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Changes of photosynthesis and carbon metabolism in *Typha angustifolia* L grown in conditions of nitrate nitrogen overload

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Abstract – Nitrates may induce alterations in NO-signaling system and change photosynthesis in plants. Significant reduction of $^{14}CO_2$ fixation was noted at concentration of 3.96 mM $NaNO_3$ in an aquatic macrophyte (*Typha angustifolia* L.). Assimilation of $^{14}CO_2$ seven days after the introduction of nitrates did not differ between control and experimental samples. There were changes in distribution of ^{14}C among products of $^{4}CO_2$ fixation 4 h after $NaNO_3$ addition, resulting in increased sugar radioactivity in experimental plants. It was suggested that the observed changes may have regulatory importance.

Keywords: aquatic macrophyte, carbon metabolism, nitrate, photosynthesis

Introduction

The role of C / N proportion in the regulation of plant metabolism has been actively studied in recent years. It is known that during interaction between carbon and nitrogen metabolisms the start of trigger systems occurs; this may have an effect on the physiological and biochemical processes in plants (Corruzzi and Bush 2001). The functional role of the nitrate ion for the regulation of C / N status in plants has already been demonstrated (Krapp and Stitt 1995).

With respect to of flax plants (*Linum usitatissimum*) it has been shown that introduction of nitrate solution (50 mM) to the apoplast of plant bine may result in the inhibition of the

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outflow of assimilates from leaves after 30 min as well as accumulation of ^{14}C- sucrose in donor leaves and an increase of the ascending transport of photosynthetic products (BATASHEVA et al. 2007). The blocking of ^{14}C- sucrose export from leaves was found at the level of the transition of phloem terminals to vessel fascicles; vacuolization of cells-satellites in phloem terminals was noted (ABDRAKHIMOV et al. 2008).

Introduction of sodium nitroprusside, a generator of NO, (in concentrations of 2 orders lower) into plant apoplast induced similar changes (BATASHEVA et al. 2010). This suggests the participation of NO-signaling in the rearrangement of physiological and biochemical changes in plants with increasing nitrate concentrations. Then, it was proposed that the entry of nitrate into plants results in the formation of nitrogen oxide by enzymatic or nonenzymatic ways from the nitrate ion; NO may activate NO-signaling system and trigger genetically determined alterations of plant metabolism (KHAMIDULLINA et al. 2011).

Probably, the ability of NO to increase the content of callose (β-1,3- glucan) in a leaf (PARÍS et al. 2007) influences the blocking of sucrose transport at the level of footstalk (and, likely, root) phloem. The data on the ability of salicylic acid to induce a synthesis of NO (ZOTTINI et al. 2007) and to inhibit callose destruction confirms indirectly the proposed mechanism (SEROVA et al. 2006). It may also mean that inhibition of callose destruction may be mediated by the action of NO.

Plants with both symplast and apoplast type of phloem sugar loading have their carbon metabolism changed due to nodifications of assimilate transport and leaf cell ultrastructure (KHAMIDULLINA et al. 2011). The importance of NO-signaling systems in animals was convincingly presented (GLYAN'KO et al. 2009). This allows the conclusion that the action of nitrates on metabolism via NO-signaling system may have a universal character. In this connection, it was important to test the reaction of an aquatic macrophyte (*Typha angustifolia* L.) to increased concentration of $NaNO_3$ in the environment. The choice of the plant was governed by the fact that it is the important provider of exometabolites participating in the regulation of structure and functions of aquatic organisms (RATUSHNYAK and ABRAMOVA 2011).

The hypothesis of the present study was to check whether nitrogen overload may result in both morphological and physiological alterations in aquatic macrophyte.

Materials and methods

To detect the intensity of photosynthesis and to evaluate the distribution of ^{14}C in leaf cells in vivo, we used the radioactive indicators method. Plants of *Typha angustifolia* L. were taken from Sredniy Kaban Lake (55°44'29" N.L., 49°09'37" E.L.) and placed into experimental tanks (V = 30 L) according to the recommendations of (TSIRTSIS and KARYDIS 1997, XU et al. 1999): each tank contained 10 plants that were cultivated in natural lighting at 24–26 °C. The plants were transferred to experimental tanks with sediments and natural water. Data from this experimental design may be extrapolated to natural ecosystems (TSIRTSIS and KARYDIS 1997, XU et al. 1999). To assess quantitatively the content of exometabolites of *T. angustifolia* in its natural environment, water samples were taken from environment a month after the beginning of the experiment. The liquid was evaporated gradually (2 mL) at 30 °C, and then the pellet was supplied with scintillation liquid ZHS-1 to assess radioactivity (RATUSHNYAK and ABRAMOVA 2011). Biomass of the macro-

phyte was presented as g of dried substance per 1 m^2. The number of bines and the average plant height were detected, then the plants were segregated into underground and above ground parts, and their dried and wet mass was registered. Detection of nitrate ions was performed using photometric assay with salicylic acid. The assay is based on interaction between nitrate ion with salicylic acid with formation of a yellow complex compound with 410 nm absorption spectrum. The drange of detected concentrations was 0.1 to 100 mg dm^{-3}. The grade dependence was stated according to a set of standard solutions with concentrations of 0.1 to 10 mg dm^{-3}. To detect concentration of nitrate ions in water samples, the tested solution (10 mL) was mixed with 2 ml of salicylic acid, and then dried. After cooling, 2 ml of sulphuric acid was added to the mixture and stored for 10 min. After that, 10–15 mL of distilled water and 15 mL of a complex solution (NaOH with Rochelle salt) were added. Then, this solution was used for photometric analysis at 410 nm. Two weeks later (a period of adaptation), a solution of sodium nitrate (final concentration 0.39 and 3.96 mM) was added to the plant root area. Unexposed plants served as controls. Four hours and seven days later, the medial part of a leaf from the medial plant tier (1.5–2.0 cm^2) was exposed to photosynthetic chamber with $^{14}CO_2$ (0.03%) and 3 and 4 min at natural illumination. Then, this part of the leaf was fixed with boiling ethanol (80%), the fixed leaves were ground with ethanol (60%), and the content of ^{14}C was detected in the homogenized samples. For each sample, total volume was measured and radioactivity was analyzed in 0.1 mL volume. Radioactivity was measured using a Delta-300 counter (Tracor Analytic, USA). Spirit-water fraction was analyzed using 2D paper (FN-3) chromatogram and autoradiography on AGFA radiographic film. Start dots of chromatogram were coated with sample concentrate corresponding to 100,000 pulses per min. A liquid scintillation detector Tri-Carb B2810TR (Perkin Elmer, USA) was used to detect the amount of ^{14}C in the samples. All data are presented in 5-fold replication, mean and standard errors are indicated in tables and figures. The level $p<0.05$ was considered to indicate significance.

Results

It was revealed that *T. angustifolia* plants showed an activation of production processes under conditions of nitrate overload. We detected an increase of total biomass, number of bines and average heights ($P<0.05$, Tab. 1). In parallel with morphological changes, we detected little change in the intensity of $^{14}CO_2$ fixation 4 h after the introduction of $NaNO_3$

Tab. 1. Dried biomass of underground and above ground parts of *T. angustifolia* (per 1 m^2) in conditions of nitrate overload

Variants	Number of bines	Average height (cm)	Total biomass of dried matter (g)	Overground biomass		Underground biomass	
				Dried matter (g)	% of total biomass	Dried matter (g)	% of total biomass
Control	14	140±7	246±11	79±4	32±4	166±8	67±4
$N_{0.396}$	17	150±7	346±14	122±6	35±4	224±11	64±4
$N_{3.96}$	22	162±8	459±22	246±10	53±3	213±9	46±3

(0.39 mM) to the root area of *T. angustifolia* ($P<0.05$, Fig. 1). A significant reduction of $^{14}CO_2$ fixation was noted at 3.96 mM $NaNO_3$ concentration ($P<0.05$). Assimilation of $^{14}CO_2$ seven days after introduction of nitrates did not differ between control and experimental samples (Fig. 2). There were changes in distribution of ^{14}C among products of $^{4}CO_2$ fixation 4 h after $NaNO_3$ addition; this resulted in increased sugar radioactivity in experimental plants ($P<0.05$, Fig. 3).

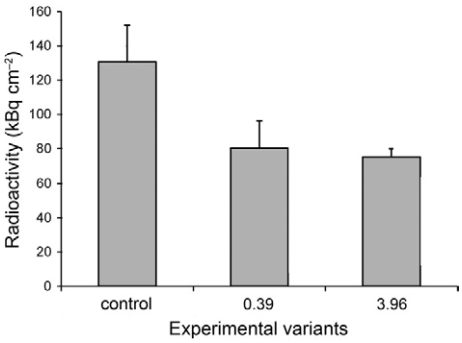

Fig. 1. Radioactivity of $^{14}CO_2$ in leaves of *T. angustifolia* L. measured after nitrate nitrogen addition (4 h before the experiment).

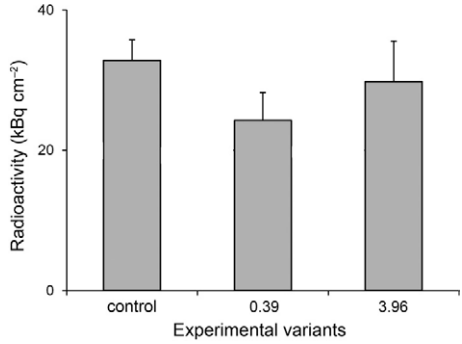

Fig. 2. Radioactivity of $^{14}CO_2$ in leaves of *T. angustifolia* L. measured after nitrate nitrogen addition (7 days before the experiment).

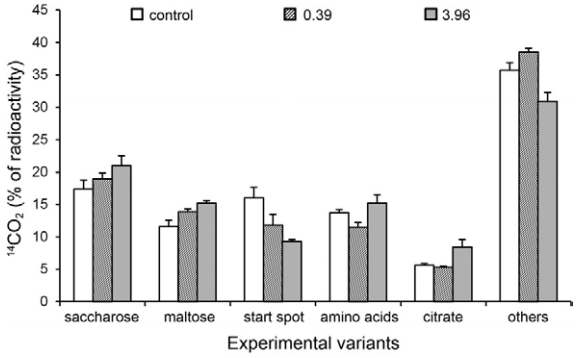

Fig. 3. The action of nitrate nitrogen 4 h after its introduction on the distribution of ^{14}C among products of 3-min exposition. Radioactivity corresponds to ethanol-water- soluble fraction.

Discussion

Our study indicated that nitrogen overload may induce morphological and physiological changes in the aquatic macrophyte *T. angustifolia.* Significant reduction of $^{14}CO_2$ fixation was noted at a concentration of 3.96 mM. Since in symplast plants the rate of assimilate efflux is lower than in apoplast plants (KHAMIDULLINA et al. 2011), it is possible to suggest that the influence of nitrates was not distributed far from the root zone of the exper-

imental plants since the height of the plants was 1.5–1.7 m and the experimental part of the leaf functioned normally. Probably, the 7 day period favored the utilization of nitrates by aquatic hydrobionts, and carbon dioxide metabolism was at a stationary level. This was supported by hydrochemical data (RATUSHNYAK and ABRAMOVA 2011). It was previously reported (RATUSHNYAK 2002) that the excretory activity of macrophytes was raised after the addition of nitrogen to the environment. An increase in plant excretion results in enhancement (primarily at the periphytic area) of bacterial biomass for 3 days; these are basic destroyers of polluting compounds; these microbes may be competitors for biogenic elements of hydrobionts of other taxonomic groups, firstly phytoplankton (RATUSHNYAK et al. 2008, 2009). Also reported was the predominance of saprophytes (15.5-fold domination in open biotopes), denitrite bacteria (10–1000-fold domination), nitrite bacteria (38–100-fold domination) after the addition of nitrate nitrogen (3.96 mM); these bacteria participate actively in processes of organic destruction that facilitate their action in the hydroecosystem (RATUSHNYAK and ABRAMOVA 2011). Interestingly, increase of nitrate (a basic substrate of inorganic nitrogen for plants) concentration did not result in elevated synthesis of amino acids: previous studies showed that nitrate increases the amount of ^{14}C within amino acids in experiments with terrestrial (BATASHEVA et al. 2007, KHAMIDULLINA et al. 2011) and aquatic plants (RATUSHNYAK et al. 2010). Most likely this difference is connected with different exposition times and concentrations of nitrate ion used. At the same time, signal systems may inhibit enzyme synthesis in Calvin's cycle. This was confirmed by a significant reduction of ^{14}C content at the starting spot at chromatogram where mainly spirit-water soluble proteins are concentrated (primarily, ribulosodiphosphatcarboxylase) (ANDREEVA 1982).

Increase of ^{14}C in sucrose and maltose (transport products of photosynthesis in the symplast plant, *T. angustifolia*) (WEISE et al. 2004, CHIA et al. 2004, LU and SHARKEY 2004) may be considered as difficulties with sugar export from the experimental part of a leaf. Very likely the induction of callose synthesis (under the action of nitrate entering the underground organs of the plants) reduced the carrying capacity of phloem. As a result, the sieve tube located above started to overfill with sugars.

These data allow the suggestion that the action of nitrates has a trigger mechanism. A sudden increase of nitrate concentration in roots opposes the stationary abilities of enzyme systems to reduce nitrates to amino groups. As a result, NO, nitric oxide may be formed as a product of the incomplete reduction of nitrate; NO may trigger NO-signal system. The triggering of the NO-signal system may occur during endogenous increase of the nitrate concentration in leaves or organs. There are many ways for nitric oxide to be generated from nitrite, both enzymatic and nonenzymatic (NEILL et al. 2003). The detected changes are characteristic of both concentrations of nitrate (Fig 3). The increased radioactivity of citrate suggests the activation of respiration activity in connection with the forthcoming metabolic changes. It should be noted here that the used concentration of $NaNO_3$ was 25 times lower than those for terrestrial plants (BATASHEVA et al. 2007, ABDRAKHIMOV et al. 2008) and 2.5 times lower than the nitrate concentration used usually in nutrient solutions for hydroponic plant cultivation.

The detected effects may have a regulatory character; the interaction between downward flow of sugars from leaves and the upward flow of nitrates with transpiration water may occur at different distances from the zone at which nitrate is introduced to the plant. Synthesis of callose is the first reaction to the appearance of NO (PARIS et al. 2007); this

compound may occlude pores in cribriform lamellas of phloem. This contradicts to the transport of saccharose from leaves to roots and may result in repletion of phloem vessels with sugars (including those labeled with ^{14}C). As a result, an increased radioactivity of saccharose and maltose was detected in the experimental part of a leaf that had received labeled ^{14}C.

Thus, it was revealed in this study that nitrogen overload may result both in morphological and physiological alterations. This compound induced growth of the aquatic macrophyte and altered assimilation of carbon dioxide and transport of ^{14}C-products. Although NO measurement was not performed, we discuss its possible formation since sodium nitroprusside and nitrates may have similar effects, according to literature data (WILDT et al. 1997, KHAMIDULLINA et al. 2011).

References

ABDRAKHIMOV, F. A., BATASHEVA, S. N., BAKIROVA, G. G., CHIKOV, V. I, 2008: Dynamics of ultrastructure changes in sheet plate fiber flax with braking transport assimilate by nitrate-anion. Tsitologiia 50, 700–710.

ANDREEVA, T. F., 1982: Photosynthesis and nitrogen metabolism in plants (In Russian). Moscow, Nauka.

BATASHEVA, S. N., ABDRAKHIMOV, F. A., BAKIROVA, G. G., CHIKOV, V. I., 2007: Action of nitrates introduced with transpiration water flow on the transport of assimilates (In Russian). Plant Physiology 54, 421–431.

BATASHEVA, S. N., ABDRAKHIMOV, F. A., BAKIROVA, G. G., ISAEVA, E. V., CHIKOV, V. I., 2010: Action of NO donor – nitroprussid sodium on photosynthesis and ultrastructure of fibre flax leaves (In Russian). Plant Physiology 57, 398–403.

CHIA, T., THORNEYCROFT, D., CHAPPLE, A., MESSERLI, G., CHEN, J., ZEEMAN, S. C., SMITH, S. M., SMITH, A. M. 2004: A cytosolic glucosyltransferase is required for conversion of starch to sucrose in *Arabidopsis* leaves at night. Plant Journal 37, 853–863.

CORRUZZI, G., BUSH, D. R., 2001: Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiology 125, 61–64.

GLYAN'KO, A. K., MITANOVA, N. B., STEPANOV, A. V., 2009: Physiological role of nitric oxide (NO) at vegetative organisms. Journal of Stress Physiology and Biochemistry 5, 33–52.

KRAPP, A., STITT, M., 1995: An evolution of direct and indirect mechanisms for the »sink--regulation« of photosynthesis of spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady–state transcript levels after cold girdling of source leaves. Planta 195, 313–323.

KHAMIDULLINA, L. A., ABDRAKHIVOV, F. A., BATASHEVA, S. N., FROLOV, D. A., CHIKOV, V. I., 2011: Influence of nitrate introduction to bine apoplast on photosynthesis and transport of assimilates in symplast and apoplast plants (In Russian). Plant Physiology 58, 420–426.

LU, Y., SHARKEY, T. D., 2004: The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. Planta 218, 466–473.

NEILL, S. J., DESIKAN, R., HANCOCK, J. T., 2003: Nitric oxide signalling in plants. New Phytologist 159, 11–35.

PARÍS, R., LAMATTINA, L., CASALONGUÉ, C. A., 2007: Nitric oxide promotes the wound--healing response of potato leaflets. Plant Physiology and Biochemistry 45, 80–86.

RATUSHNYAK, A. A., 2002: Ecological-physiological aspects of regulation of homeostasis of aquatic biosystems of various organization levels with participation of phytohydrocenosis. PhD Thesis, University of Nizhniy Novgorod.

RATUSHNYAK, A. A., 2008: The investigation of exometabolism of some aquatic macrophytes. Global Journal of Environmental Research 2, 62–65.

RATUSHNYAK, A. A., SHAGIDULLIN, R. R., ANDREEVA, M. G., RATUSHNYAK, A. YU., TRUSHIN, M. V., 2009: The influence of *Daphnia magna* microcosm on its resistance to deltamethrin. American-Eurasian Journal of Agricultural and Environmental Sciences 6, 257–261.

RATUSHNYAK, A. A., ABRAMOVA, K. I., SHAGIDULLIN, R. R., ANDREEVA, M. G., TRUSHIN, M. V., 2010: Ecologic plasticity of *Typha angustifolia* under the action of nitrate-nitrogen. World Applied Sciences Journal 8, 1032–1035.

RATUSHNYAK, A. A., ABRAMOVA, K. I., 2011: Autoecological fundamentals of algaecide and sanative activity of helophytes. LAP LAMBERT Academic Publishing, Saarbrucken.

SEROVA, V. V., RALDUGINA, G. N., KRASAVINA, M. S., 2006: Inhibition of callose hydrolysis by salicylic acid interferes with tobacco mosaic virus transport. Doklady Biochemistry Biophysics 406, 36–39.

TSIRTSIS, G., KARYDIS, M., 1997: A methodological approach for the quantification of eutrophication processes. Environmental Monitoring and Assessment 48, 193–215.

WEISE, S. E., WEBER, A. P. M., SHARKEY, T. D., 2004: Maltose is the major form of carbon exported from the chloroplast at night. Planta 218, 474–482.

WILDT, J., D. KLEY, A. ROCKEL, P. ROCKEL, and H. J. SEGSCHNEIDER, 1997: Emission of NO from several higher plant species, Journal of Geophysical. Research 102(D5), 5919–5927.

XU, F-LIU, JØRGENSEN, S. E., TAO, S., 1999: Ecological indicators for assessing freshwater ecosystem health. Ecological Modelling 116, 77–106.

ZOTTINI, M., COSTA, A., DE MICHELE, R., RUZZENE, M., CARIMI F., LO SCHIAVO, F., 2007: Salicylic acid activates nitric oxide synthesis in *Arabidopsis*. Journal of Experimental Botany 58, 1397–1405.