Regulation of Physiological and Biochemical Processes in an Intact Plant Is Determined by Interaction of Flows of Substance Bulk Transfer

Chikov Vladimir

Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

Email address:

vichikov@bk.ru

Abstract: The analysis of characteristic features of photosynthesis alterations, assimilate transport, growth function, and plant leaf ultrastructure under the changes in sink-source relations (SSR) between photosynthetic and sink organs is carried out, as well as the level of nitrogen (nitrate) nutrition. Under modeling conditions, there is shown NO-triggering mechanism of inhibition of assimilate export from leaves which becomes involved with the increase in the plant apoplast nitrate level. The concept that the plant metabolism regulation under the changes of environmental conditions is realized through the interaction of counter flows of nitrates and photoassimilates. This interaction involves changing in the degree of reduction of absorbed nitrates resulting in NO formation. By activating the callose synthesis, NO plugs pores in sieve tubes and inhibits sugar transport along the phloem. Numerous genes are activated under the effect of NO signal system. Out of all enzymes synthesized due to the expression of these genes, there function and change the metabolism only the ones for which cofactors and substrates resulting from SSR disturbance exist. Such reorganization of metabolism occurs every new photoperiod in accordance with new levels of assimilates and nitrates in plants.

Keywords: Photosynthesis, Nitric Oxide, Apoplast, Regulation

1. Introduction

Recently the role of C/N ratio in the regulation of plant metabolism is discussed by many researchers. Most researches in this field are focused on independent particular reactions of nitrogen metabolism, enzyme systems and their genetic providing. There are proposed various mechanisms of integration of regulatory processes in intact plants, such as turgor (Walch-Liy, Filleur, Gan, Forde, 2005) and concentration of inorganic phosphate or individual carbon metabolites (Paul, Pellny, 2003), protein carriers (Ainsworth, Bush, 2011), and phytohormones (Paul, Foyer, 2001). The phloem along which the movement of messenger molecules occurs is also proposed to be the integrating structure (Turgenon, Wolf, 2009; Ainsworth, Bush, 2011). The large importance is conferred to NO signal system (Lamattina, Polacco, 2006). However, all these mechanisms are not integrated into the general system of physiological and biochemical process regulation on the level of the intact plant. The origin of the trigger mechanism launching the metabolism reorganization resulting from environmental changes is not yet obvious.

On the other hand, the integration of the physiological processes of the intact plant is well traced while considering SSR between photosynthetic and assimilate sink organs (Mokronosov, 1972; Moorby, 1977; Chikov, 1987; Paul and Foyer, 2001). In this case the mechanism of photosynthesis regulation by assimilate acceptors is quite clear: it occurs by means of overloading of a plant by photosynthetic products. However the nature of the signal for switching on the metabolism rearrangement for both source and sink of assimilates is still obscure. At the same time the coupling of the root system with the shoot organs might be an integrant factor.

The present paper proposes the feasible version of integration of various aspects of regulation of physiological and biochemical processes in an intact plant. The relationship between major flows of bulk matter transfer in plants is

considered as a consolidating factor. Among all transferred along the plants compounds, three of them can be distinguished with respect to their intensity: the upward movement of transpiration water; descending phloem transport of photosynthetic sugars (from leaves to roots); and ascending flow of nitrates from roots to shoots.

2. Donor-Acceptor Relations Between Photosynthetic and Assimilate Sink Organs

Numerous studies of SSR convince of the fact that photosynthesis is determined by the leaf exportation function. However the results of studies of the effect of SSR disturbance on leaf photosynthesis were often contradictory. For example, Mokronosov, Ivanova (1971) showed that the reduction of the leaf surface, instead of assumed assimilate deficit and photosynthesis increase in the remained leaves, resulted in a long term photosynthesis stimulation only in case of minor impact (removal of 20% of leaves). The removal of 50% and more of the leaf surface caused brief stimulation followed by continuous photosynthesis depression.

In another set of experiments Chikov (1987) showed that the decrease in the accepting capacity of sink organs, which caused the assimilate redundancy in plants, reduced the photosynthesis of mature leaves – assimilate source - only during the first two days, and then the process activated. Thus the overloading of a leaf with photosynthetic products did not only slow down the photosynthesis, but also stimulated photooxydative processes (Lenz, 1977; Hall, Brady, 1977; Chikov, Isfandiarov, 1978; Chikov, Yargunov et al., 1982). And vice versa, the intensification of assimilate efflux reduced the Warburg effect (stimulation of photosynthesis by oxygen) (Chikov, 1987). The changes in Warburg effect were accompanied by congruent changes in glycolate metabolism during photosynthesis (Chikov, Bulka, Yargunov, 1985). These data allowed the conclusion (Chikov, 1987) about the importance of photooxydative processes in SSR regulation between photosynthetic and assimilate sink organs.

The inconsistence of the results of experiments with SSR disturbance were explained (Chikov, 1987) by traits of interaction between the photosynthetic apparatus and root system. As it turned out (Chikov, Chemikosova et al., 1984; Chikov, 1987), the assimilate transport influx into roots depended on the activity of other assimilate acceptors. For example, in experiments using wheat, the influx of radioactively labeled photosynthetic products into roots during the reproductive period was inversely related to dry matter accumulation in spikes (Chikov, 1987). During the blooming period when qualitative changes in spike caryopses took place without any significant dry matter accumulation, the assimilate efflux into roots increased compared to booting phase. After blooming the developing caryopses attracted the assimilate flow into spikes. The transport of photosynthetic

products into spikes increased, and the one into roots decreased multiply. Chikov, (1987), concluded that spikes and roots competed for assimilates. It was confirmed in experiments when a part of a spike was removed, which resulted in multiple increase in the assimilate supply of roots. This increase resulted in the intensive root growth. It is characteristic, that there increased the growth of not only the roots, but also other organs including the truncated spike (Chikov, 1987).

SSR disturbance resulted in changes in the direction of carbon photosynthetic metabolism (CPSM). Under the photosynthesis inhibition by any factor, the synthesis of sucrose reduced, and the inclusion of ^{14}C into aminoacids increased relatively $^{14}CO_2$ fixation (Tarchevskii, 1965; Chikov, Bulka, Lozovaya, 1972; Chikov, Bulka, Yargunov, 1985; Chikov, 1987). A conclusion could be drawn that the top position of all regulatory mechanisms of SSR belonged to the correlation between nitrogen and carbon in a plant, but not the carbohydrate status (Paull & Pellny, 2003). As mentioned above, the SSR disturbance altered the correlation between the leaf gas exchange constituents (Lenz, 1977; Hall, Brady, 1977; Chikov, Isfandiarov, 1978; Chikov, Yargunov et al., 1982). It affected the direction of CPSM in leaves-sources. The removal of a part of sink organs (cotton balls) reduced the inclusion of ^{14}C from $^{14}CO_2$ into sucrose and intensified its inclusion into the products of glycolate metabolism (serine, glycine glycolate). The intensive synthesis of glycolate metabolism products at the saturating concentration of CO_2 was observed after feeding of plants with the nitrate nitrogen (Chikov, Bakirova, 1999).

Moyse (1980), supposed, that the metabolism of phosphoglycolate, which was synthesized in the course of RBPC/O reaction in mature leaves, was accomplished via the closed path (p-glycolate →glycolate → glycine → serine → glycerate → PGA) and its carbon returned to the Calvin cycle in the form of PGA. It means that, during the stimulation of photorespiration, the pressure of the ^{14}C-carbon flow trended from glycolate towards PGA. If this was the case, then the stimulation of the carbon flow via the glycolate pathway should cause the increase in the ratio of the radioactivity of glycine/serine and serine/glycerate.

The validity of such approach was confirmed by calculation of these factors using the data of other researchers (increase in CO_2 concentration (Mokronosov, 1981), and decease in O_2 (Voskresenskaya, Wiil, Grishina, Pärnik, 1970)). At the same time, if carbon, serine, and glycine did not partially return to Calvin cycle, but were used for protein synthesis, the index serin/glycerate should decline. In the experiments with cotton plant (Chikov, 1987), the removal of cotton balls resulted in the increase in the index glycine/serine and decrease in the index serine/glycerate. It means that, during the suppression of the assimilate efflux from the leaf and activation of its growth function, less amount of carbon products of the glycolate pathway (at least serine) returned to the Calvin cycle for further sugar synthesis, and their larger amount was used for synthesis of proteins of chloroplasts and the cell in general. In this case,

the interaction of photorespirational metabolism with dark respiration increased (Bykova, Keerberg, Pärnik, 2005). It is quite obvious, that at the same time there had to take place the expression of genes by corresponding enzymes, but the triggering mechanism is not clear so far.

Since the hindrance of sugar export from leaves results in the redundant accumulation in mesophyll cells of Calvin cycle intermediates – sugar phosphates (including eritrosophosphate as well), it creates the substrate background for activation of shikimic pathway of metabolism of products of photosynthesis (Fig. 1). Due to the condensation of eritrosophosphate with phosphoenolpyruvate the chorism acid is synthesized. The latter, in the course of

further transformations, gives rise to cyclic compounds – precursors of complex aminoacids, auxins, and phenol inhibitors (Henkes, Sonnewald, Badur, Flachmann, Stitt, 2001). The synthesis of these compounds can be a part of the system of hormone cell metabolism reorganization and leaf growth function. In the case of sugar redundancy, the sucrose hydrolysis process might take place in assimilate sinks. The synthesized hexoses (and first of all, glucose) begin to enter redundantly the cytoplasm of adjacent non-assimilating cells, where their carbon can also metabolize along the shikimic pathway with formation of hormone compounds via the oxidative pentosophosphate cycle.
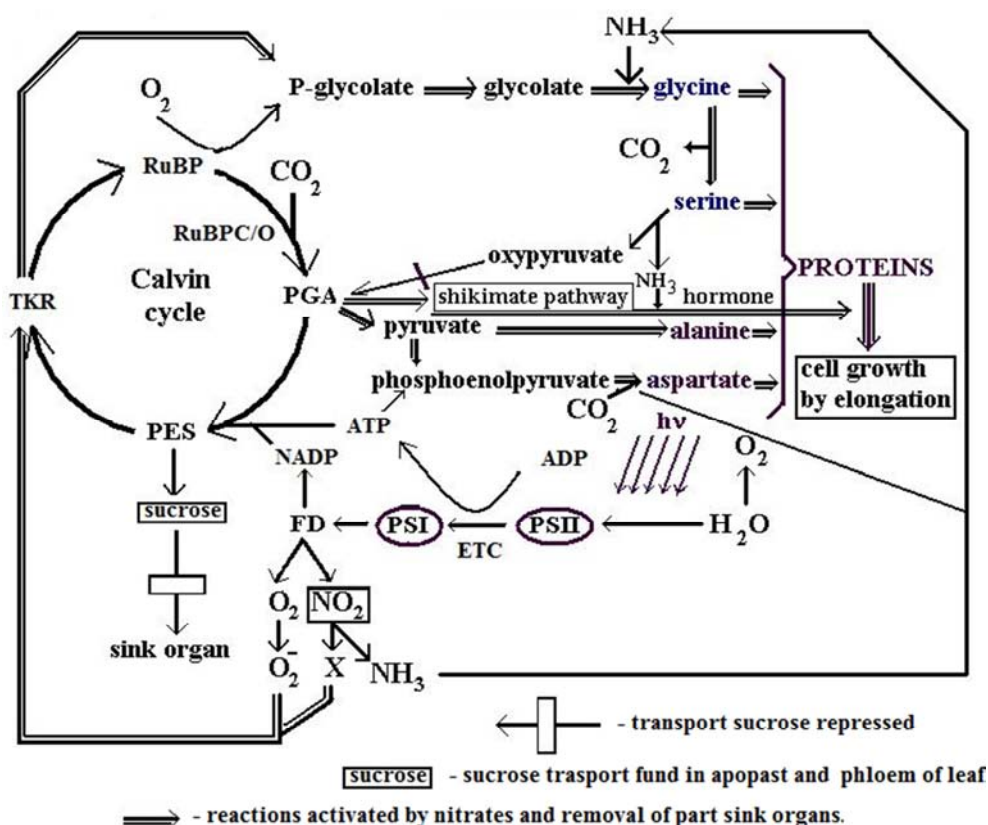
Figure 1. *Scheme of regulation of carbon photosynthetic metabolism (thick arrows: reactions activated by nitrogen and assimilate inhibition).*

ETC – electron transport chain; FD – ferredoxin; PES – phosphorous esters of sugars; PS – photosystem; RuBP – ribulose 1, 5 –bisphosphate; RuBPC/O – ribulose-1, 5-bisphosphate carboxylase/oxigenase; X – unknown oxidizer. (Chikov, Abdrakhimov, Bakirova, Batasheva, 2007).

3. Mechanisms of Assimilate Transport in Plants

It is currently accepted, that the main mechanism of the distant transport of photosynthetic products is the transfer of transport sugars along the phloem (Kursanov, 1976; 1984; Ainsworth, Bush, 2011). Only the ways of sugars loading into phloem vessels are discussed (Rennie & Turgeon, 2009; Slewinski & Braun, 2010). At the same time Minchin & McNaughton (1987) disputed this point of view. The authors fed a lupine leaf in the light with labeled [11]CO_2. Simultaneously the probes controlled the emerging of [11]C

above the leaf-source and in roots. It was found that the dynamics of the emerging of the radioactive label above the leaf-source had two components (fast and slow), and below the source there was only a slow one. Below the ^{11}C-assimilate leaf-source the ^{11}C-label appeared in 20-30 minutes, and above the leaf-source the part of ^{11}C-labeled compounds appeared during the first minutes, and another part – also in 20-30 minutes. The authors explained the obtained results by the escape of labeled assimilates into the stem apoplast and their fast transportation along the xylem dragged by the transpiration water upwards (the fast component). In their opinion, the labeled assimilates moved downwards only along the phloem (the slow component)

(Chikov, Avvakumova et al., 2001).

These experiments confirmed the conclusions by Minchin & McNaughton (1987). 5 minutes after $^{14}CO_2$ assimilation, much more ^{14}C-label occurred in the upper part of the shoot compared to the lower part. For 5 minutes ^{14}C-compounds reached the tip of the plant having traveled 25-30 cm. Only water moves along the plant at such high speed. Being dragged by water and having entered the apoplast of "alien" mature leaves, assimilates were not utilized, and they were reloaded from the apoplast into the phloem companion cells of these leaves-donors, but at the same time they were sinks for "alien" assimilates, and were reexported. As a result, the concentration of the labeled carbon in mature leaves above the donor decreased during the following 24 hours, and in young growing leaves (the tip of the shoot) it increased.

Since "alien" assimilates being retransported along the phloem, might again escape into the apoplast, then, obviously, a certain total pool of photosynthetic products from all leaves was formed in the apoplast of the whole plant, and it could be redistributed between organs, according to their transpiration. Therefore, the total vector of movement of radioactively labeled photosynthetic products along the apoplast was directed upwards.

Multiply repeated reentrance into the mature leaf phloem of assimilates, that escaped to the apoplast, eventually promoted their movement down the vessels. As a result, 24 hour later they were found to a greater degree in the lower part of the shoot. Thus, the resulting descending assimilate flow from leaves depended on the intensity of sugar escape into the apoplast and their ascending movement dragged by the transpirational water flow upwards.

So, besides the phloem (from leaves to roots) assimilate transport, there exists a fast ascending transfer of the photosynthetic products dragged by transpirational water. This transport obviously can significantly hinder the descending sugar movement along the phloem. The intensity of this process, probably, depends on sugar concentration in the phloem. The larger the concentration, the higher sugar concentration gradient exists between the phloem and the xylem, and the larger amount of sugar escapes to the apoplast. Assimilates which escaped into the apoplast are dragged upwards with water. Besides, the ascending assimilate flow shifts the ratio C/N in the intact plant, as the intensity of water transpiration from leaves increases. By diverting sugars from transportation to roots, the ascending transpirational flow intensifies nitrate absorption, but fails to reduce them all, and the larger portion of NO_3^- has an opportunity to affect the metabolism of shoots.

This concept was checked by Chikov, Salyakhova, et al. (2012). They studied the distribution of ^{14}C-assimilates throughout the organs of a potato plant, grown under various conditions of illumination (25%, 50%, and 95% of the total sunlight). $^{14}CO_2$ was inserted into the upper plate of the complex potato leaf from the middle level (as shown in Fig. 2). The export of assimilates from the leaf-donor correlated well with the descending transport of assimilates (Fig. 2).
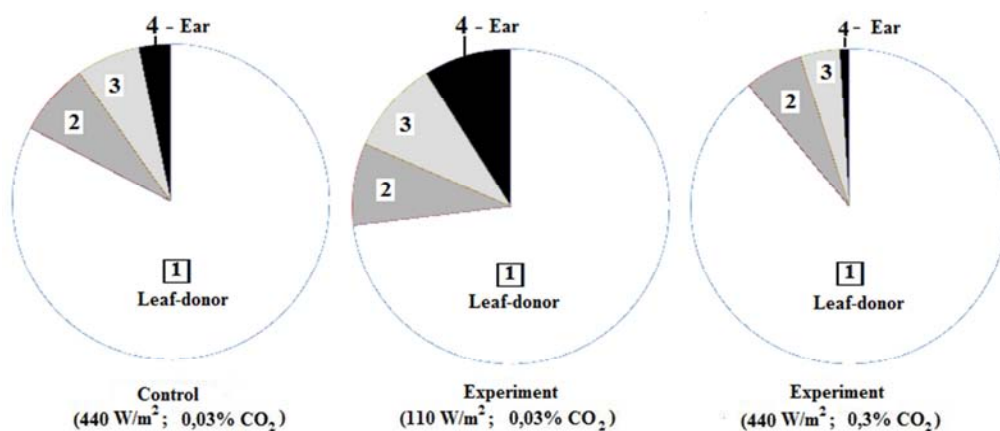
Figure 2. Effect of changes in illumination and CO_2 concentration (at the time of fixation of $^{14}CO_2$) on the distribution (%) of radioactively labeled assimilates among different organs of wheat in 1 h after 1 min $^{14}CO_2$ fixation under different conditions. Irradiance and CO_2 concentration of each treatment were, 440W/m^2 and 0.03% for the control, 110W/m^2 and 0.03% for the lowered illumination, and 440W/m^2 and 0.3% for the increased CO_2 treatment. (Batasheva, Isaeva, Chikov and Ratushnyk, 2011).

The ratio of radioactivity found above ^{14}C-donor leaf to the one below it was the largest at the maximal illumination, and was minimal at the average illumination. The difference with respect to this factor exceeded 3 times. The treatment with the low illumination had an intermediate position. Thus, the ascending transport of ^{14}C-assimilates dragged with transpirational water flow increased at the high illumination, when the overheating of leaves occurred and transpiration increased, and this hindered the transfer of photosynthetic products into tubers and roots. As a result, the relative influx of ^{14}C into tubers at the high illumination was almost twice lower than at the average and low illumination. It should be noted, that the plants at the low and high illumination had actually the same high concentration of labeled carbon in the remaining plates of the leaf-donor of ^{14}C, this factor being minimal for the plants at the average illumination.

These data can be explained under the assumption that three mechanisms of assimilate transport are working in plants simultaneously. The first one is the conventional phloem transport, which needs energy to load sugars into the

phloem against the concentration gradient. The second one (described above) is the efflux of assimilates into the intercellular space (apoplast) and their ascending dragging by transpirational water. This mechanism hinders the transport along the sieve tubes downwards and increases drastically the energy demands of the phloem transport. The third mechanism is the diffusional uniform outspread of assimilates along the symplast in all directions. The photosynthesis is known to respond fast to changes in environmental conditions (particularly, light intensity and CO_2 concentration) (Chikov, 1996). The abrupt decrease in illumination promoted the intensification of export of assimilated from a leaf by reducing their amount. On the contrary, if during the $^{14}CO_2$ assimilation the concentration of carbon dioxide in the chamber increased, then the photosynthesis increased immediately, and the export of the photosynthates relatively decreased (Batasheva, Isaeva, Chikov and Ratushnyak, 2011). Brief changes in conditions of photosynthesis also affected the transport of the products of photosynthesis to the terminal sink organs (Figure. 2).

Depression of the illumination increased the influx of ^{14}C-products of photosynthesis into the spike, and the increase of CO_2 concentration decreased it. In experiments with fast changes under the conditions of formation of photosynthetic products, the stability of the steady-state current along the conductive system from a leaf to sink organs was shown (Batasheva, Isaeva, Chikov and Ratushnyak, 2011). A certain steady-state current of photo assimilates from leaves to sink organs is formed. In case of SSR disturbance, the system tries, within the available operative opportunities (reserved pool of transported compounds and enzyme activity), to restore the existing equilibrium. If these opportunities are insufficient, the violation of the proportion between mass compound transfer currents in the whole plant occurs, the most significant among them being the counter currents of sugars and nitrates.

4. Interaction of Nitrogen and Carbon Metabolism

The important element of the plant mineral nutrition is nitrogen, which affects the growth processes and formation of crop. However nitrogen fertilizers might cause negative effects either. One of them is the excessive growth of leaves under the conditions of the intensive nitrogen nutrition. It was conveniently explained by the increased synthesis of nitrogen-containing compounds from fresh products of photosynthesis, and, as a result, little sugar is left for its export from leaves. However, the study of ^{14}C distribution among the labeled photosynthetic products of leaves after the increase in the level of nitrogen nutrition showed that sugar synthesis and export was negatively affected only by nitrate nitrogen (Chikov, 1987; Chikov, Chemikosova, Nesterova, Zernova, 1988; Chikov, Bakirova, 1999). Under these conditions the ratio sucrose/hexose radioactivity reduced significantly. The analysis of composition of labeled products

of photosynthesis in the apoplast showed (Chikov, Avvakumova et al., 2001), that, in nitrate plants, the ratio sucrose/hexose in the apoplast decreased several times more crucially than in mesophyll cells.

To find out the reasons for the changes in the export function of the leaves, model experiments were carried out. Using a special device (Batasheva, Abdrakhimov et al., 2007), urea or KNO_3 solutions were fed to the cut off common flax shoot. The analysis of distribution of ^{14}C-assimilates over the plant from leaves and middle part of the shoot showed substantial difference between tested plants. Nitrates suppressed drastically the movements of assimilates downwards (along the phloem) and stimulated their ascending movements, the labeled assimilates being "accumulated" in the stem zone of the donor shoot part (Batasheva, Abdrakhimov et al., 2007). The impression was created that in the "nitrate" plants, the assimilates were not only poorly exported from mesophyll cells, but they also moved slowly along the phloem, being accumulated in the stem zone of the donor shoot part. The compounds that get into the phloem are known to escape into the apoplast in the course of movement, and their ability to move along the phloem to a large distance is determined by the possibility of their return to phloem tubes [Ayre, Keller, Turgeon, 2003].

It should be noted that the similar pattern of photosynthesis and assimilate transport response to nitrate solutions supplied to the apoplast was observed for the symplast plants as well (Khamidullina, Abdrakhimov et al., 2010) It points to the similarity of mechanisms of regulation by nitrates of physiological and biochemical processes in various plant types. All these effects, observed when nitrates were supplied to the plant apoplast (suppression of the assimilate efflux, enhancement of the ascending assimilate transport, and changes in companion cells ultrastructure), could be reproduced (Batasheva, Abdrakhimov et al., 2010) when the shoot apoplast was supplied with the solution of the NO-nitroprusside generator at concentrations 100-1000 times less than nitrates. The parity of the effect of nitrate ion in substrate concentration, and of nitroprusside in catalyzing concentration allowed the authors to conclude that all physiological effects of nitrates are triggered by NO-signal system, which is activated during the interaction between the descending sugar flow and ascending nitrate flow (Batasheva, Abdrakhimov et al., 2010; Chikov, Batasheva, 2012).

The nitrate ion is not only the substrate of assimilation, but also plays an important signaling role (Crawford, 1995). In shoots, nitrate is able to trigger the response reactions both by induction of synthesis of physiologically active compounds (e.g. cytokinines), and independently – as a result of NO formation.

In the case of the excess on nitrite in plant cells, nitrite reductase can reduce it further to NO due to one-electron transfer, the amount of NO invariably exceeding the demands for signaling (Meyer et al., 2005). Moreover, in the case of nitrite escape into the apoplast, there is possible its non-enzymic reduction to nitrogen oxide (Bethke et al., 2004).

The participation of callose in the inhibition of assimilate transport along the phloem under cold conditions was shown by Majumder et al. (1967). The data that salycilic acid effect resulted in the increased synthesis of nitrogen oxide (Zottini et al., 2007) and inhibition of callose decay (Serova et al., 2006) testified in favor of this mechanism.

5. Interaction of Sugar and Nitrate Flows - the Trigger of Metabolism Reorganization

On the basis of the above discussion, it can be supposed that NO signaling system might be one of the important triggering mechanisms of the reorganization of physiological and biochemical processes. Nitrate ion is known to be not only the substrate for amino acid synthesis, but has also an important signaling function (Crawford, 1995). Nitrate reduction is limited by the level of reductant in the cytosol (Kaiser et al., 2000). The increase in the nitrate concentration in cytosol in the terminal regions of xylem loading results in formation of the product of its incomplete reduction – nitrogen oxide (NO). Nitrate reductase is supposed to be the main source of NO production (Meyer et al., 2005). In the case of nitrite redundancy in the cytosol, when its reduction in the chloroplasts is limited, nitrate reductase reduces it in the cytosol to NO. Moreover, when nitrite escapes to the apoplast, it can be reduced to NO by non-enzymatic means (Neill, Desikan, Hancock et al., 2003; Bethke et al., 2004).

The absorption of nitrate, its reduction, and fixing in the whole plant is determined by the leaf-root interaction (Stitt et al., 2002). The absorption of nitrates by roots and their transportation to shoots occur mainly in the light (Matt, Geiger, Walch-Liu, Engels, Stitt, 2001), the photosynthesis being efficiently regulated by the activity of the processes in the root system. The regulation is performed metabolically by means of changes in amino acid synthesis activity in roots and leaves from 'fresh" products of photosynthesis (Chikov, Bakirova, Batasheva, Sergeeva, 2005). The incomplete nitrate reduction under the conditions of incoordination (at least during the initial period of time) of their amount with the potentials of reducing enzymes promote the emerging of NO which triggers the metabolism reorganization by means of NO-signal system. This disproportion might appear both in roots and leaves and between these end points as well.

The degree of nitrate reduction in roots, leaves or in tissues between them depends on these initial conditions. At various concentrations of NO_3^- in soil solution the interrelation leaf-root is formed differently. At low nitrate concentration they are completely reduced and assimilated in roots. As the nitrate concentration in soil solution increases (it might happen as a result of supply with nitrogen fertilizer or enhancement of the nitrifying activity of soil microbial flora) initially the steady-state level of root cell reducing system won't be able to assimilate new amount of nitrates. As a result NO appears in the root cells. Initiation of the NO signal system triggers the process of formation of new roots and the

increase in the absorbing surface. The root response to the increase in the nitrate concentration is demonstrated in (Chikov, Batasheva, 2012) on potato plants. In their experiments, the mass of absorbing roots intensively increased as the nitrate concentration increased. At the low nitrate concentration carbohydrate products of photosynthesis accumulated in tubers. The exuberant root growth also happened when only a part of roots were placed into nutrient media with high concentration of nitrates.
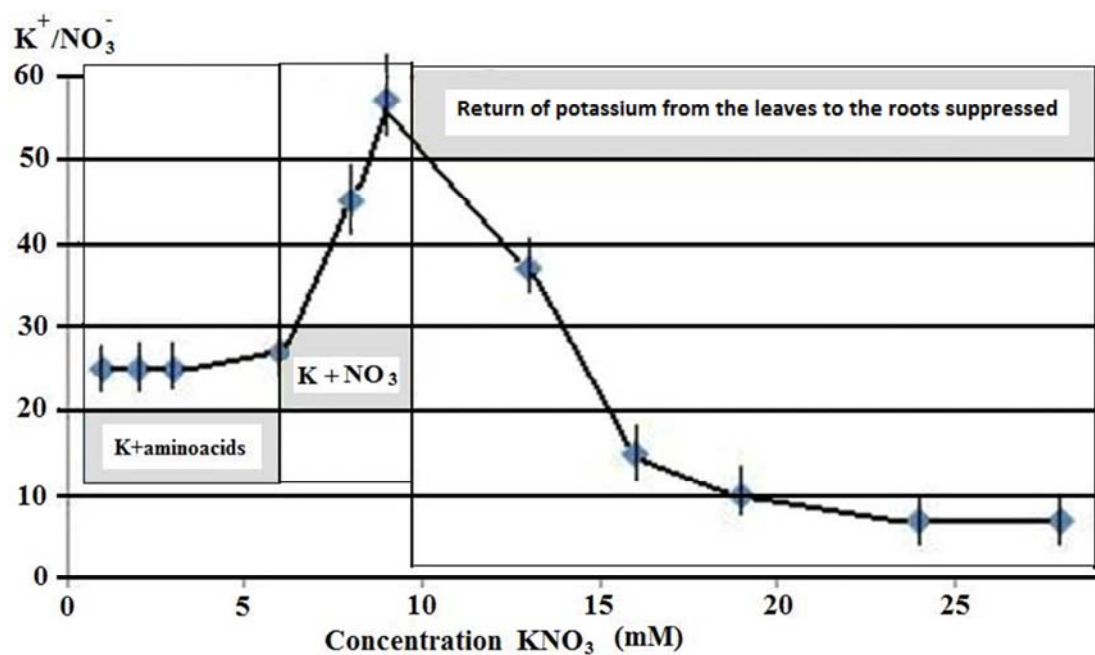
At low NO_3^- concentration in the nutrient (soil) solution, nitrate is completely reduced in roots, and its concentration in the root xylem sap is minimal (on figure 2 - zone K+aminoacids) The nitrogen gets to leaves from roots in the form of amino acids. With the increase in NO_3^- in solution, NO_3^- and K^+ begin to supply shoots along the xylem (zone $K+NO_3$). In mesophyll cells nitrate is utilized, and K^+ returns to roots together with the malate anion (Lips, 1997; Peuke, Jeschke, Hurtung, 2002) to receive a new portion of nitrate.

Since the concentration of compounds in the phloem is much larger than in the apoplast (including its stem and root parts), then, when the shoot is cut off, the potassium, which returns to roots along the phloem, turns out to be present in the root xylem sap flowing out of the stub, and the ratio K^+/NO_3^- increases (fig. 3). Just this is the reason that the ratio K^+/NO_3^- in the root xylem sap is very high (more than 20) at any nitrate concentration in the nutrient solution. NO can be synthesized from nitrate in the stem apoplast, including the non-enzymatic path [Bethke et al., 2004], which induces callose synthesis (Paris, Lamattina, Casalongue, 2006). Callose plugs pores in the phloem sieve tubes and impedes sucrose transport along the phloem. This process results in inhibition of assimilate efflux from leaves. However, the nitrates from roots continue to enter leaves in the increasing amount and are accumulated in vacuoles. As a result the ratio K^+/NO_3^- falls to the level, lower than that which existed for the equivalent replacement of nitrate by potassium (fig. 3 zone "return of potassium from the leaves to the roots suppressed").

The nitrate nitrogen which is supplied to shoots will reduce in leaves. Similar to roots, the amount of nitrate will be larger than the reducing potential of cells and there will emerge NO which will trigger NO-signal system, this time in shoots. As a result of the excitation of genetic apparatus by the NO-signaling system, there will begin the synthesis of new enzymes and hormones. The results by Trevisan et al. (2012) suggest that prolonged nitrate depletion may induce post-transcriptionally the expression of target genes by repressing the transcription of specific miRNAs.

As it was described above, the additional substrates for their synthesis and functioning are synthesized (when SSR are disturbed) by utilization of assimilates by mesophyll cells themselves, including through the shikimic pathway. This triggers formation of new structures and tissues in shoots. Thus, a conclusion can be drawn that the observed jump in the relation K/NO_3 in figure. 3, probably, reflects the moment of switching the metabolic processes in shoots resulting from interaction of nitrate and sugar counter flows.

Figure 3. *Dependence of the ratio K/NO in xylem sap of sea blite (Suaeda altissima (L)) shoots on KNO₃ concentration in the nutrient solution. We calculated it using the data from Khalilova (2008).*
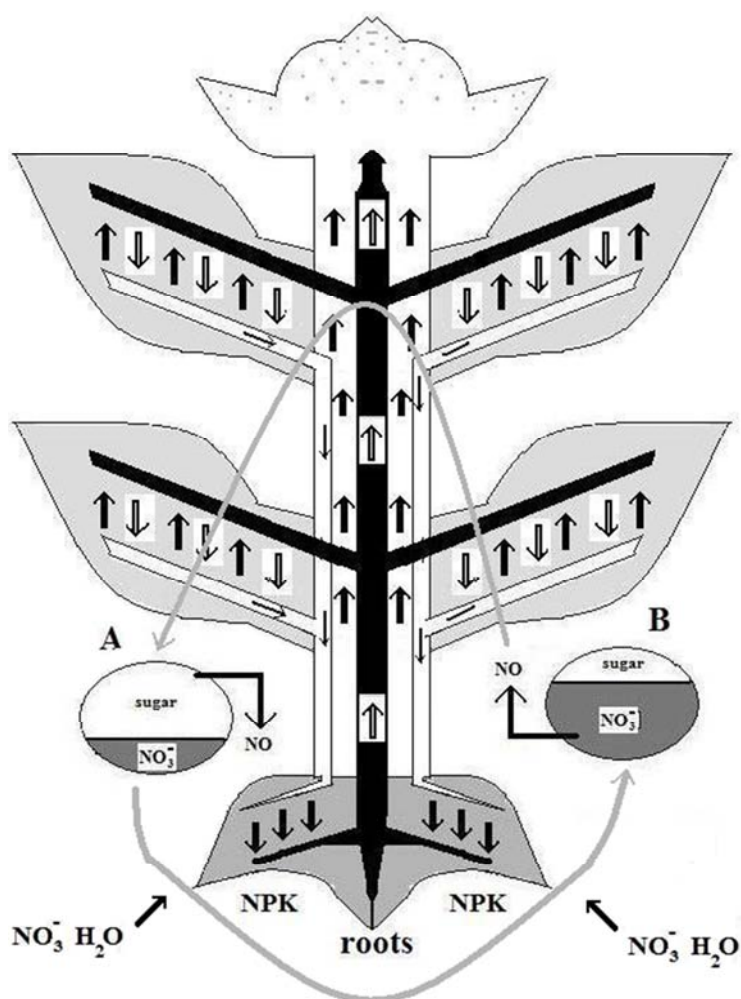
Figure 4. *The scheme of the assimilate circulation mechanisms in plants and metabolism adaptation initiation in response to environmental changes.*

Situations:

A - Surplus of products of photosynthesis. Growth of roots and absorption of nitrates increase.

B - Deficiency of products of photosynthesis. Nitrates are transported upwards. New vegetative shoots are forming.

Designation:

⇑ – water movement;

↑ ↑ – assimilate movement.

‖ -phloem

The interaction of nitrate and sugar counter flows, as it is clear nowadays (fig. 4), can happen on different stages. Initially the processes of metabolism reorganization start, leading to formation of new structures and tissues in roots, and then the similar process develops at different levels in shoots instigating new formation of structures from the lower part of the shoot to the top. Therefore new auxiliary buds start to form in the lower part of the shoot.

From figure. 4 the following order of events is supposed:

A – Excess of sugars in leaves transporting into roots. This means intensification of nitrate uptake and reduction → NO arrival into roots → stimulation of the de novo synthesis and root growth → excess of nitrates in roots → intensification of the nitrate transport into shoots → (B);

B - Excess of nitrates in roots transporting into shoots leads → arrival of NO into shoots → inhibition of sugar export from leaves along the phloem → stimulation of formation of apical points and shoots → increase in leaf area → increase in net photosynthesis → excess of sugars s is transported into roots → (A).

Thus, any change in plant existence conditions results in the disturbance of the ratio sugar/nitrate flows, which triggers new metabolic processes either in roots, or in shoots (as shown in Fig. 4) by means of NO signal system. These changes then affect the metabolism of the plant another part. The current sugar/nitrate ratio can move along the plant upwards or downwards triggering changes in the metabolism of the shoot appropriate canopy. The fact that potassium ions do not return from leaves into roots for the new portion of nitrate ions results in KNO_3 accumulation in the vacuoles of mesophile cells and, therefore, instigates them to proceed the growth by extension.

Various SSR disturbances affect the interaction leaf-root only during the next 24 hours (Chikov, Bakirova, Batasheva, Sergeeva, 2005). The changes in the photosynthetic carbon metabolism at SSR disturbances might be quite significant (Chikov, 1987). They remain in the leaf the next day as well (Chikov, Bakirova, Batasheva, Sergeeva, 2005). In our experiments SSR disturbance affected the composition of labeled compounds in the bleeding sap. The decrease in the assimilate flow to roots (plant defoliation) resulted in the decrease in the amount of labeled compounds in the root xylem sap next morning, and the increase in the assimilate flow to roots (removal of sink organs) increased the latter. Its composition also changed. Defoliation diminished the content of malate in the root xylem sap by 5-6 times.

However, already the next day, the amount of labeled products of photosynthesis in the root xylem sap decreased, and the difference between treatments diminished. A conclusion can be drawn that the functional time unit for the plant metabolism reorganization is one photoperiod. Each morning the physiological and biochemical processes in plants are regulated in accordance with the existing by this time compound mass transfer level.

If the factor, disturbing SSR, continued its effect (e.g., light day elongation), then every new day the increasing amount of sugars stimulated the root growth and new formation of shoots. On the contrary, if every new day the light day period shortened and the amount of assimilates decreased, then more and more nitrates entered plant shoots. At the increasing deficit of the products of photosynthesis, there will be observed qualitative changes in metabolism, such as formation of not vegetative organs, but reproductive ones with a new capability both to accept products of photosynthesis and to consume nitrogen (see Fig. 4).

The proposed mechanism of plant metabolism rearrangement under the changes in growth conditions does not cancel the participation of many other, more particular, regulation mechanisms, which can be found in literature in a great number. They can be used both on the photosynthetic apparatus level (Noctor and Foyer, 1998; 2000; Paul and Foyer, 2001), and during phloem unloading (Rosche et al. 2000; Weichert et al., 2010), and on the level of phloem loading (Leggewie et al., 2003), and, probably, they can affect the process of assimilate transport along the phloem itself. However, any particular changes in regulation of the processes of photosynthesis, assimilate transport or accumulation will meet resistance of the system of homeostasis of physiological and biochemical processes of the intact plant.

Abbreviations

SSR - sink-source relations

References

[1]   Ainsworth EA, Bush DR. 2011. Carbohydrate export from the Leaf: A highly regulated process and target to enhance photosynthesis and productivity. *Plant Physiology*. 155, 64-69.

[2]   Ayre BG, Keller F, and Turgeon, R. 2003. Symplastic continuity between companion cell and the translocation stream: long-distance transport is controlled by retention and retrieval mechanisms in the phloem. *Plant Physiology* 131, 1518-1528.

[3]   Batasheva S, Abdrakhimov F, Bakirova G, Isaeva E, Chikov V. 2007. Effect of nitrates supplied with the transpiration flow on assimilate translocation. *Rus. J. Plant Physiology* 54, 373-380. [in rus].

[4]   Batasheva S, Abdrakhimov F, Bakirova G, Isaeva E, Chikov V. 2010. Effects of sodium nitroprusside, the nicric oxyde donor, on photosynthesis and ultrastructure of common flax leave blades. *Rus. J. Plant Physiology* 57, 376-381. [in rus].

[5]   Batasheva S, Isaeva E, Chikov V, and Ratushnyk A. 2011. The Influence of Suddenly Changing Quantity of Produced Photosynthetic Products on its Export from Donor-Leave. *Middle-East Journal of Scientific Research*. 10, 188-190.

[6]   Bethke PC, Badger MR, Jones RL. 2004. Apoplastic synthesis of nitric oxide by plant tissue. *The Plant Cell* 16. 332-341.

[7]   Chikov VI. 1987. Photosynthesis and Assimilate Transport. Moscow: Nauka. [in rus].

[8]   Chikov VI. 1996. Photorespiration. *Soros Educate Journal*. (11) 2-8. [in rus].

[9]   Chikov VI, Abdrakhimov FA, Bakirova GG, Batasheva SN. 2007. The role of sink-source relationship between different organs in regulation of photosynthesis and productivity. *Acta Horticulturae, Proceedings of the International Symposium on Source-Sinc relationsheps in Plants*. (835) 87-98.

[10] Chikov, V. I., Abdrakhimov, F. A., Batasheva, S. N., and Khamidullina L. A.: Characteristics of Photosynthesis in Maize Leaves (C4 Plants) upon Changes in the Level of Illuminance and Nitrate Nutrition. − Russ. J. Plant Physiol. 63: 620-625, 2016b. (In Rus).

[11] Chikov V. I., Akhtyamova G. A., Batasheva S. N., D. S. Dyurbin, M. Ш. Tagirov M. Sh., Blokhin V. I. Root formation in the pre-heading period in different morphobiotypes of barley // Niva Tatarstana. 2017. N. 2. P. 27-29. (In Rus).

[12] Chikov VI, Avvakumova NY, Bakirova GG, Belova LA, Zaripova L. 2001. Apoplastic transport of ^{14}C-photosynthates measured under drought and nitrogen supply. *Biollogia Plantarum* 44 (4), 517-521.

[13] Chikov VI, Bakirova GG. 1999. Relationship between carbon and nitrogen metabolisms in photosynthesis. The role of photooxidation processes. *Photosynthetica* 37 (4), 519-527.

[14] Chikov VI, Bakirova GG, Batasheva SN, Sergeeva AA. 2005. Effect of Defoliation or Excision of Growing Axillary Shoots on the Composition of Labeled Products od Photosynthesis in the leaves and Xylem Sap of Kidney Bean. *Rus. J. Plant Physiology* 52, 459-462. [in rus].

[15] Chikov VI, Batasheva SN. 2012. The role of C to N balance in the regulation of photosynthesis function. *Function Advances in Photosynthesis - Fundamental Aspects ISBN 978-953-307-928-8 Edited by: Mohammad Mahdi Najafpour Publisher:* InTech, 273-298.

[16] Chikov V*I*, Bulka ME, Lozovaya VV. 1972. Effect of insecticides on ^{14}C distribution in the products of photosynthesis. Sov. *J. Plant Physiology* 19, 190-193. [in rus].

[17] Chikov VI, Bulka ME, Yargunov, VG. 1985. Effects of fruit removal on photosynthctic metabolism of ^{14}C in cotton leaves. Sov. J. Plant Physiol. 32, 1055-1063. [in russ].

[18] Chikov VI, Chemikosova SB, Bakirova GG, Gazizova NI. 1984. Influence of removal of a portion of spike or leaves on assimilate transport and photosynthetic productivity in spring wheat. Sov. J. Plant Physiol 31 475-481. [in russ].

[19] Chikov V, Chemikosova S, Nesterova T, Zernova O. 1988. Peculiarity of photosynthesis and export function of leaf under Enhanced Nitrogen Nutrition. *Photosynthesis and productive process. Ed. Mokronosov, A. T. Sverdlovsk. Russia*. 145-154. [in rus].

[20] Chikov VI, Isfandiyarov NI. 1978. Effects of the rate of assimilate efflux on the ratio between some components of plant gas exchange. *Abst. All-Union Conf. "Biochemical and Biophysical Mechanisms of Substance Transport in Plants and its Regulation". Gorky.* 182. [in russ].

[21] Chikov, V. I., Mikhailov, A. L., Timofeeva, O. A., and Khamidullina, L. A2016a.. Photosynthetic carbon metabolism in potato leaves under changes in light intensity. − Russ. J. Plant Physiol. 63: 70–76, (In Rus).

[22] Chikov VI, Salyakhova GA, Safiullina GF, Zamalieva FF. 2012. Photosynthesis, assimilates transport and productivity in potato plants of the nevskii variety grown under different insolation. *Agricultural biology*. No 1, 72-77. (in rus).

[23] Chikov VI, Yargunov VG, Fedoseeva, Chemikosova SB. 1982. Influence of the regulationship between production and consumption of photosynthates on the operation of photosynthetic apparatus in plants. Sov. J. Plant Physiology (Moscow) 29 (6) 1141-1146. [in russ].

[24] Crawford NM. 1995. Nitrate: nutrient and signal for plant growth. *The Plant Cell.* 7, 859-868.

[25] Eickenbusch JD, Beck E. 1973. Evidence for involvement of 2 types of reaction in glycolate formation during photosynthesis in isolated spinach chloroplasts. *FEBS Lett.* 31, 225-228.

[26] Foyer CH, Parry M, Noctor G. 2003. Markers and signals associated with nitrogen assimilation in higher plants. *Journal of Experimenta Botany* 54, 585-593.

[27] Fritz C, Mueller C, Matt P, Feil R, Stitt M. 2006. Impact of C-N status on the amino acids profile in tobacco sourse leaves. *Plant, Cell and Environment* 29, 2055-2076.

[28] Hall AJ, Brady CJ. 1977. Assimilate source-sink relationships in Capsicum annuum L II Effects fruiting and defloration on the photosynthetic capacity and senescence of the leaves. *Aust J Plant Phisiol* 4, 771-783.

[29] Henkes S, Sonnewald U, Flachmann R, Stitt M. 2001. A small decrease of plastid transketolase expression in antisence tobacco transformants has dramatic effects on photosynthesis and pheniltropanoid metabolism. *The Plant Cell.* 13, 535-551.

[30] Kaiser WM, Kandlbinder A, Stoimenova M, Glaab J. 2000. Discrepancy between nitrate reduction rates in intact leaves and nitrate reductase activity in leaf extracts: what limits nitrate reduction in satu? *Planta* 210, 801-807.

[31] Khamidullina LA, Abdrakhimov FA, Batasheva SN, Frolov DA, Chikov VI. 2011. Effect of nitrate infusion into the shoot apoplast on photosynthesis and assimilate transport in symplastic and apoplastic plants *Rus. J. Plant Physiology* 57, 484-490. [in rus].

[32] Khalilova LA. 2008. The ways of transport Cl$^-$ in the whole plant sistem halophyte Suaeda antissima (L.) *Thesis of Dissertation. Institute of plant physiology*, Moscow. Russia. [in rus].

[33] Krapp A, Saliba-Colombani V, Daniel-Vedelr F 2005. Analisis of C and N metabolisms and of C/N integrations using quantitative genetics. *Photosynthesis Research* 83, 251-263.

[34] Kursanov AL. 1976. Assimilate transport in plants. Nauka, Moscow. 1976. [in rus].

[35] Kursanov AL. 1984. Endogenous regulation of assimilate transport and source-sink relation in plant. *Soviet J. Plant Physiology.* 31, 579-595. [in rus].

[36] Lamattina L. & Polaco J. C. 2006. Nitric Oxide in Plant Growth. Springer-Verlag Berlin Heidelberg.

[37] Leggewie G, Kolbe A, Lemoine R, Roessner U, Lytovchenko A, Zuther E, Kehr J, Frommer WB, Riesmeier JW, Willmitzer, et al 2003. Overexpression of the sucrose transporter So SUT1 in potato results in alterations in leaf carbon partitioning and in tuber metabolism byt has little impact on tuber morphology. *Planta* 217, 158-167.

[38] Lenz F. 1977. Einflüβ der Frucht auf Photosynthese und atmung. *Ztschr. Pflanzenernahr. Und Bodenk.* 140, 51-61.

[39] Lips SN. 1997. The role of InorganicNitrogen Ions in Plant Adaptation Process. *Rus. J. Plant Physiology* 44, 487-498. [in rus].

[40] Lawlor D. W. Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities // Journal of Experimental Botany. 2013. Volume 64, Issue 1. Pp. 83-108.

[41] Majumder SK, Leopold AC. 1967. Callose formation in response to low temperature. *Plant and Cell Physiol.* 8, 775-778.

[42] Matt P, Geiger M, Walch-Liu P, Engels C, Krapp A, Stitt M. 2001. Elevated carbon dioxide increases nitrate uptake and nitrate reductase activity when tobacco is growing on nitrate, but increases ammonium uptake and inhibits nitrate reductase activity when tobacco is growing on ammonium nitrate. *Plant Cell and Environment* 24, 1119-1137.

[43] Meyer C, Lea US, Provan F, Kaizer WM, Lillo C. 2005. Is nitrate reductase a major player in the plant NO (nitric oxide) game? Photosynth. Res. 83, 181-189.

[44] Minchin PEH. & McNaughton GS. 1987. Xylem transport of recemtly fixed carbon within Lupin. Aust. Plant Physiol. 14, 325-329.

[45] Mokronosov AT. 1972. Photosynthetic Function in the System of the Whole Plant. *Theoretical Basics for Photosynthetic Productivity. Nichiporovich, A. A. Ed., Moscow.* Nauka. 355-361. [in rus].

[46] Mokronosov AT. 1981. Developmental aspects of Photosynthesis. *Moscow. Nauka.* [in russ].

[47] Mokronosov AT, Ivanova NA. 1971. Specialities of Photosynthetic Function at the Partial Defoliation of Plants *Sov. Journal of Plant Physiology. (Moscow).* 18, 668-676. [in rus].

[48] Moorby J. 1977. Integration and regulation of translocation within the whole plant. *Symp Soc Exp Biol.* 31, 425-454.

[49] Moyse A. 1980. La photorespiration: differents aspects de la respiration des vegetaux a la lumiere. *Physiol. Veg.* 18, 543-565.

[50] Neill SJ, Desikan R. & Hancock JT. 2003. Nitric oxide signaling in plants. *New Physiologist* 159, 11-35.

[51] Noctor G, Foyer CH. 1998. A re-evaluation of the ATP: NADPH budget during C3 photosynthesis. A contribution from nitrate assimilation and its associated respiratory activity? *Journal of Experimental Botany* 49, 1895-1908.

[52] Paris R, Lamattina L, Casalongue CA 2007. Nitric oxide promotes the wound-h.

[53] Paul MJ, Foyer CH. 2001. Sink regulation of photosynthesis. *Journal of Experimental Botany* 52, No 360, 1383-1400.

[54] Paul MJ, Pellny TK. 2003. Carbon metabolitefreedback regulation of leaf photosynthesis and development. *Journal of Experimental Botany* 54, *(382), Regulation of Carbon Metabolism Special Issue,* 539-547.

[55] Peuke AD, Jeschke WD, Hurtung, WW. 2002. Flows of elements, Ions and abscisic acid in Ricinus communis and Site of Nitrate Reduction under Potassium Limitation. *Journal of Experimental Botany* 53, pp. 241-250.

[56] Rennie EA, Turgeon R 2009. A comprehensive picture of phloem loading strategies. *Proc Natl Acad Sci.* USA 106, 14162-14167.

[57] Rosche E, Blackmore D, Tegeder M, Richardson T, Schroeder H, Higgins TJV, Frommer WB, Offler CE, Patrick JW. 2000. Seed-specific overexpression of a popate sucrose transporter increases sucroseuptake and growth rates of developing pea cotyledons. *Plant J.* 30, 165-175.

[58] Slewinski TL, Braun DM. 2010. Current perspectives on the regulation of whole-plant carbohydrate partitioning. *Plant Sci*ence 178, 341-349.

[59] Stitt M, Müller C, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A. 2002. Steps towards an integrated view of nitrogen. *Journal of Experimental Botany* 53, (370), *Inorganic Nitrogen Assimilation Special Issue,* 959–970.

[60] Tarchevsky IA. 1965. Relationship between Photosynthetic Phosphorylation, CO_2 assimilation and Other Functions of Chloroplasts and Photosynthesizing Cells. *In: Biochemistry and Biophysics of Photosynthesis. Krasnovskii A. A. Ed. Moscow.* 305-319. [in rus].

[61] Turgenon R, Wolf S. 2009. Phloem transport: cellular pathways and molecular trafficking. *Annu Rev. Plant Bioogy* 60, 207-221.

[62] Voskresenskaya NP, Wiil YA, Grishina GS, Pärnik TR. 1970. Effect of oxygen concentration and light intensity on the distribution of labeled cfrbon in photosynthesis products in bean plants. *Photosynthetica* 4, (1) 1-8.

[63] Walch-Liy P, Filleur S, Gan Y, Forde BG. 2005. Signaling mechanisms integrating root and shoot responses to changes in the nitrogen supply. *Photosynthesis Research* 83, 239-250.

[64] Wardlaw JF, Moncur L. 1976. Source, Sink and Hormonal Control of Translocation in Wheat. *Planta* 128 (2) 93.

[65] Webb JA, Gorham PR. 1965. The Effect of Node Temperature on Assymilation and Translocation of ^{14}C in Squash. *Can. Journal of Botany* 43, 1009-1017.

[66] Zottini M, Costa A, Michele RD, Ruzzene M, Carimi F. 2007. Salicylic acis activates nitric oxide synthesis in Arabidopsis. *Journal of Experimental Botany* 58, 1397-1405.