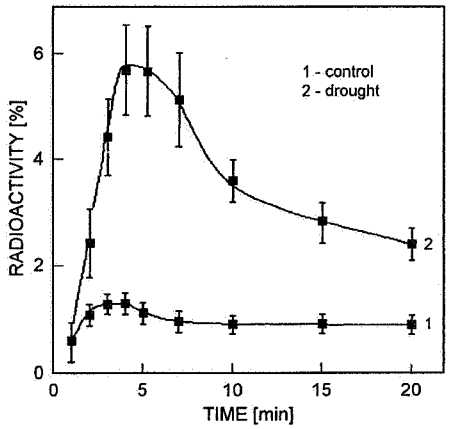

Fig. 2. Dynamics of the output of ^{14}C-photosynthates in leaf apoplast of a bean after 15-second photosynthesis in $^{14}CO_2$ [% of total leaf radioactivity].

Then after 5 min a plant was cut off, divided in three sections, equal in length, and the labelled products of photosynthesis were extracted from each with the help of the pressure chamber. The experiments have confirmed an efflux of photosynthates from cells in apoplast of shoot. In this experiment, the labelled carbon was much more in the top part of shoot, than in bottom. It correlated with the large contents of ^{14}C in apoplast of these section of shoot. In apoplast 3 - 7 times more ^{14}C in the top section than in bottom.

In direction to the top part of shoot ^{14}C was transported with a rate exceeding 10 m h^{-1}, while downwards it about 0.4 - 0.5 m h^{-1}. Latter rate was equal to the known rate of transport of photosynthetic products on phloem (Kursanov 1976). The fast transfer of labelled carbon to the top part of shoot (first of all, in leaves) suggests to their output in apoplast during movement on a stem phloem, from where they are transported with transpiration stream upwards, because only water moves with such rate in plants. It is known, that the transpiration rate is 100 and more times higher than that of photosynthesis.

Table 1. The ^{14}C distribution [% of total plant radioactivity] along the plant (5 min or 24 h after 2 min $^{14}CO_2$ pulse in the middle part of shoot) as affected by nitrogen nutrition (* - all parts together). Means ± SE; radioactivity in parentheses [kBq].

Plant parts		Non-fertilized plants 5 min	24 h	N-fertilized plants 5 min	24 h
Parts above	top	0.9 ± 0.04 (17.6)	2.8 ± 0.5 (34.8)	0.3 ± 0.04 (13.6)	2.3 ± 0.3 (60.8)
^{14}C-donor part	leaves	3.8 ± 0.60 (74.0)	1.0 ± 0.2 (12.4)	4.0 ± 0.25 (180.8)	1.0 ± 0.2 (26.4)
	cortex	1.8 ± 0.08	1.1 ± 0.3	2.3 ± 0.30	1.7 ± 0.3
	wood	1.2 ± 0.06	2.9 ± 0.5	0.4 ± 0.05	2.8 ± 0.3
	wood/cortex	0.67	2.65	0.17	1.65
	apoplast	0.7 ± 0.04		0.3 ± 0.02	
^{14}C-donor part	leaves	72.6 ± 0.40	50.3 ± 0.9*	73.5 ± 0.60	56.7 ± 2.1*
	cortex	14.5 ± 0.30		13.3 ± 0.20	
	wood	1.4 ± 0.20		2.1 ± 0.10	
	apoplast	1.7 ± 0.10		1.9 ± 0.10	
Parts below	leaves	0.9 ± 0.10 (17.6)	0.6 ± 0.2 (7.6)	1.1 ± 0.10 (49.6)	0.5 ± 0.1 (13.2)
^{14}C-donor part	cortex	0.3 ± 0.05	7.0 ± 0.9	0.4 ± 0.05	7.3 ± 0.7
	wood	0.2 ± 0.02	21.8 ± 2.2	0.3 ± 0.02	19.2 ± 2.0
	wood/cortex	0.67	3.11	0.75	2.63
	apoplast	0.1 ± 0.07		0.1 ± 0.03	
	roots		12.5 ± 0.4		8.5 ± 1.2

Table 2. Distribution of ^{14}C [% of the water-ethanol-soluble fraction] among labelled products of photosynthesis in leaves and apoplast of top and donor parts of the flax after 5 min of $^{14}CO_2$ assimilation by a middle part of shoot. Means ± SE.

	Compounds	Upper part leaves	apoplast	^{14}C-donor part leaves	apoplast
Non-fertilized plants	sucrose	73.5 ± 0.6	89.7 ± 0.2	60.9 ± 0.5	89.6 ± 0.5
	fructose	2.2 ± 0.2	0.3 ± 0.2	1.0 ± 0.1	0.3 ± 0.0
	glucose	2.6 ± 0.2	0.3 ± 0.1	2.6 ± 0.1	0.4 ± 0.1
	amino acids	10.0 ± 0.6	4.1 ± 0.2	15.1 ± 0.3	3.9 ± 0.4
	malate	3.2 ± 0.2	2.2 ± 0.1	4.0 ± 0.1	2.1 ± 0.1
	other compounds	8.5 ± 0.2	3.4 ± 0.8	16.4 ± 0.7	3.7 ± 0.1
	sucrose/hexoses	15.0	149.5	16.9	128.0
N-fertilized plants	sucrose	61.5 ± 0.3	77.1 ± 0.5	57.1 ± 1.0	76.2 ± 1.1
	fructose	1.6 ± 0.2	1.0 ± 0.2	1.5 ± 0.1	1.0 ± 0.1
	glucose	3.7 ± 0.1	1.1 ± 0.2	2.7 ± 0.1	1.0 ± 0.1
	amino acids	15.7 ± 0.5	11.7 ± 0.5	17.2 ± 0.6	11.1 ± 0.7
	malate	4.7 ± 0.7	3.4 ± 0.4	5.1 ± 0.3	3.7 ± 0.4
	other compounds	12.8 ± 0.7	5.7 ± 0.4	16.4 ± 0.3	7.0 ± 0.4
	sucrose/hexoses	11.6	36.7	13.6	38.1

In the top part of shoot most of all labelled photosynthates transported in the mature leaves, which transpirated more than others. ^{14}C was much less transported to young leaves with poorly developed stomata. An elevated nitrogen nutrition of plant has reduced the content of labelled photosynthates in apoplast of the top part of shoot (Table 1). As a result, transport of ^{14}C to a top became less.

The analysis of distribution of ^{14}C along a plant in 24 h after $^{14}CO_2$ assimilation has shown (Table 1), that the most part of labelled carbon exported from donor part of shoot, has appeared already in the bottom part of a plant. For this period the content of ^{14}C in a top has increased, but in growth-finished leaves has decreased. The reduction of a radioactivity in mature leaves of the top section of shoot was observed not only in relative units, but also in kBq (Table 1). From all quantity of the labelled carbon which has transported to top leaves in the first minutes, in 24 h remained no more than 17 %. 24 h after $^{14}CO_2$ absorption, the most part of the exported labelled carbon appeared in wood (especially in the bottom part of shoot), thus there was an increase of the wood/cortex relation. At once after $^{14}CO_2$ assimilation it was much less than 1.0, and in 24 h it was more than 1.5 - 2.0.

Contents of apoplast and leaves were analysed for inclusion of ^{14}C in different labelled low-molecular substances. The results of the analysis have shown, that the main part of ^{14}C in apoplast contained in sugars, first of all in sucrose (Table 2). It is remarkable, that distribution of ^{14}C among the labelled substances was nearly identical in apoplast as in top, as in donor section of shoot. It suggest about structural similarity of substances entering the apoplast of leaves and stems.

Discussion

The labelled photosynthates transported to top, mature leaves are utilized in them to a small degree, and in the most part are transported repeatedly in phloem of leaves-acceptors of ^{14}C-photosynthates and reexported. Being transported on stem phloem downwards, these photosynthates can, apparently, enter the apoplast again. So it is possible to conclude, that photosynthates circulate on a plant, falling downwards on phloem vessels, and upwards with transpiration water. Probably assimilate circulation in apoplast is of significance in forming flax fiber and its polysaccharide composition, so far as the own flax stem photosynthates are not transported in other plant parts and are used where they were synthesized (Gorshkova *et al.* 1996, Chikov *et al.* 1997).

The distribution of ^{14}C among products of photosynthesis in leaves and apoplast differs essentially. In leaves, much more radioactivity is contained in amino acids and hexoses, but less in sucrose. The free labelled hexoses can be formed as result hydrolysis of newly synthesized ^{14}C-sucrose. It is known, that invertase is present in various compartments: in cytosole, vacuole, and in apoplast. The increase in activity of any of them results in use of sucrose carbon for the synthetic processes inside assimilating cells, and also for accumulation of starch and soluble sugars in mesophyll cells (Sonnewald *et al.* 1991). The ratio of radioactivity of labelled sucrose and hexoses is a sensitive parameter of influence of the most different effects on photosynthesis. Any effect, supressing photosynthesis or lowering assimilate export, results in decrease of sucrose synthesis and decrease of the relation of radioactive sucrose/hexoses (Chikov 1987).

The leaves of plants grown in conditions of the enhanced nitrate nutrition have relatively low export function (Tarchevskiĭ *et al.* 1973). According to our data (Table 2) the enhanced nitrate feed of plants also has resulted in decrease of sucrose/hexoses relation, and more significant in apoplast. It allows to conclude, that in conditions of the enhanced nitrate nutrition the activity of only apoplast invertase increases. Proceeding from this, it is possible to state a hypothesis on the mechanism of inhibiting action of a nitrate feed on outflow of photosynthates from a leaf. The essence is in the following: a relative decrease of export of photosynthates from a leaf is caused not by decrease of sucrose synthesis, which is a transport product of photosynthesis, but by hydrolysis in apoplast of the sucrose already formed. The subsequent utilization of hydrolysis products (hexoses) in mesophyll cells has result in the intensified growth of leaf tissue. The activation of apoplast invertase in conditions of the increased nitrate feed, probably, occurs as a result of decrease of pH of the apoplast liquid, since the optimum of this enzyme is in acidic pH area (Kursanov 1976). The local decrease of pH in apoplast can be a result of enhancing of H^+ATPase functioning on a surface of the cells-companions of the phloem endings, that was well shown in the original work (Bouchepillon *et al.* 1994).

There are data about NH_3 gas exchange between a leaf and a external space (Mattsson *et al.* 1997). The emission of NH_3 increased with increasing leaf temperatures and irradiance. Compensation points for NH_3 were different for wild-type and mutant plants. Compensation points for NH_3 were estimated on the basis of apoplastic pH and NH_4 concentrations. Thus, NH_3 take part in formation pH in apoplast. This influences invertase activity probably too.

The participation of apoplast in photosynthesis

regulation is possible through other mechanisms. It is shown (Lu *et al.* 1995), that sucrose excreted to apoplast can move with transpiration and as a result of water evaporation its concentration in the apoplast liquid surrounding the stomatal guard cells can raise. The latter can be the initial factor in the mechanism of stomatal closing, since the water potential of an external solution can exceed that of stomatal guard cells.

The analysis of the abscisic acid (ABA) contents in chloroplasts, mesophyll cells and leaf apoplast has allowed the authors of work (Rensburg *et al.* 1996) to express doubt on the chloroplast origin of ABA. The data, received by them suggest rather a root origin of this phytohormone.

References

Bouchepillon, S., Fleuratlessard, P., Fromont, J.C., Serrano, R., Bonnemain, JL.: Immunolocalization of the plasma membrane H^+-ATPase in minor veins of *Vicia faba* in relation to phloem loading. - Plant Physiol. **105**: 691-697, 1994.

Chikov, V.I.: Fotosintez i Transport Assimilyatov. [Photosynthesis and Assimilate Transport.] - Nauka, Moskva 1987. [In Russ.]

Chikov, V.I., Bakirova, G.G., Ivanova, N.P., Nesterova, T.N., Chemikosova, S.B., Gorshkova, T.A., Lozovaya, V.V.: [Assimilation of labeled carbon by different parts of fiber flax plant and its distribution.] - Fiziol. Biokhim. kul't. Rast. **29**: 93-99, 1997. [In Russ.]

Chikov, V.I., Bulka, M.E., Yargunov, V.G.: [Effect of a removal of reproductive organs on photosynthetic $^{14}CO_2$ metabolism in cotton leaves.] - Fiziol. Rast. **32**: 1055-1063. 1985. [In Russ.]

Gorshkova, T.A., Wyatt, S.E., Salnikov, V.V., Gibeaut, D.M., Ibragimov, M.P., Lozovaya, V.V., Carpita, N.C.: Cell-wall polysaccharides of developing flax plants. - Plant Physiol. **110**: 721-729, 1996.

Kursanov, A.L.: Transport Assimilatov v Rastenii. [Assimilate Transport in a Plant.] - Nauka, Moskva 1976. [In Russ.]

Kursanov, A.L.: [Endogenous regulation of assimilate transport and source-sink relation in plants.] - Fiziol. Rast. **31**: 579-595, 1984. [In Russ.]

Li, Z.-C., McClure, J.W.: Soluble and bound apoplastic protein in isozymes of peroxidase, esterase and malate dehydrogenase in oat primary leaves. - J. Plant Physiol. **136**: 398-403, 1990.

Lu, P., Zhang, S.Q., Outlaw, W.H., Riddle, K.A.: Sucrose: a solute that accumulates in the guard-cell apoplast and guard-cell simplast of open stomata. - FEBS Lett. **362**: 180-184, 1995.

Mattsson, M., Hausler, R.E., Leegood, R.C., Lea, P.J., Schjoerring, J.K.: Leaf-atmosphere NH_3 exchange in barley mutants with reduced activities of glutamine synthetase. - Plant Physiol. **114**: 1307-1312, 1997.

Sonnewald, U., Brauer, M., von Schaewen, A., Stitt, M., Willmitzer, L.: Transgenic tobacco plants expressing yeast-deriver invertase in either the cytosol, vacuole or apoplast: a powerful tool for studying sucrose metabolism and sink/source interaction. - Plant J. **1**: 95-106, 1991.

Tarchevskiĭ, I.A., Ivanova, A.P., Biktemirov, U.A.: [Transport of assimilates in wheat and the effect of mineral nutrition on this process.] - Tr. biol.-pochv. Inst., dal'nevost. nauch. Tsentr Akad. Nauk SSSR **200** (123): 174-1789, 1973. [In Russ.]

Van Rensburg, L., Kruger, H., Breytenbach, J., Coetzee, J., Van der Merwe, C. F., Van Aswegen, G., Kruger, G.H.: Immunogold localization and quantification of cellular and subcellular abscisic acid, prior to and during drought stress. - Biotech. Histochem. **71**: 38-43, 1996.