ISSN 1021-4437, Russian Journal of Plant Physiology, 2016, Vol. 63, No. 1, pp. 70–76. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © V.I. Chikov, A.L. Mikhailov, O.A. Timofeeva, L.A. Khamidullina, 2016, published in Fiziologiya Rastenii, 2016, Vol. 63, No. 1, pp. 76–82.

RESEARCH PAPERS

Photosynthetic Carbon Metabolism in Potato Leaves under Changes in Light Intensity

V. I. Chikov[a], A. L. Mikhailov[b], O. A. Timofeeva[b], and L. A. Khamidullina[a]

[a] *Kazan Institute of Biochemistry and Biophysics, Kazan Research Center, Russian Academy of Sciences, ul. Lobachevskogo 2/31, P.O. B 30, Kazan, Tatarstan, 420111 Russia*

e-mail: vichikov@bk.ru

[b] *Kazan Federal University, Kazan, Russia*

Received April 21, 2015

Abstract—Photosynthetic assimilation of $^{14}CO_2$ was examined in leaves of potato (*Solanum tuberosum* L.) plants that were grown under direct sunlight and then transferred to 50% irradiance for various periods. The rate of $^{14}CO_2$ assimilation correlated with light intensity: the photosynthetic rate reduced by 52% after 5-day shading and by 70% after 30-min shading. In all shaded and shade-adapted plants, the sucrose/hexose ratio decreased by a factor of 3.5–4.1; furthermore, the radioactivity of glycolate cycle metabolites and the serine/glycine ratio were lowered. In plants shaded for 5 days or 30 min, the radioactivity of aspartate and malate was higher than at continuous high irradiance, especially in plants shaded for 30 min, whereas a sudden illumination of the shaded plants reduced the radioactivity of these substances. We suppose that low irradiance averted the reentry of glycolate path carbon into the Calvin cycle and redirected this carbon source for the production of four-carbon acids that acidified the apoplast. This acidification activated the apoplastic invertase, which enhanced sucrose hydrolysis and hindered the sucrose export from the leaf. Hydrolysis of sucrose promoted the increase in osmolarity of the apoplastic solution, this increase being stronger at close distances to the stomatal pores where water is intensely evaporated. The increase in osmolarity of extracellular medium led to closing of stomata and the suppression of photosynthesis.

Keywords: Solanum tuberosum, irradiance, stomata, invertase, photosynthetic carbon metabolism, regulation

DOI: 10.1134/S1021443716010040

INTRODUCTION

The problem of interaction of light and dark processes in regulation of photosynthesis, as it is posed in the literature, is mostly confined to the light-dependent production of energy equivalents (ATP and NADPH) and their use for sucrose synthesis in the Calvin cycle. Relations of photoprocesses in chloroplasts to nitrogen assimilation [1, 2] and dark respiration [3] are frequently discussed. Strong positive correlation between the glycine/serine ratio and photorespiration, noted in some studies [4], indicates the hindrance to the carbon flow in the glycolate pathway. It has long been observed [5] that a variety of adverse treatments inhibiting photosynthesis produce monotonic responses of photosynthetic carbon metabolism. Sucrose synthesis declines with a concurrent increase in production of glucose, fructose, aspartate, malate, and alanine. Such a response of photosynthesis was termed a nonspecific one. Shading belongs to this group of treatments [5, 6]. In early studies, the nonspecific response was tentatively related to photophosphorylation and ATP deficiency arising under unfavorable conditions [5]. This was the first attempt of integrating photochemical processes and carbon metabolism with the aim to explain not only variable photosynthetic rates but also the flexibility of carbon metabolism pathways.

Later, it was found that the decreased level of ^{14}C in sucrose under the action of adverse factors was related to specific features of label incorporation into this substance [7]. Under short exposures of leaves to $^{14}CO_2$-enriched atmosphere, the incorporation of the label into sucrose was diminished under unfavorable conditions. By contrast, the labeling of sucrose (expressed in percents of the total radioactivity of soluble compounds) increased in the post-photosynthetic period and even exceeded the reference level (regardless of whether the leaf was illuminated or not). This phenomenon was explained by the gradual (over 30 min) accumulation of sucrose in the phloem endings of the leaf and by the inhibition of sucrose export from the leaf [8]. The "noncarbohydrate" photosynthetic metabolism was also enhanced under enriched nitrogen nutrition [9]. Therefore, further studies of the nonspecific photosynthetic response to the action of unfavorable factors were focused on elucidating the interactions of nitrogen and carbon metabolisms [10, 11]. We found that the noncarbohydrate route of pho-

Abbreviations: PGA—phosphoglyceric acid.

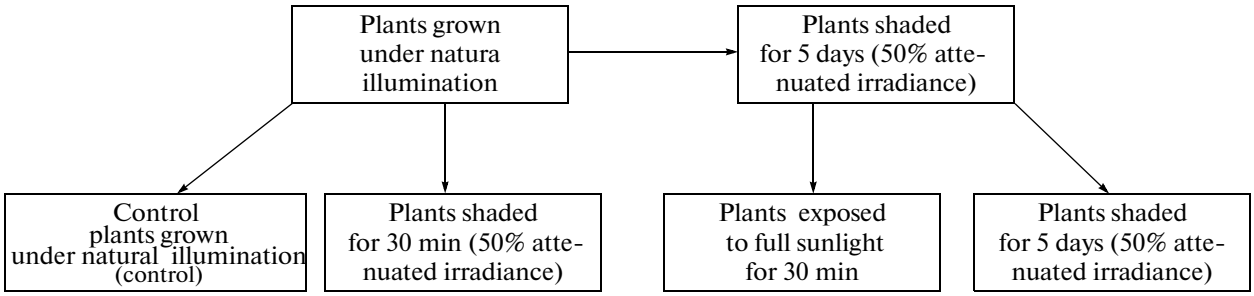

Fig. 1. Scheme of experimental treatments to study the influence of irradiance on photosynthesis and photosynthetic carbon metabolism.

tosynthesis was enhanced at the elevated nitrate component in plant nitrogen [12], whereas the use of urea as a nitrogen source did not cause such effects.

The reduction in the sucrose/hexose ratio in plants grown under increased nitrate nutrition was found related to the activity of apoplastic invertase [13]. Studies on the role of apoplastic invertase in regulation of photosynthetic gas exchange implied the involvement of this enzyme in the function of stomata [14]. Inhibition of invertase activity by means of RNA interference led to unusual changes of stomatal conductance at lowered light intensities. In wild-type plants, the attenuated irradiance or darkening resulted in the synchronous decline of photosynthesis and transpiration. However, when the plants with the suppressed gene of apoplastic invertase were subjected to similar conditions, the inhibition of photosynthesis was paralleled by the increase in transpiration. It should be noted that Gulyaev was the first to draw attention to the effect of light and carbon dioxide on the stomatal conductance [15].

Based on the reports [16, 17] that the stomatal aperture depends on the sucrose concentration in the guard cell apoplast, we reconsidered the "effect of the suppressed invertase gene" [14]. Judging from the diminished sucrose/hexose ratio in plants exposed to reduced irradiance [6], hydrolysis of sucrose was enhanced (albeit for yet unknown reasons). Hence, the osmolarity of extracellular fluid in the mesophyll apoplast probably increased during hydrolysis, because two moles of low-molecular-weight sugars (glucose and fructose) replaced one mole of sucrose. The osmolarity of the apoplastic fluid is expected to be additionally elevated in the vicinity of the stomatal pores where water is evaporated. Because of the increased osmolarity in the apoplast, the guard cells lost their turgor, thereby closing the stomata. In the gene-modified plants, sucrose synthesis decreased presumably at low irradiance, and inhibition of apoplastic invertase prevented sucrose hydrolysis. Thus, the loading of sucrose into the phloem terminals could proceed. As a result, the osmolarity of the apoplastic fluid decreased in the transformed plants after shading. In these conditions, the guard cell turgor increased and caused the opening of stomata.

Since all these phenomena become evident on the background of reduced irradiance, when the energy supply for carbon metabolism was drastically reduced, it was reasonable to assume that these events were triggered by changes in the rate of phosphoglyceric acid (PGA) reduction in the Calvin cycle. Thus, special experiments have been planned to examine the photosynthetic carbon metabolism in leaves at different irradiances (solar irradiance and 50% shading).

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L. cv. Nevskii) plants propagated in vitro in test tubes were transferred into polyethylene boxes filled with a mixture of peat and humus and grown under natural daylight irradiance (1681 μmol/(m^2 s)). In the beginning of flowering, when the sink organs, tubers started their development, one group of plants was shaded with gauze to reduce the irradiance by 50% for 5 days before the experiment. On the day of experiment, a group of shaded plants was divided, and one half was exposed to full sunlight for 30 min. The group of sun-grown plants was also divided and one half was shaded for 30 min. Thus, we obtained four groups of plants that differed in the history of irradiance changes (Fig. 1). Photosynthesis in all plants was evaluated at those irradiances at which the plants were exposed on the time of measurement (in direct sunlight or at 50% shading).

Photosynthesis was measured with the apical blade of a mature source leaf sampled from the middle leaf layer. The leaf sample placed in a leaf-clip chamber was fed with $^{14}CO_2$ for 3 min, fixed with boiling ethanol, and assayed for the rate of photosynthesis and ^{14}C incorporation into the products. The 3-min duration of leaf incubation in the presence of $^{14}CO_2$ was selected, because this incubation period ensured a half-saturated incorporation of ^{14}C into the photosynthates [18]. This means that information on synthesis of individual metabolites can be obtained by measuring specific radioactivity of these compounds.

The leaf feeding with $^{14}CO_2$ was conducted in a closed system with a 10-liter gas holder at a final CO_2 concentration of 0.03%. To this end, the gas holder was first filled with CO_2-free air (air passed through

Table 1. Assimilation of $^{14}CO_2$ by leaves of potato plants (cv. Nevskii) under changes in illumination conditions

Treatment	Irradiance during measurement of photosynthesis, μmol/(m^2 s)	Photosynthetic rate, kBq/(cm^2 min)	% of the control value
Continuous sunlight	1681	17.74 ± 2.05	100
50% shading for 5 days before the experiment	840	8.45 ± 0.94	47.6
50% shading for 30 min	840	5.32 ± 1.05	30.0
Transfer to full sunlight after 5-day shading	1681	18.42 ± 1.14	103.8

Experimental conditions during $^{14}CO_2$ fixation were the same as prior to measurements.

the absorbent with an alkali hydroxide). Next, the CO_2 concentration was adjusted to 0.04% using dry radioactive or nonradioactive bicarbonate; after completing the experiment, the CO_2 concentration dropped to nearly 0.03%. Specific radioactivity of labeled $^{14}CO_2$ was 4 MBq/L before leaf incubation, O_2 concentration was 21%, and temperature was 26°C on the day of experiments. A possible decline in photosynthetic parameters during gradual CO_2 depletion in the gas holder was accounted for by the overall variability of the mean values.

The photosynthetic assimilates were analyzed using two-dimensional paper chromatography with two solvents. We used the mixture of butanol, water, and formic acid (75 : 12 : 13) for separation in the first direction and water-saturated phenol for separation in the second direction. The radioactivity of samples, as well as of individual chromatographic spots corresponding to labeled photosynthates was measured with a Delta-300 scintillation counter (Tracor Analytic, United States).

All experiments with labeled carbon (^{14}C) were performed in 5–6 replicates, with two assays per replicate. The results were processed statistically using Microsoft Excel. Significant differences between the mean values were revealed with the Student *t*-test at $P < 0.05$.

RESULTS AND DISCUSSION

The rate of $^{14}CO_2$ fixation after 3-min exposures depended directly on light intensity. After 5-day shading, the plant photosynthesis was reduced by 52% compared to photosynthesis in full sunlight (Table 1). However, when the plants were shaded for 30 min prior to measurements, photosynthesis was reduced by 70%. On the other hand, if the shaded plants were exposed to direct sunlight, the rate of photosynthesis increased sharply. When the plant leaves adapted to full sunlight were suddenly transferred to shade, the photosynthetic rate dropped lower than that in plants adapted to shade for 5 days. Furthermore, the transfer of shaded plants to full sunlight caused a rapid increase in $^{14}CO_2$ fixation rate, up to the levels exceeding the photosynthetic rate in control plants adapted to full sunlight.

Analysis of ^{14}C partitioning among the photosynthates in plant leaves revealed significant differences between the treatments in the formation of carbohydrate and noncarbohydrate compounds (Table 2). Lighting conditions had a significant impact on synthesis of sucrose. For example, the highest incorporation of ^{14}C into sucrose compared to labeling of low-molecular-weight substances was observed in plants exposed to full sunlight. The content of ^{14}C-labeled sucrose was reduced after prolonged (5 day) shading, but the amount of labeled free sugars (sucrose, glucose, fructose, and oligosaccharides) with respect to radioactivity of all labeled compounds was elevated in plants leaves exposed to this treatment. The lowest rate of sucrose formation (and carbohydrates in general) was observed after short (30 min) shading of plants adapted to full sunlight. Considering that the plants of this treatment exhibited the lowest rate of $^{14}CO_2$ assimilation (30% of the rate in light-adapted plants), we conclude that synthesis of sugars in these plants was three times lower than in the control plant group.

In all plants shaded prior to or during measurements, irrespective of whether they had been adapted or not adapted to full sunlight, the sucrose/hexose ratio decreased by 3.6–4.1 times (Table 3). The largest decrease in this ratio was observed after sudden shading of plants for 30 min, while the smallest decrease was noted after the transfer of plants adapted to shade for 5 days to full sunlight for 30 min.

In all treated plants (except for untreated sun-grown plants), the radioactivity of glycolate pathway products was decreased; the serine/glycine ratio was lower in plants once exposed to shade than in the plants adapted to full sunlight (Table 2). The incorporation of ^{14}C into four-carbon molecules (aspartate, malate) was altered in different directions, depending on the irradiance during $^{14}CO_2$ fixation (Table 2). For example, the radioactivity of these substances was higher in plants shaded for 5 days and 30 min than in the control plants (especially after sudden shading). Conversely, after the transfer of plants shaded for 5 days to full sunlight, this ratio dropped below the

Table 2. Effect of irradiance changes on partitioning of ^{14}C among the products of 3-min photosynthesis in potato leaves (% of ethanol--water-soluble radioactive substances)

Substance	Untreated (sunlight-adapted)	50% Shading for 5 days	50% Shading for 30 min	Transfer to full sunlight for 30 min after 5-day shading
Sucrose	36.2 ± 3.6	25.8 ± 3.6	16.7 ± 3.9	35.4 ± 1.5
Glucose	2.7 ± 0.6	8.3 ± 2.7	5.2 ± 1.0	7.4 ± 0.9
Fructose	2.4 ± 0.7	4.9 ± 1.1	4.4 ± 0.6	3.6 ± 0.4
Glycolate	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Glycine	1.0 ± 0.3	1.2 ± 0.2	1.0 ± 0.3	1.8 ± 0.3
Serine	12.1 ± 1.8	6.9 ± 1.0	8.2 ± 0.8	9.6 ± 0.8
Glycolate pathway products, total	13.3	8.5	9.5	11.7
Aspartate	5.4 ± 0.7	6.6 ± 1.0	16.4 ± 3.5	3.4 ± 0.6
Malate	2.8 ± 0.4	5.4 ± 1.6	7.6 ± 1.2	1.5 ± 0.4
Four-carbon substances	8.2	12.0	24.0	4.9
Alanine	12.9 ± 0.9	12.5 ± 0.6	21.4 ± 1.8	12.4 ± 0.8
Glycerate	6.3 ± 0.7	6.2 ± 1.1	3.8 ± 1.1	9.9 ± 1.5
Pigments	1.8 ± 0.3	2.4 ± 0.2	1.8 ± 0.4	0.3 ± 0.1
Oligosaccharides	9.5 ± 1.2	13.2 ± 3.1	8.4 ± 2.1	5.1 ± 0.3
Carbohydrates, total	50.8 ± 1.6	52.2 ± 3.6	34.7 ± 5.9	51.5 ± 2.4
Other substances	6.7	6.2	4.7	9.3

Table 3. Effect of irradiance changes on the proportions of various photosynthetically produced labeled substances

Groups	Untreated (sunlight-adapted)	50% Shading for 5 days	50% Shading for 30 min	Transfer to full sunlight for 30 min after 5-day shading
Sucrose/hexoses	7.10	1.95	1.74	3.22
Serine/glycine	12.10	5.75	8.20	5.33
Glycolate pathway products/(malate + aspartate)	1.62	0.71	0.40	2.39

control level. This was possibly caused by the accumulation of aspartate and malate pools at the stage of preliminary shading.

As a result (Table 3), the ratio of labeled glycolate pathway metabolites and four-carbon compounds decreased almost twofold in plants shaded for 5 days and dropped nearly fourfold in plants shaded for 30 min. On the other hand, when shaded plants were transferred to full sunlight, this ratio increased approximately 1.5 times compared to that in control plants exposed to solar irradiance.

Specific changes in ^{14}C-labeling of alanine are remarkable (Table 2). In all the treatments, except for plants exposed to 30-min shading, the proportion of ^{14}C incorporated into alanine was nearly the same as in control plants, whereas the alanine radioactivity in leaves transferred suddenly to shade increased almost twofold. It is known that alanine is produced from PGA. Therefore, its rapid massive production immediately after the decrease in irradiance might be due to the decrease in PGA reduction and the respective rapid redirection of total PGA flux toward the formation of alanine. Since alanine is a transportable amino acid [19], the altered rates of alanine synthesis are likely to reflect the shift of C/N ratio in the photoassimilates exported from the leaf. A particularly large increase in alanine synthesis is apparently associated with large options for its export from leaves to other plant parts.

Thus, the most dramatic changes in photosynthetic carbon metabolism occurred upon the sudden

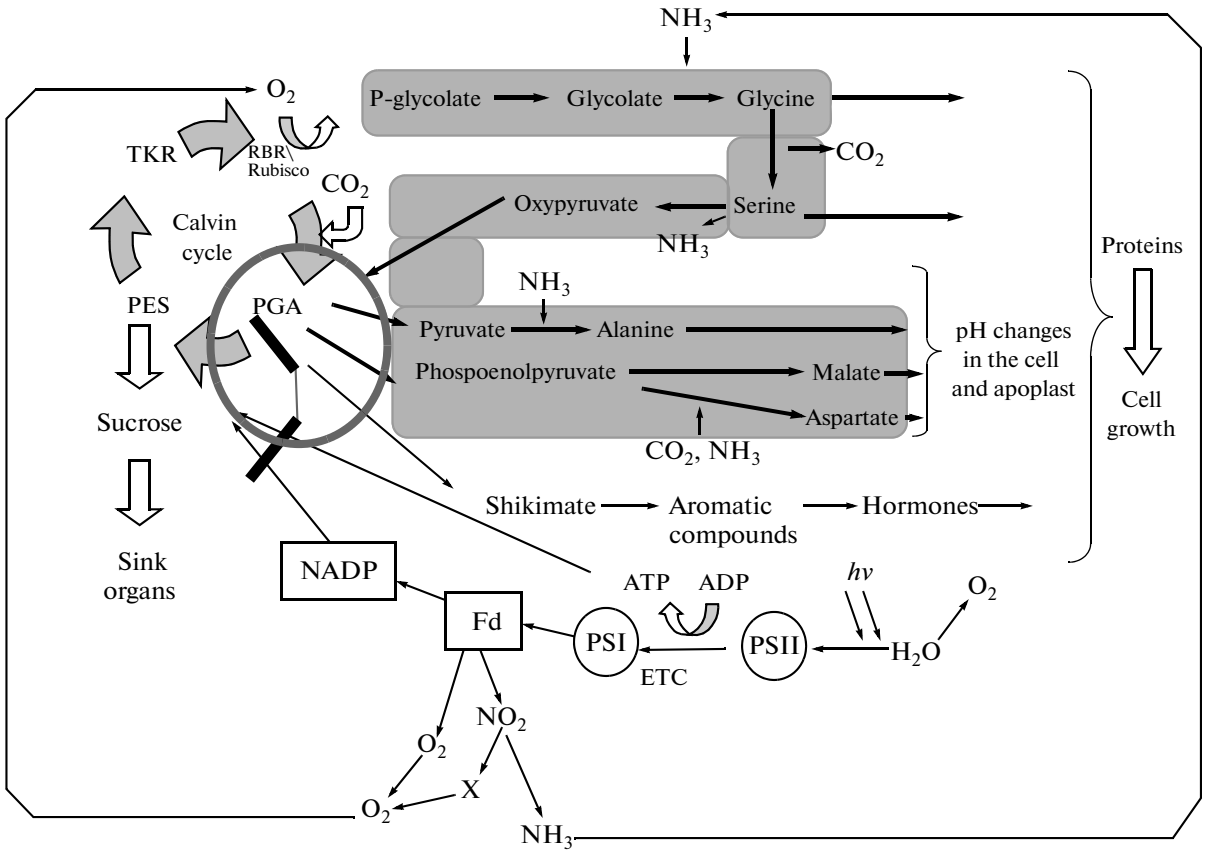

Fig. 2. Schematic view of regulation of photosynthetic carbon metabolism under changes in irradiance ([12] with supplements). Fd—ferredoxin; PGA—phosphoglyceric acid; PSI and PSII—photosystems I and II; Rubisco—ribulose bisphosphate carboxylase/oxygenase; PES—phosphoric esters of sugars; TKR—transketolase reaction (glycolate formation); ETC—photosynthetic electron-transport chain; X—unidentified oxidant (probably hydroxylamine) that triggers the transketolase mechanism of glycolate formation. Shaded area depicts the carbon flux via the glycolate pathway with the production of either sugars or acids.

decrease in irradiance. Even larger distinctions between the treatments could be expected if we extrapolate the trends in photosynthetic rate and individual metabolite formation to the moment of stepwise decrease in irradiance. This means that the regulatory processes are triggered immediately after lowering the irradiance, because of the partial depletion of the metabolites involved in chloroplast photochemical reactions.

One concluding note is that sufficiency of NADPH and ATP is a critical requirement for PGA reduction in the photosynthetic carbon metabolism. Therefore, we assume that PGA is a key element in metabolic rearrangements upon changes in irradiance. The successful PGA reduction is critical because (1) it enables the major outflow of photosynthetic assimilates from the leaf (export of sugars) and (2) it promotes the reentry of the glycolate path carbon into the Calvin–Benson cycle.

The increase in irradiance facilitates ATP and NADPH production in chloroplasts, thus providing conditions for the effective reduction of PGA to sugars. High irradiance enhances the photosynthetic rate, thus increasing the carbon flow through the leaf, including a very powerful flow along the glycolate pathway (Fig. 2). It is worth reminding that the glycolate pathway processes half of the total assimilated CO_2 carbon. The carbon flowing through this path is partly converted into carbon dioxide (in the reaction: 2 glycine → serine), which manifests itself in photorespiration constituting 25–30% of the apparent photosynthetic CO_2 uptake [20]. A clear correlation between the high content of amino acids and NADPH content was also observed by Dutilleul et al. [21] under all photoperiods tested.

The increased yield of carbohydrates during photosynthesis at high irradiance indicates that the glycolate pathway products are also successfully reduced to produce sugars. This means that the connectivity of the glycolate pathway to the Calvin–Benson cycle increases with the irradiance. Conversely, at lowered irradiance and the resulting deficiency of NADPH produced in chloroplasts, the return of carbon from the glycolate pathway products into the pentose phosphate cycle diminishes, so that the majority of these compounds are converted into organic acids and amino acids.

During long-term adaptation to low light conditions (5-day shading in our case), when the production of a transportable photosynthate (sucrose) is retarded, the plant employs alternative means for maintaining the export function of the leaf. One possibility is the formation of oligosaccharides and their export from the leaf via the symplast to other plant parts [22]. This mechanism is particularly pronounced during drought conditions [23]. The activation of the symplastic transport of assimilates in low light apparently involves specific structures and metabolic pathways. On the other hand, this transport ceases rapidly after the increase in photon flux density, as evidenced by the lack of the oligosaccharide enhanced production when the plants were shaded suddenly (for 30 min).

The abundant accumulation of intracellular organic and amino acids apparently lowers not only the cytoplasmic pH but also the apoplastic pH of mesophyll cells. A large scale of photosynthate production is well illustrated by gas exchange data obtained with leaves upon switching a light on and off [20]. Within 5–10 min after switching off the light, CO_2 is released at the rate that constitutes approximately 25% of the photosynthetic rates prior to darkening. These products are oxidized metabolites of the Calvin–Benson cycle, which passed through the glycolate pathway. The burst of CO_2 emission after switching off the light is directly related to the extent of loading of the Calvin cycle pools; shortly after repeated lighting, a decline in the burst of CO_2 emission was evident [20].

Acidification of the apoplast activates the apoplastic invertase and the hydrolysis of sucrose. In turn, hydrolysis elevates the osmolarity of the apoplastic fluid, this increase being larger at shorter distances to the stomatal pores. The increase in osmolarity of the medium around the guard cells results in the drop of their turgor, which is followed by closing of stomata, increased resistance to CO_2 diffusion, and by suppression of photosynthesis.

Thus, the leaf is endowed with a regulatory complex that integrates chloroplast photochemical events, photosynthetic carbon metabolism, apoplastic invertase, and the stomatal function into a single mechanism. We assume that this mechanism is sensitive to changes in light intensity, nitrogen (nitrate) nutrition, and growth rates of sink organs, thus optimizing the photosynthetic function of the whole plant. In all these cases, the key trigger mechanism is presumably the disposal of photochemical reaction products in chloroplasts. With the increase in leaf nitrate concentration, nitrite starts competing for electron acceptance from electron-transport chain in chloroplasts. As a result, the photosynthetic carbon metabolism responds to shading, and, consequently, all downstream changes develop as described above. When the assimilate export from the leaf is hindered (e.g., upon the decrease in the number or activity of sink organs) the sucrose loading into the overfilled phloem will be retarded. The increase in sucrose concentration in the apoplast would lead to the *substrate activation of invertase*, the increased hydrolysis of sucrose, and the subsequent closing of the stomata. Large amounts of hexoses produced by hydrolysis would reenter the mesophyll cells and interfere with the metabolism of triose phosphates released from the chloroplasts. These events would activate Mehler reaction and produce glycolate in the transketolase reaction. The carbon of glycolate pathway metabolites would be unable to reenter the Calvin–Benson cycle; it would contribute to the pool of acids, with the subsequent influence on invertase and the stomatal guard cells. In addition, the accumulation of hexoses in the cytoplasm in the absence of inorganic phosphate reentry into the chloroplasts would intensify starch synthesis.

It should be noted that the 5-day exposure of plants to low light might be insufficient for the complete adaptation to shade conditions. The plant adaptation to changes in irradiance might depend, to a certain extent, on the increase in apoplastic concentration of acids at the expense of acid sequestration in the vacuoles of mesophyll cells.

REFERENCES

1. Gandin, A., Denysyuk, M., and Asaph, B., Cousins disruption of the mitochondrial alternative oxidase (AOX) and uncoupling protein (UCP) alters rates of foliar nitrate and carbon assimilation in *Arabidopsis thaliana, J. Exp. Bot.*, 2014, vol. 65, pp. 3133–3142.
2. Eichelmann, H., Oja, V., Peterson, R.B., and Laisk, A., The rate of nitrite reduction in leaves as indicated by O_2 and CO_2 exchange during photosynthesis, *J. Exp. Bot.*, 2011, vol. 62, pp. 2205–2215.
3. Gandin, A., Duffes, C., Day, D.A., and Cousins, A.B., The absence of alternative oxidase AOX1A results in altered response of photosynthetic carbon assimilation to increasing CO_2 in *Arabidopsis thaliana, Plant Cell Physiol.*, 2012, vol. 53, pp. 1627–1637.
4. Novitskaya, L.L., Trevanion, S.J., Driscoll, S., Foyer, C.H., and Noctor, G., How does photorespiration modulate leaf amino acid contents? A dual approach through modelling and metabolite analysis, *Plant Cell Environ.*, 2002, vol. 25, pp. 821–835.
5. Tarchevskii, I.A., Relationship between photosynthetic phosphorilation and assimilation of CO_2 and other functions of chloroplasts and photosynthetic cells, in *Biokhimiya i biofizika fotosinteza* (Biochemistry and Biophysics of Photosynthesis), Krasnovskii, A.A., Ed., Moscow: Nauka, 1965, pp. 305–319.
6. Tarchevskii, I.A., Kurmaeva, S.A., and Vdovina, A.I., Change the direction of photosynthesis in plants transplanted under the forest canopy, *Sov. Plant Physiol.,* 1962, vol. 47, pp. 1366–1368.
7. Chikov, V.I. and Nikolaev, B.A., The effect of drought on post-photosynthetic conversion of ^{14}C in bean leaves, *Sov. Plant Physiol.*, 1975, vol. 4, pp. 587–590.
8. Chikov, V.I., Evolution of notions about relationships between photosynthesis and plant productivity, *Russ. J. Plant Physiol.*, 2008, vol. 55, pp. 130–148.

9. Karpilov, Yu.S. and Nedopekina, I.F., The products of photosynthesis in tomato and their effect on the formation of nitrogen–phosphorus nutrition, *Tr. Moldav. Nauch. Issled. Inst. Oroshaemogo Zemledeliya Ovoshchevodstva*, 1965, pp. 35–43.

10. Chikov, V.I., *Fotosintez i transport assimilyatov* (Photosynthesis and Transport of Assimilates), Moscow: Nauka, 1987.

11. Chikov, V.I. and Batasheva, S.N., The role of C to N balance in the regulation of photosynthetic function, in *Advances in Photosynthesis – Fundamental Aspects*, Najafpour, M.M., Ed., InTech., 2012, pp. 273–298. doi 10.5772/28084

12. Chikov, V. and Bakirova, G., Relationship between carbon and nitrogen metabolisms in photosynthesis. the role of photooxidation processes, *Photosynthetica*, 1999, vol. 37, p. 519.

13. Chikov, V.I., Bakirova, G.G., Avvakumova, N.Y., Belova, L.A., and Zaripova, L.M., Apoplastic transport of ^{14}C photosynthates measured under drought and nitrogen supply, *Biol. Plant.*, 2001, vol. 44, pp. 517–521.

14. Chikov, V.I., Akhtyamova, G.A., Batasheva, S.N., Mikhailov, A.L., Khamidullina, L.A., and Timofeeva, O.A., Effect of silencing of the apoplastic invertase gene on photosynthesis in tomato, *Russ. J. Plant Physiol.*, 2015, vol. 62, pp. 39–44.

15. Gulyaev, B.I., Reaction of stomata to changes in the intensity of light and CO_2 concentration, *Fiziol. Biokh. Kul't. Rast.*, 1979, vol. 11, no. 6, pp. 593–600.

16. Lu, P., Outlaw, W.H., Jr., Smith, B.G., and Freed, G.A., A new mechanism for the regulation of stomatal aperture size in intact leaves, *Plant Physiol.*, 1997, vol. 11, pp. 109–118.

17. Outlaw, W.H., Jr. and Vlieghere-He, X.D., Transpiration rate. An important factor controlling the sucrose content of the guard cell apoplast of broad bean, *Plant Physiol.*, 2001, vol. 126, pp. 1716–1724.

18. Mokronosov, A.T., *Ontogeneticheskii aspekt fotosinteza* (Ontogenetic Aspects of Photosynthesis), Moscow: Nauka, 1981.

19. Kursanov, A.L., *Transport assimilyatov v rastenii* (Transport of Assimilates in Plants), Moscow: Nauka, 1976.

20. Chikov, V.I., Photorespiration, *Soros. Obraz. Zh.*, 1996, no. 11, pp. 2–8.

21. Dutilleul, C., Lelarge, C., Prioul, J.-L., de Paepe, R., Foyer, C., and Noctor, G., Mitochondria-driven changes in leaf and status exert crutial influence on the control of nitrate assimilation and the integration of carbon and nitrogen metabolism, *Plant Physiol.*, 2005, vol. 139, pp. 64–78.

22. Turgeon, R., Symplastic phloem loading and the sink–source transition in leaves: a model, in *Recent Advances in Phloem Transport and Assimilate Compartmentation*, Bonnemain, J.L., et al., Eds., Nant: Quest, 1991, pp. 18–22.

23. Taji, N., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi, K., and Shinozaki, K., Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana, Plant J.*, 2002, vol. 29, pp. 417–423.

Translated by A. Bulychev