ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 4, pp. 404-410. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © E. I. Galeeva, T. V. Trifonova, A. A. Ponomareva, L. V. Viktorova, F. V. Minibayeva, 2012, published in Biokhimiya, 2012, Vol. 77, No. 4, pp. 512-520.

Nitrate Reductase from *Triticum aestivum* Leaves: Regulation of Activity and Possible Role in Production of Nitric Oxide

E. I. Galeeva, T. V. Trifonova, A. A. Ponomareva, L. V. Viktorova, and F. V. Minibayeva*

Kazan Institute of Biochemistry and Biophysics, Kazan Science Center, Russian Academy of Sciences, ul. Lobachevskogo 2/31, 420111 Kazan, Russia; fax: (843) 292-7347; E-mail: minibayeva@mail.knc.ru; fminibayeva@yahoo.com

Received November 11, 2011
Revision received December 30, 2011

Abstract—Nitrate reductase (NR) and peroxidase (POX) are important enzymes involved in the metabolism of reactive oxygen (ROS) and nitrogen species in leaves of wheat (*Triticum aestivum* L.) seedlings. It has been confirmed that NR activity in wheat leaves depends on the light conditions and the presence of nitrates during the cultivation of the seedlings, and it is regulated by the molybdenum cofactor and phosphorylation. In the present study, confocal microscopy and EPR spectroscopy studies showed that the addition of nitrite, a product of NR, increased the level of nitric oxide (NO). This increase was prevented by the addition of sodium azide, an inhibitor of NR. The results suggest that in wheat leaves one of the key functions of NR is the formation of the signaling NO molecule. Cultivation of green plants under conditions of prolonged (4 days) darkness, a strong stress factor for photosynthesizing cells, decreased the activity of NR. Moreover, darkness induced significant elevation of the POX activity that was prevented by the addition of nitrate to the growth medium. It is proposed that the changes in light conditions result in the competition between nitrate- and ROS-metabolizing activities of POX in leaves, and a possible interaction between NR and POX controls the levels of NO and ROS in the leaf tissue.

DOI: 10.1134/S0006297912040128

Key words: reactive oxygen species, nitrate reductase, nitric oxide, peroxidase, *Triticum aestivum* L.

Reduction of nitrates is an important stage of the nitrogen cycle in nature. Nitrate reductase (NR) belongs to the widespread class of molybdenum-containing enzymes and catalyzes the first step in the reaction chain of the reduction of nitrates to nitrites [1]. Interest in the physiological role of NR is rapidly growing since this enzyme is involved in the formation of nitric oxide (NO) in plants [2-4]. It is known that NO, being one of the messengers, plays an important role in signal transmission in plants, regulating a wide spectrum of physiological and biochemical reactions [5]. In particular, NO takes part in the regulation of the plant cell cycle as well as plant differentiation and morphogenesis. It is involved in the transduction of signals stimulating synthesis of such phytohormones as ethylene and auxin [6, 7]. Some information has accumulated concerning the role of NO in the response reactions of plants to stresses of biotic and abiotic nature [8-11]. For example, NO controls expression of a number of genes involved in the response reaction of plants to the invasion of pathogens [6], increases the level of cGMP and salicylic acid [3], and suppresses lipid peroxidation and degradation of RNA under the influence of herbicides [12].

The mechanisms of NO production in plants are now being discussed. It is suggested that there are several possible ways of NO formation, both of enzymatic and nonenzymatic nature. Proteins that resemble the family of NO synthases of animal cells and involved in the conversion of L-arginine into L-citrulline yielding NO have been found in plants [10, 13, 14]. NO synthase activity was revealed in extracts of higher plants [3]. However, the presence of true NO synthase in plants is still disputed. P-protein of the glycine-decarboxylase complex is capable of producing NO, but its amino acid sequence has little in common with mammalian NO synthase [15]. The protein AtNOS 1 isolated from *Arabidopsis thaliana* in 2003 is of special importance [16]. In terms of some biochemical

Abbreviations: DAF-FM, 4-amino-5-methylamino-2,7-difluorofluorescein diacetate; DDTC, sodium diethyldithiocarbamate; DEANOate, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine (sodium salt); NO, nitric oxide; NR, nitrate reductase; POX, peroxidase; ROS, reactive oxygen species.

* To whom correspondence should be addressed.

characteristics, this protein is similar to the animal NO synthase. However, the ability of AtNOS 1 to catalyze the formation of NO from arginine is challenged since a number of researchers failed to reveal citrulline and NO as reaction products [9]. A protein homologous to mammalian NO synthase and the gene encoding such a protein have not yet been found [17]. Nevertheless, the treatment of plants with inhibitors of NO synthase decreases NO accumulation and the related biological effects [7, 9]. Another enzymatic source of NO production can be NR catalyzing formation of NO from nitrite in an NADPH-dependent reaction. Interestingly, only 1% of the nitrate reductase activity is used for NO production. However, the amount of NO produced by NR is comparable with that produced in the NO synthase reaction [3]. Besides, NO may be formed during the reaction catalyzed by the nitrite-reductase localized in the cytoplasm membrane [18], as well as non-enzymatically in acidic media by the reduction of nitrites in the presence of a reducing agent [19].

An NO molecule can interact with other compounds, for example, with reactive oxygen species (ROS) yielding short-living highly reactive intermediates that have both toxic and regulatory properties [8]. It is supposed that NR itself can produce ROS. This enzyme catalyzes the one-electron transfer from NAD(P)H to O_2 in the molybdopterin site yielding superoxide anion [2]. It can be assumed that the interaction between NO and ROS in plant cells is much more pronounced under stress conditions, particularly under change in light regime. In contrast to the natural changes in the illumination during daytime, long darkness is a strong stress factor for the photosynthesizing cells that decreases the intensity of the formation of light-dependent macromolecules and accelerates their degradation [20], as well as induces a number of defensive reactions including those mediated by reactive nitrogen and oxygen species. Among the enzymes metabolizing ROS, a special role is played by peroxidase (POX) involved in both utilization and production of ROS.

The present work is devoted to investigation of the changes in the NR activity in leaves of *Triticum aestivum* L. under different conditions (light regime, addition of different substrates and effectors), as well as revealing of possible role of NR in NO formation. To study the interaction between NO and ROS, the changes in POX activity were investigated in the light and in the dark in the presence of different nitrate concentrations.

MATERIALS AND METHODS

In the present work, we used seedling leaves of fall wheat (*T. aestivum* L.), variety Dar Zernograda, grown in water at 23°C, 12-h illumination at 100 W/m^2. On the sixth day of the germination, the seedlings were divided into two groups, one of which was grown under the same conditions (light variant), and the other was incubated in the dark (dark variant) for 4 days. Samples for determination of the enzymatic activity were taken every day. Leaves (1 g) were fixed with liquid nitrogen and homogenized in 0.05 M sodium phosphate buffer, pH 8.0, with subsequent centrifugation (4000*g*, r = 5.5 cm, 20 min) at 4°C.

The activity of NR (EC 1.7.1.1) in the supernatant was measured by the accumulation of nitrite [21]. The reaction mixture contained 0.2 ml of the supernatant, 0.6 ml of 0.05 M sodium phosphate buffer, pH 8.0, and 0.1 ml of 0.1 M KNO_3. As the electron donor, 0.1 ml of 5 mM NADH was added into the test sample (the blank sample contained phosphate buffer). The mixture was incubated for 12 min at 30°C, and then the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. After the proteins were removed by centrifugation (10,000*g*, r = 4.5 cm, 10 min), the supernatant was mixed with 1 ml of Griss reagent, and the solution was incubated for 20 min. The amount of the formed product was determined spectrophotometrically at 527 nm using a Perkin Elmer spectrophotometer (USA). The content of hydrogen peroxide was determined using xylenol orange at 560 nm [22].

The activity of POX (EC 1.11.1.7) in the supernatant was measured spectrophotometrically at 590 nm using benzidine as a substrate (ε_{590} = 34,000 $M^{-1}\cdot cm^{-1}$) [23, 24]. The reaction mixture contained 0.5 ml of the extract, 0.5 ml of 1 mM H_2O_2, 0.5 ml of 6 mM benzidine, and 1.5 ml of 0.2 M acetate buffer, pH 5.3. Protein content was determined by the Bradford method [25].

The content of NO was determined by EPR using a spin trap (0.5 mg/g of sodium diethyldithiocarbamate (DDTC), 3.7 mg/g of ferrous sulfate, and 18.75 mg/g of sodium citrate). DDTC interacts with ferrous citrate yielding insoluble complex that interacts with NO forming a stable complex $(DDTC)_2$-Fe^{2+}-NO monitored by EPR spectroscopy. The method allows selective detection of NO [26]. Leaves of 8-day-old seedlings (0.5 g) were homogenized in a mortar in the presence of the spin trap. The EPR spectra of the resulting samples were recorded using a Bruker EMX spectrometer of the X frequency range (Germany) at 77°K. The relative amount of the formed complex $(DDTC)_2$-Fe^{2+}-NO was estimated from the integral intensity of the EPR signal [27].

NO in the leaves was visualized using the fluorescent dye 4-amino-5-methylamino-2,7-difluorofluorescein diacetate (DAF-FM) by confocal laser microscopy [28]. The seedlings were grown in water, and after 5 days, some of them were placed in a 50 mM KNO_2 solution for 2 days. The leaves of the 7-day-old seedlings were incubated in the corresponding solutions for 2 h, then they were placed for 40 sec into a 10 μM DAF-FM solution in 10 mM Tris-HCl, pH 7.0, containing 1 mM KCl and 1 mM $CaCl_2$, and then incubated in the dark for 20 min. The samples were washed free of the dye with the indicat-

ed buffer 4 times, and then de-esterified in the dark for 1 h. The samples were examined using an LSM-510 META confocal microscope (Carl Zeiss, Germany) with an argon laser (excitation at 495 nm, emission at 515 nm).

Each experiment was made in three replications, and each analytical procedure was made in triplicate. Results were analyzed using Student's *t*-test with control for the normalcy of distribution. Data are presented as mean values and standard errors of the mean. The differences were considered to be significant at $P < 0.01$. The following chemicals were used: NADH from BDH (Great Britain); Coomassie G-250, DEANOate, xylenol orange, and DDTC from Sigma (USA); DAF-FM from Invitrogen (USA). The other chemicals were of chemically pure grade (Russia).

RESULTS AND DISCUSSION

In wheat, nitrate reductase activity is observed in both leaves and roots, but in leaves it is more pronounced [29, 30]. In the bushing phase, 75-97% of the total nitrate reductase activity is revealed in leaves [31]. In our experiments, the nitrate reductase activity in leaves of the wheat seedlings grown in the light without a source of nitrogen (nitrate) was low (Fig. 1). The addition of 10 mM KNO_3 to the growing medium of the 6-day-old seedlings increased the activity of NR 3-fold in 1 day, reaching the maximal value (13-fold increase compared to the control) in 3 days with subsequent gradual decrease in the activity. It is known that nitrate increases the level of nitrate reductase mRNA [32]. It can be assumed that the addition of exogenous nitrate to the growing medium not only increases the activity of the existing NR, but induces *de novo* synthesis of this protein. Besides the nitrate-dependent regulation, the content of NR and its activity is controlled by light, sugars [32, 33], and other factors, in particular, by reversible phosphorylation [31]. The presence of Mg^{2+} cations is important for the regulation of the nitrate reductase activity at the posttranslational level. Nitrate reductase phosphorylated at the conserved serine residue can interact with 14-3-3 proteins in the presence of Mg^{2+} cations, which are necessary for the inactivation of the enzyme [4]. In our experiments, in plants pretreated with 0.05 mM $MgSO_4$ during 3 days before the addition of nitrate, a decrease in the nitrate reductase activity was observed on the fourth day after 10 mM KNO_3 was added compared to the plants growing in the presence of 10 mM KNO_3 all the time (Fig. 1). It can be assumed that Mg^{2+}-dependent inactivation of NR in intact plants requires some time interval for the manifestation of the specific action of Mg^{2+} ions.

One of the factors important for the catalytic activity of NR is the presence of molybdenum in the active site of the enzyme [1]. While investigating the structure and properties of molybdenum-containing enzymes, tungsten (an antagonist of molybdenum) is often used (tungsten model) [1, 34]. To reveal the role of the molybdenum

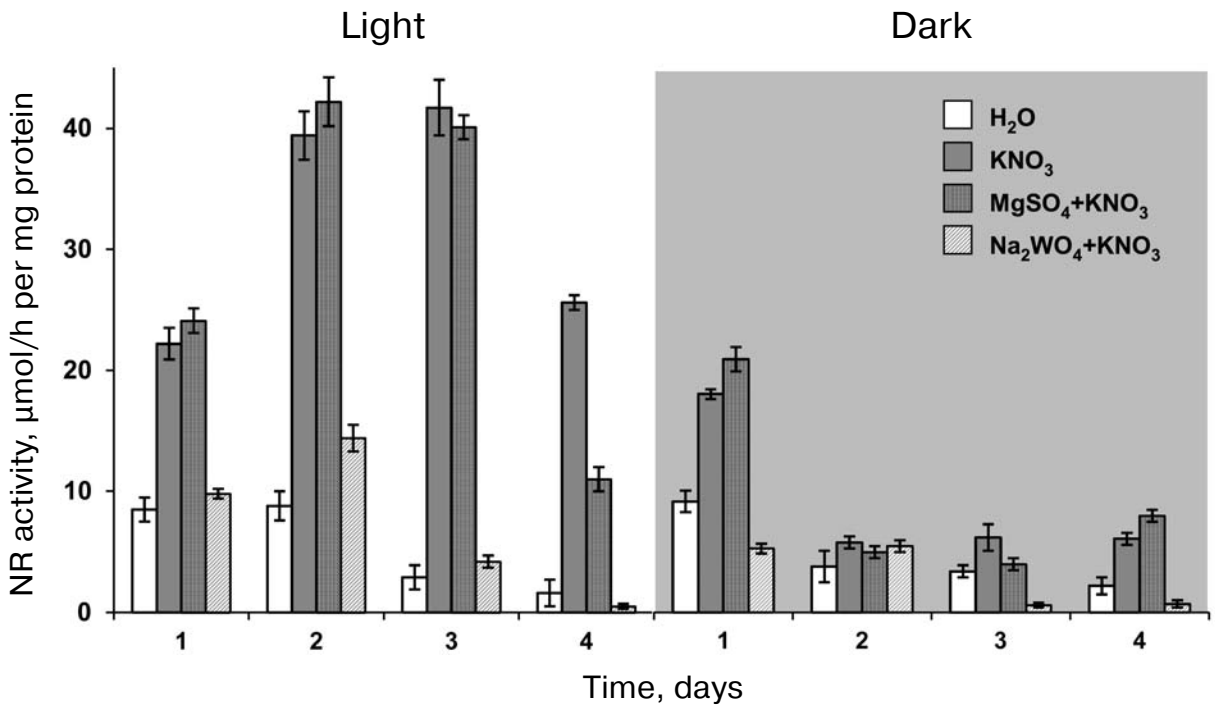

Fig. 1. Nitrate reductase activity in wheat seedlings grown in the light and in the dark in the presence of 10 mM KNO_3, 0.05 mM $MgSO_4$, and 0.1 mM Na_2WO_4.

cofactor in the regulation of the nitrate reductase activity, the growing medium for the seedlings was supplemented with tungsten (Na_2WO_4) that competed with molybdenum for the binding in the active site of the enzyme [35]. Incubation of the plants in 0.1 mM Na_2WO_4 for 3 days before the addition of nitrate significantly suppressed the activity of NR (Fig. 1). Previously, it was shown that tungstate also significantly decreased the activity of NR in leaves of bean [34] and tobacco plants [35]. Inhibiting of the activity of NR in the presence of tungstate (Fig. 1) indicates the importance of the molybdenum cofactor in the enzyme functioning. Besides, the observed decrease in the activity of NR in the presence of tungstate may be due to the inhibition of protein phosphatases [36] that increases the level of phosphorylation of NR [37].

Illumination is one of the key factors of the regulation of NR, especially in photosynthesizing tissues. The activity of NR was shown to change rapidly depending on the illumination, according to different reports, within 5-10 [34] or 40 min [32]. A long period of darkness is a strong stress factor for green tissues of plants leading to the depression of the activity of photosynthesizing systems and enzymes. Actually, the activity of NR in leaves of wheat seedlings grown in the dark without inducers was lower than that of the seedlings grown in the light (Fig. 1). The addition of nitrate under the dark incubation resulted in much less increase in the nitrate reductase activity than that observed in the light (2-fold in 1 day) (Fig. 1). Such a drop in the nitrate reductase activity could be due to the exhaustion in the dark of the products of photosynthesis that are necessary for the enzyme functioning (electron donors, sugars, etc.). Sugars are involved in the posttranslational regulation of the nitrate reductase activity, inhibiting the activity of protein kinases and thus maintaining NR in the active (less phosphorylated) state [34]. In contrast, according to literature data [37], phosphorylation of NR (including that at the serine residue) accelerates in the dark. This facilitates the interactions between NR and 14-3-3 proteins [4] and inactivation of the enzyme, thus preventing the accumulation of toxic nitrites in the dark or while using inhibitors of photosynthesis. In the dark, the addition of $MgSO_4$ had virtually no effect on the observed low activity of NR, supporting the assumption that acceleration of the phosphorylation in the dark suppresses the nitrate reductase activity (Fig. 1). Incubation of the plants in 0.1 mM Na_2WO_4 for 3 days before the addition of nitrate, the same as in the light, completely suppressed the activity of NR (Fig. 1). Thus, the observed decrease in the activity of NR in leaves in the dark compared to that in the light is due to a complex of factors preventing the maintenance of the active state of the enzyme.

Constitutive and inducible NRs of higher plants are similar in structure, but they differ in physicochemical properties [38]. The leaf tissue of most of higher plants including wheat contains nitrate-inducible NR exhibiting maximal activity at pH 7.5 and using NADH as an electron donor [39]. This is supported by data of the present work on the significant stimulation of NR activity after the addition of nitrate and inhibition of NR in the presence of tungstate. However, some plants have constitutive nitrate reductases. For example, in soybean plants two constitutive nitrate reductases (NADPH-dependent c_1NR exhibiting maximal activity at pH 6.5 and NADH-dependent c_2NR with maximal activity at pH 6.5) and also inducible NADH-dependent NR (iNR) with maximal activity at pH 7.5 [38] were found. Interestingly, tungstate inhibiting the activity of iNR does not affect the activity of cNR [40]. In *A. thaliana*, inducible NR is encoded by two genes, *NIA1* and *NIA2*. The gene *NIA2* is responsible for 90% of the total NR activity, while 10% of the activity is provided by the product of *NIA1* [41]. The genes *NIA1* and *NIA2* were also found in tobacco plants [42]. According to Boissom et al. [39], the inducible NR of wheat is encoded by two genes, *NADH-NAR1* and *NADH-NAR2*.

It is considered that inducible NR is a key component for NO production in plant cells. For example, in *A. thaliana* the NR encoded by *NIA1* is involved in NO formation in guard cells, as well as during cold acclimation [41]. To confirm the role of NR of wheat leaves in NO production, EPR spectroscopy was employed. After the addition of 50 mM KNO_2 to a leaf homogenate, in the EPR spectrum of the paramagnetic complex $(DDTC)_2$-Fe^{2+}-NO at $g = 2.038$, the characteristic for NO triplet hyperfine structure was detected that was due to the hyperfine interaction of the magnetic moment of the unpaired electron ($S = 1/2$) with the magnetic moment of the nitrogen nucleus ($I = 1$) (Fig. 2). The addition of 1 mM NaN_3, an inhibitor of ferrous-containing enzymes including NR, suppressed the KNO_2-induced signal. The use of the synthetic NO inducer DEANOate (0.5 mM) also resulted in NO production, but to a less extent than using nitrite. It is known that the formation of NO in the presence of nitrite can be due to the enzyme nitrite reductase. However, according to literature data, such nitrite-NO-reductase is located in the plasma membrane of root cells but not in leaf cells [18]. The nitrite reductase of chloroplasts reduces nitrite to ammonia, but its participation in NO production has not been demonstrated yet [18, 43, 44]. Oppositely, the one-electron reduction of nitrite by NR yielding NO has been described, this indicating the manifestation of the nitrite-reducing activity of NR [43]. We assume that in our experiments nitrite as a substrate can stimulate the activity of nitrite reductase, but the product of this reaction must be ammonia rather than NO. Interestingly, the KNO_2-induced NO signal was much less pronounced in the plants grown in medium with supplementation of nitrate (10 mM KNO_3) during the last two days than in plants grown in water (table). Presumably, the KNO_2-induced formation of NO is competitively inhibited by nitrate due to the fact that nitrate

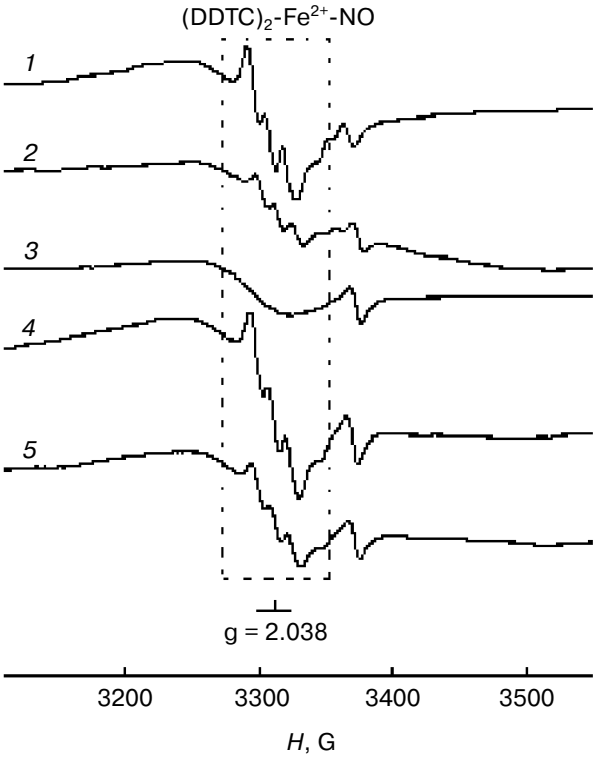

Fig. 2. EPR signal of mononitrosyl complex $(DDTC)_2$-Fe^{2+}-NO in leaves of wheat seedlings: *1*) standard sample; *2*) 0.5 mM DEANOate; *3*) H_2O; *4*) 50 mM KNO_2; *5*) 50 mM KNO_2 + 1 mM NaN_3.

and nitrite may have the same binding site in the NR molecule [4, 45] or to be transferred to the enzyme by the same transporters [1].

Further confirmation of the KNO_2-induced formation of NO was obtained by its imaging using laser confocal microscopy. The incubation of leaves in a 50 mM KNO_2 solution induced the fluorescence of DAF-FM (Fig. 3). The addition of NaN_3 significantly suppressed the fluorescence. Thus, the results indicate that NR can be involved in the production of NO in wheat leaves.

Biologically active molecules interacting with NO can function as NO sensors. They include heme-containing proteins, such as hemoglobin and guanylate cyclase producing cGMP, as well as thiol-containing proteins. NO can react with ROS yielding intermediate products, for example, peroxynitrite that is much more reactive than NO and superoxide anion. Peroxynitrite is involved in the inactivation of aconitases, oxidation of thiols [6]. In the response to different signals, NO and ROS are often formed simultaneously and in the same place. Changes in the NO content may control the level of ROS in the cells and vice versa [9]. However, the mechanisms of the interrelation between NO and ROS in plants subjected to stress conditions have not been ascertained.

Among the enzymes metabolizing ROS, the key enzyme is POX, one of the enzymes of dual purpose involved in both generation and utilization of ROS. POX is an oxidoreductase with wide substrate specificity and various mechanisms of oxidative reactions. There are some data on the participation of POX in the nitrogen metabolism of plant cells through the reduction of nitrates [46]. In our experiments, the activity of POX in the leaves of the seedlings grown in the light was constant for 4 days (Fig. 4). The addition of 10 mM KNO_3 and 0.05 mM $MgSO_4$ did not greatly affect the activity of POX (Fig. 4) and content of H_2O_2 (data not shown). The effect of darkness in the absence of nitrate suppressed the NR activity in the leaves (Fig. 1) and resulted in oxidative stress, which was detected by an increase in POX activity. The increase in the POX activity in the dark is presumably caused by the necessity of the detoxification of the enhanced content of ROS. Besides, it has been shown that under stress conditions POX can exhibit oxidase activity [47]. Under conditions of long-term dark, the presence of nitrate in the growing medium prevented significant growth in the activity of POX (Fig. 4) but resulted in the accumulation of H_2O_2 (51% increase by the 4th day). Presumably there is a competition between the nitrate- and ROS-metabolizing functions of POX under stress conditions. It can be assumed that when the POX activity towards oxidation of benzidine and reduction of H_2O_2 is lower, the ability of the enzyme to reduce nitrates is activated. According to [48], POX can be involved in the nitrogen metabolism under certain conditions as an additional way of reduction of nitrates. The molecular mechanisms of the influence of nitrates on catalytic properties of POX are virtually unstudied. It is suggested that the active site (heme group) of POX can interact with both H_2O_2 and KNO_3 [48]. Besides, there are some data indicating that nitrate inhibits formation of compound E_1 (intermediate semi-oxidized enzyme form) via binding to the heme "pocket" of POX [49, 50]. Thus, the data demonstrate high peroxidase activity in wheat leaves under stress conditions, in particular, under long-term dark. The decrease in the POX activity in the presence of

Integral intensity of the EPR signal of mononitrosyl complex $(DDTC)_2$-Fe^{2+}-NO in leaves of wheat seedlings grown in water and in 10 mM KNO_3 solution

Conditions	Signal intensity, %	
	H_2O (control)	KNO_3 (10 mM)
Without additions	<5	<5
+ KNO_2	100	15
+ KNO_2 + NaN_3	35	<5

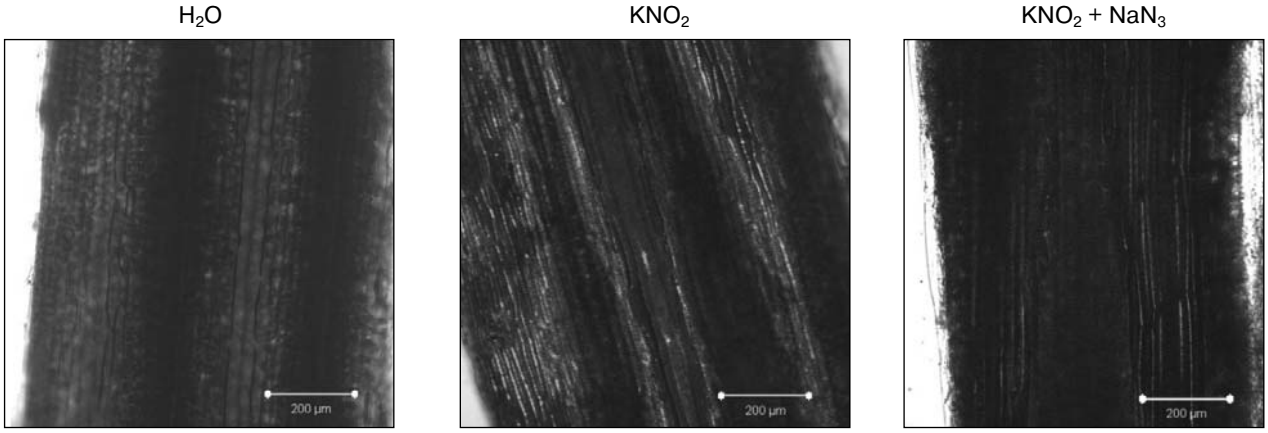

Fig. 3. Visualization of NO in wheat leaves using fluorescent dye DAF-FM (excitation/emission at 495/515 nm; the scale bar corresponds to 200 μm).

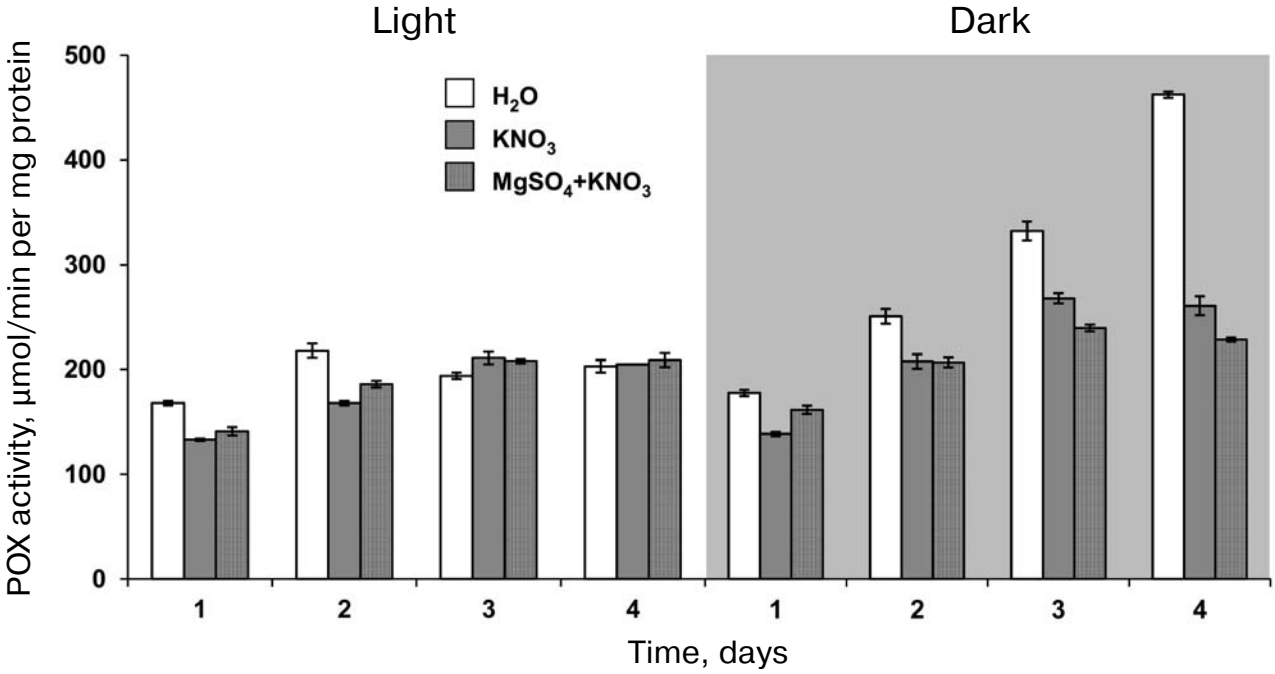

Fig. 4. Peroxidase activity in leaves of wheat seedlings grown in light and in dark in the presence of 10 mM KNO_3 and 0.05 mM $MgSO_4$.

nitrate could be due to the competition between H_2O_2 and nitrate and, as a consequence, to the induction of the nitrate-reducing activity of the enzyme. The hypothesis concerning the participation of POX in the formation of NO seems to be interesting [51], but it requires additional investigation.

The results demonstrate that NR and POX are important enzyme systems involved in the metabolism of reactive nitrogen and oxygen species. The activity of NR in leaves is controlled by light conditions during growth of seedlings, the presence of nitrates, molybdenum cofactor, and phosphorylation. The possibility of the participation of NR of wheat leaves in the production of NO has been confirmed. The changes in the activities of NR and POX under the change of light regimes (light/long-term dark) and under the addition of nitrates suggest a possible cooperative participation of these enzymes in the control of the level of NO and ROS in the photosynthesizing organs of plants.

Authors are grateful to the colleagues from Zavoisky Physico-Technical Institute (Kazan) V. V. Andrianov, A. A. Sukhanov, and V. V. Iyudin for their assistance in determination of NO by EPR spectroscopy.

This work was supported by the Russian Foundation for Basic Research (11-04-93962), by the Program of President of Russian Federation "Support of Leading Scientific Schools" (NSh. 825.2012.4), and by the Federal Program of the Ministry of Education and Science of Russian Federation (14.740.11.0797).

REFERENCES

1. Morozkina, E. V., and Zvyagil'skaya, R. A. (2007) *Biochemistry (Moscow)*, **72**, 1151-1164.
2. Yamasaki, H., and Sakihama, Y. (2000) *FEBS Lett.*, **468**, 89-92.
3. Rockel, P., Strube, F., Rockel, A., Wildt, J., and Kaiser, W. M. (2002) *J. Exp. Bot.*, **53**, 103-110.
4. Kaiser, W. M., Weiner, H., Kandlbinder, A., Tsai, C.-B., Rockel, P., Sonoda, M., and Planchet, E. (2002) *J. Exp. Bot.*, **53**, 875-882.
5. Tarchevskii, I. A. (2002) *Signal Systems in Plant Cells* [in Russian], Nauka, Moscow.
6. Wilson, I. D., Neill, S. J., and Hancock, J. T. (2008) *Plant Cell Environ.*, **31**, 622-631.
7. Kolupaev, Yu. V., and Karpets, Yu. V. (2009) *Visnik Kharkiv Nats. Agr. Univ.*, **3**, 6-19.
8. Dubovskaya, L. V., Kolesneva, E. V., Knyazev, D. M., and Volotovskii, I. D. (2007) *Fiziol. Rast.*, **54**, 847-855.
9. Neill, S., Bright, J., Desikan, R., Hancock, J., Harrison, J., and Wilson, I. (2008) *J. Exp. Bot.*, **59**, 25-35.
10. Glyan'ko, A. K., Mitanova, N. B., and Stepanov, A. V. (2009) *Zh. Stress-Fiziol. Biokhim.*, **5**, 34-52.
11. Hong, J.-K., Yun, B-W., Kang, J-G., Raja, M. U., Kwon, E., Sorhagen, K., Chu, C., Wand, Y., and Loake, G. J. (2008) *J. Exp. Bot.*, **59**, 147-154.
12. Beligni, M. V., and Lamattina, L. (2000) *Planta*, **210**, 215-221.
13. Gueto, M., Hernandez-Perera, O., Martin, R., Bentura, M. L., Rodrigo, J., Lamas, S., and Golvano, M. P. (1996) *FEBS Lett.*, **398**, 159-164.
14. Guo, F. Q., and Crawford, N. M. (2005) *Plant Cell*, **17**, 3436-3450.
15. Chandok, M. R., Ytterberg, A. J., van Wijk, K. J., and Klessig, D. F. (2003) *Cell*, **113**, 469-482.
16. Guo, F. Q., Okamoto, M., and Crawford, N. M. (2003) *Science*, **303**, 100-103.
17. Sakihama, Y., Nakamura, S., and Yamasaki, H. (2002) *Plant Cell Physiol.*, **43**, 290-297.
18. Stohr, C., Strube, F., Marx, G., Ullrich, W. R., and Rockel, P. (2001) *Planta*, **212**, 835-841.
19. Bethke, P. C., Badger, M. R., and Jones, R. L. (2004) *Plant Cell*, **16**, 332-341.
20. Tarchevskii, I. A. (2001) *Metabolism of Plants under Stress* [in Russian], Fen, Kazan.
21. Basra, A. S., Dhawan, A. K., and Goyal, S. S. (2002) *Planta*, **215**, 855-861.
22. Gay, C., and Gebicki, J. M. (2000) *Anal. Biochem.*, **284**, 217-220.
23. Viktorova, L. V., Maksyutova, N. N., Trifonova, T. V., and Andrianov, V. V. (2010) *Biochemistry (Moscow)*, **75**, 95-100.
24. Senchuk, V. V., and Grintsevich, V. E. (2004) *Biochemistry (Moscow)*, **69**, 201-207.
25. Bradford, M. M. (1976) *Anal. Biochem.*, **72**, 248-254.
26. Vanin, A. F., Mordvintcev, P. I., and Kleschev, A. L. (1984) *Stud. Biophys.*, **107**, 135-143.
27. Ingrem, D. (1972) *Electron Spin Resonance in Biology* [Russian translation], Mir, Moscow.
28. Groppa, M. D., Rosales, E. P., Iannone, M. F., and Benavides, M. P. (2008) *Phytochemistry*, **69**, 2609-2615.
29. Shirshova, E. D., Klyuikova, A. I., and Alekhina, N. D. (1986) *Biol. Nauki*, **1**, 76-82.
30. Polevoi, V. V. (1989) *Physiology of Plants* [in Russian], Vysshaya Shkola, Moscow.
31. Klimenko, S. B., Peshkova, A. A., and Dorofeev, N. V. (2006) *Zh. Stress-Fiziol. Biokhim.*, **2**, 50-55.
32. Galangau, F., Daniel-Vedele, F., Moureaux, T., Dorbe, M.-F., Leydecker, M.-T., and Caboche, M. (1988) *Plant Physiol.*, **88**, 383-388.
33. Kaiser, W. M., and Huber, S. C. (2001) *J. Exp. Bot.*, **52**, 1981-1989.
34. Peive, Ya. V., Ivanova, N. N., Ovcharenko, G. A., and Shirshinskaya, M. G. (1975) *Fiziol. Rast.*, **22**, 527-536.
35. Deng, M., Moureaux, T., and Caboche, M. (1989) *Plant Physiol.*, **91**, 304-309.
36. Rayapureddi, J. P., Kattamuri, C., Chan, F. H., and Hegde, R. S. (2005) *Biochemistry*, **44**, 751-758.
37. Lillo, C., Meyer, C., Lea, U. S., Provan, F., and Oltedai, S. (2004) *J. Exp. Bot.*, **55**, 1275-1282.
38. Dean, J. V., and Harper, J. E. (1988) *Plant Physiol.*, **88**, 389-395.
39. Boissom, M., Mondon, K., and Torney, V. (2005) *Theor. Appl. Cenet.*, **110**, 932-940.
40. Nelson, R. S., Streit, L., and Harper, J. E. (1986) *Plant Physiol.*, **80**, 72-76.
41. Zhao, M.-G., Chen, L., Zhang, L.-L., and Zhang, W.-H. (2009) *Plant Physiol.*, **151**, 755-767.
42. Scheible, W.-R., Gonzalez-Fontes, A., Lauerer, M., Rober, B. M., Caboche, M., and Stitt, M. (1997) *Plant Cell*, **9**, 783-798.
43. Meyer, C., Lea, U. S., Provan, F., Kaiser, W. M., and Lillo, C. (2005) *Photosyn. Res.*, **83**, 181-189.
44. Rosales, E. P., Iannone, M. F., Groppa, M. D., and Benavides, M. P. (2011) *Plant Physiol. Biochem.*, **49**, 124-130.
45. Vanin, A. F., Svistunenko, D. A., Mikoyan, V. D., Serezhenkov, V. A., Fryer, M. J., Baker, N. R., and Cooper, C. E. (2004) *J. Biol. Chem.*, **279**, 24100-24107.
46. Andreeva, V. A. (1988) *The Enzyme Peroxidase* [in Russian], Nauka, Moscow.
47. Minibayeva, F., Kolesnikov, O., Chasov, A., Beckett, R. P., Luthje, S., Vylegzhanina, N., Buck, F., and Bottger, M. (2009) *Plant Cell Environ.*, **32**, 497-508.
48. Peive, Ya. V., Ivanova, N. N., and Drobysheva, N. I. (1972) *Fiziol. Rast.*, **19**, 340-347.
49. Araiso, T., and Dunford, H. B. (1980) *Biochem. Biophys. Res. Commun.*, **94**, 1177-1182.
50. Rogozhin, V. V. (2004) *Peroxidase as the Component of Antioxidant System of Living Organisms* [in Russian], Giord, St. Petersburg.
51. Vliet, A., Eiserich, J. P., Halliwell, B., and Cross, C. E. (1997) *J. Biol. Chem.*, **272**, 7617-7625.