Journal of Biological Systems, Vol. 8, No. 3 (2000) 219–235

A MULTI COMPARTMENTS MODEL OF NITRATE METABOLISM REGULATION IN PLANT ROOTS

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Received May 2000

ABSTRACT

A new concept illustrated by a corresponding mathematical model of nitrate metabolism regulation is proposed. The model is based on the root nitrate compartmentation in several functional pools: storage, metabolic and mobile (MobP) intended for translocation to shoots. Data on nitrate uptake, compartmentation, reduction in intact roots and translocation to shoots were obtained on steady state wheat seedlings grown at 25° and 12°C in the root zone. The net uptake, influx/efflux ratio, MobP size and translocation changed depending on the medium temperature. The oscillations of the net uptake rate, nitrate tissue concentration and its temperature modification were revealed. The scheme of regulation is based on the idea that net uptake through nitrate influx/efflux is under the control of the nitrate of MobP which size was dependent on the nitrate translocation into shoots. The mathematical model is represented by a system of ordinary differential equations simplified according to the time hierarchy of reactions. It has a limit cycle at definite values of parameters. The model postulates the mechanism of a positive feedback regulation of newly absorbed nitrate transfer into translocated pool formed in the root cortex. Theoretical results are verified experimentally.

Keywords: Nitrate pools, nitrate uptake, oscillation, regulation by compartmentation, mathematical model.

1. Introduction

Nitrate is the main source of nitrogen for plants and as such may be even the only one under certain conditions. Nitrate assimilation in plants involves a series of processes. The first step of the nitrate utilization is the absorption of nitrate by roots from the medium. In root cells nitrate may be reduced and then assimilated, transported to the vacuole and accumulated there, translocated to shoots and assimilated or accumulated in leaf cells [3,4,6,19]. The nitrate accumulation in plant organs is connected with the anion compartmentation in a cell and tissues. Usually ion compartmentation is considered as the distribution between cytosolic and vacuolar pools [4,9,30]. The main part of tissue NO_3^- is localized in vacuoles and only

the small amount of nitrate is presented in the cytosol cell [4,6,7]. The cytosolic nitrate is a substrate for nitrate reductase (NR) and for the subsequent N-NO_3^- assimilation. Thus, nitrate of cytosol was called "metabolic pool" (MP). The vacuolar nitrate is not available for the reduction and it was disignated as a "storage pool" (StP). However, a number of studies have demonstrated that the system of the nitrate compartmentation in plant roots is more complicated [1,16,17,20,28]. The two-compartmental scheme (MP and StP) does not reflect the real nitrate distribution in root tissues, because it does not take into account the nitrate translocated into xylem vessels and upflowed to shoots [1,17,28]. Earlier we suggested the term "mobile pool" (MobP) to distinguish this portion of the root nitrate. The latter belongs neither to MP nor to StP, but is translocated from roots to shoots [1,16,17].

The nitrate uptake and assimilation processes in root and shoot tissues are sensitive to external and internal factors and proceed under metabolic and genetic control [2–4,6,18–20]. Mathematical models play an important role as a tool for the analysis of mechanisms regulating the physiological processes. It has been found that the net uptake is regulated through the influx/efflux relationship [4,7,8,15,18,20,22,24]. Hence reported models of the nitrate uptake control [23,25] were focused mainly on the description of the nitrate transport across the plasma membrane. The use of ^{13}N, short-lived nitrogen isotope, allowed to propose the model describing quantitatively nitrate influx and efflux in roots depending on the nitrate distribution in root tissues [25]. In another model the time courses of $^{15}NO_3^-$ fluxes across the plasma membrane of root symplast were simulated. It was shown that the cytoplasmic NO_3^- and certain amino acids are involved in uptake regulation by the inhibition of influx and acceleration of efflux [23]. However the detailed studies of the nitrate utilization on the steady-state wheat plants revealed oscillatory behavior of the nitrate uptake processes and short-term fluctuations in endogenous nitrate concentration [2,14,15]. The net uptake rate and the nitrate tissue content have been shown to oscillate in the contraphase manner. It was suggested that these oscillations were related to the nitrate compartmentation in the roots and reflect the peculiarities of the regulation mechanism of nitrate absorption. The results of the investigation of nitrate uptake, reduction, compartmentation and translocation [1,2,14,15,19] were generalized in the empirical scheme [17]. A new mathematical multi-compartmental model of the nitrate uptake regulation in roots was developed and the possibility of the oscillations was simulated. The present paper deals with further development of the suggested mathematical model and the analysis of its compatibility with the observed experimental data.

2. Experimental Data. The Nitrate Utilization in Wheat Roots in Relation to the Environment Temperature

The wheat seedlings (*Triticum aestivum L.*, cv. Mironovskaya 808) were grown on the nutrient solution containing 3,0 mM KNO_3 as the nitrogen source. There were two temperature treatments: 25°/25°C and 25°/12°C in the shoot/root zone. There were no difference in root mass of 12-day old seedlings grown at two

temperature regimes , but nitrate utilization was considerably affected by the temperature conditions [2,3,19].

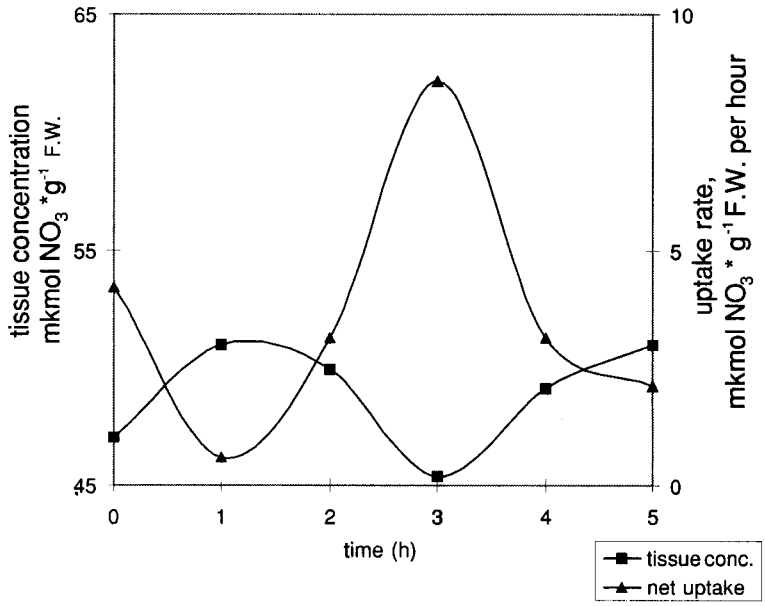
The main parameters of nitrate metabolism change are presented in Table 1 and Fig. 1. The nitrate net-uptake determined as the decrease in the NO_3^- concentration in the nutrient solution was less at 12° in the root zone as compared to 25°C. The decline in nitrate absorption at low temperature was often observed [2–4]. It is generally believed that the decrease in the nitrate net-uptake is due to the anion accumulation in root tissues followed by the NO_3^- efflux enhancement [2,4,8,9]. The low temperature during plant growth affects both fluxes but in different way: an influx decreased and an efflux increased in roots at 12°C as compared to 25°C (Table 1). The rate of NO_3^- net-uptake was changing rhythmically (Fig. 1). The amplitude of oscillations (the difference between minimal and maximal magnitudes) was higher at 25°C than at 12°C [14,15].

Table 1. Nitrate metabolism parameters in wheat roots in relation to temperature conditions (net uptake, influx, efflux, translocation to shoot: mkmol NO_3^-/g fresh weight *h; endogenous concentration and pool sizes of nitrate: mkmol NO_3^-/g fresh weight).

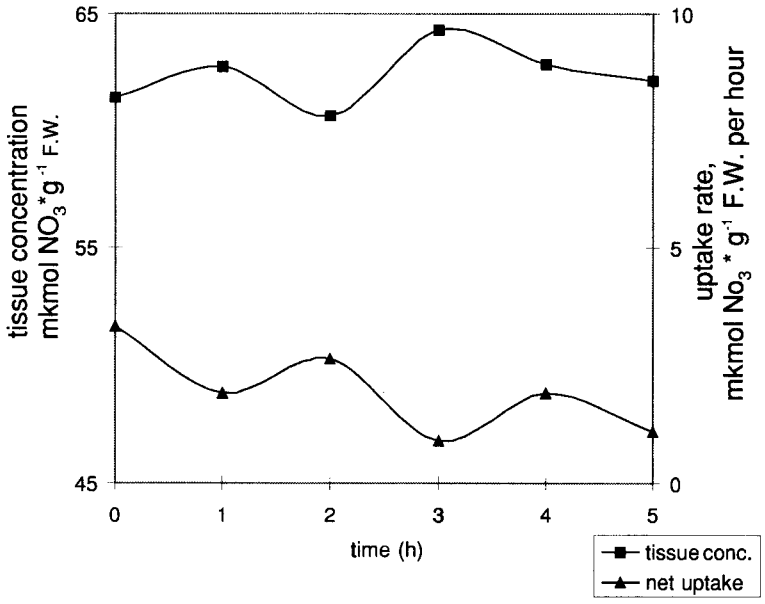
Nitrate metabolism parameters	Treatment (t°C in root zone) 25°	12°	References
Net uptake	3,81	1,93	[2,13,14]
Influx[1]	5,65	4,36	[1,14]
Efflux	1,84	2,43	[14]
Tissue concentration	48,9	62,4	[1,2,13]
Storage pool	21,6	21,6	[1,2]
Mobile pool	26,0	40,0	[1,2]
MP[2]			
NR activity	1,1	0,8	[1,2,18]
$^{15}NO_3^-$ reduction	0,9	0,9	[1,18]
Translocation to shoots[1]	4,12	2,53	[1,2,18]

[1]These processes were investigated coincidentally
[2]MP-metabolic pool

The nitrate content in roots was measured potentiometricaly in water extracts from tissues. Although at 12°C the nitrate uptake decreased the nitrate concentration in roots increased (Table 1). The measurements of tissue NO_3^- in parallel to the measurements of the uptake rate revealed the occurrence of fluctuations of tissue nitrate concentration in roots approximately with the two hour period (Fig. 1). The oscillations of nitrate concentrations and uptake proceeded in the contraphase [2,14,15]. The root StP was determined by the ≪trophic stress≫ method [1,16]. The intact plants were transferred to NO_3^--deprived medium. Over a period of 5 hours the roots lost part of nitrate but the next 5 to 10 hours the NO_3^- content remained constant. This nitrate represent the vacuolar StP. The size of StP did not



(a)



(b)

Fig. 1. The oscillations of nitrate net uptake rate and the anion tissue concentration in roots of wheat growing at 25°C (a) or 12°C (b) in the root zone [13,14].

change depending on different temperature conditions (Table 1). The NR in wheat roots was also insensitive to the effect of low temperature [2,3,19]. The MP size, determined on the basis of NR activity as well as on $^{15}NO_3^-$ reduction in situ, was very small and similar at both temperatures (Table 1). In roots at low temperature only the MobP was elevated significantly (Table 1) and therefore the net NO_3^- accumulation in tissues at 12°C has to be assigned to this pool [1,2].

In wheat plants a large part of absorbed nitrate moves from roots to shoots [3]. The nitrate translocation was determined as ^{15}N revealed in shoots of intact steady-state seedlings, exposed for a short time to nutrient solution containing $K^{15}NO_3$ [1,2,19]. At the 12°C the mean rate of nitrate translocation achieved only 60% of that at 25°C (Table 1). The decrease in the rate of nitrate flow in the middle of the light period becomes the limiting factor for the nitrate reduction in the leaves of seedlings grown at the low temperature [19]. The inhibition of the nitrate translocation brings about the nitrate accumulation in roots, or to be more exact in the MobP. Eventually the increase of endogenous nitrate concentration brings about the decrease of NO_3^- net uptake due to the influx/efflux ratio variations (Table 1) and modification of oscillation patterns (Fig. 1) in wheat seedlings at the low temperature.

3. Model and Equations

3.1. *The Scheme of Regulation of Nitrate Utilization Considered for Mathematical Model*

A new empirical scheme of nitrate uptake control linking the transport process (uptake and translocation) to the compartmentation in roots of non-starving steady-state plants has been elaborated (Fig. 2(a)). The nitrate transport across the plasma membrane is controlled through the influx/efflux ratio. It is usually believed that the nitrate availability and accompanying cations (K^+, NH_4^+) are the major exogenous effectors responsible for the regulation of nitrate fluxes. The endogenous regulation of nitrate uptake is commonly associated with tissue NO_3^- concentration, the activity of NR, reducing the absorbed nitrate, and some amino acids as the end products of N-NO_3^- assimilation [3,4,8,14,18,20,23].

The multi-compartmental scheme of the regulation of nitrate utilization (Fig. 2(a)), based on the single-cell model of a root system [5,9,24], provides for the existence of MobP and the nitrate translocation as a limiting step of NO_3^- uptake [1–3,19]. In roots of steady-state wheat seedlings the StP size was constant (Table 1) and besides its exchanges proceed too slowly in time [7,16]. The MP was very small and at different temperature treatments there was neither variation in its size (Table 1). Consequently in roots of wheat plants adapted to the environmental temperature the nitrate of StP and MP is not involved in the NO_3^- net uptake regulation via the influx/efflux mechanism. It is obvious that the changes of MobP size depending on the temperature in the root zone (Table 1) are important to the regulation of nitrate fluxes and net uptake as a whole. The nitrate accumulation

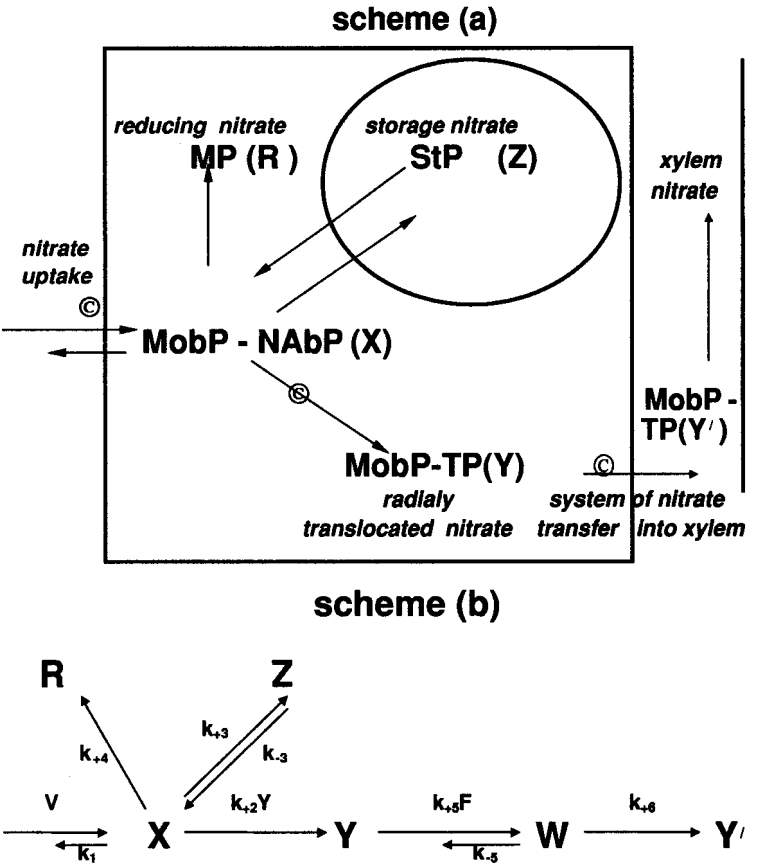


Fig. 2. The empirical (a) and kinetic (b) schemes of nitrate uptake regulation involving the polycompartmentation, transport fluxes ($\rightleftharpoons$) and the sites of control ©. The pattern of nitrate compartmentation involves the follow functional pools: metabolic (MP), storage (StP) and mobile (MobP), devided into the pool of newly absorbed NO_3^- (NAbP) and the translocational pool (TP). ***X*** (NAbP) — the pool of nitrate newly absorbed from the media; ***Y*** (***TP***) — the pool of nitrate which is translocated from roots to shoots; ***Z***(StP) — the pool of nitrate stored in vacuole; R (MP) — the pool of nitrate reduced by nitrate reductase and then assimilated; F — the carrier forming a complex ***W*** with the nitrate of ***Y*** (TP) and releasing nitrate ***Y'*** in the xylem; ***V*** — the rate constant of nitrate influx; and $\mathbf{k}_{+i}$, $\mathbf{k}_{-i}$ — the rate constants of corresponding stages of nitrate transformations.

or depletion in MobP is due to the translocation rate and hence the accumulation is associated with the processes of radial and xylem transport. Mechanisms of nitrate translocation from roots to shoots are not elucidated and the regulation of the xylem load is still to be studied [5,6,9,22,24,29].

The scheme of regulation based on the single-cell model and the pattern of the root nitrate distribution among three functional pools (MP, StP and MobP) is the simplification of the real situation [1,4,5,17,24]. The nitrate activity and concentration had been shown to be different in epidermal and cortical cells of a barley root. The nitrate activity related to the cytosol compartment was higher

in epidermal cells, whereas its concentration was higher in the cortical ones due to the anion accumulation in vacuole [30]. MP and MobP are localized in cytosol and both pools are responsible for the observed nitrate concentration level in this compartment [1,17]. It is argued that the endoplasmic reticulum (ER) represents the cytoplasmic structure where the nitrate, intended for the transfer into shoots, may be localized [5,28]. The ER of the neighboring cells are associated through the desmotubules of cell wall plasmodesmata and provides a suitable pathway for radial transfer of solutes in roots [5,9,28]. It is obvious that the nitrate of MobP in root is not ≪homogenous≫, since a portions of this nitrate are localized in the symplast as well as in the apoplast, predominantly in xylem vessels and partially in the free space of root cortex [1,20,28]. Besides, the nitrate pool providing NO_3^- efflux must exist in symplast along with the nitrate transported radialy in an ER [15,20,25]. Most likely the largest share of nitrate efflux to a medium comes from the newly absorbed nitrate pool (NAbP) localized in cytosol of epidermal cells. The NAbP also replenishes other pools such as MP and StP. In this connection in the suggested empirical model (Fig. 2(a)) the MobP is distributed between two compartments corresponding to nitrate of translocational pool (TP) localized in ER and xylem vessels and nitrate of NAbP is placed in cytosol of cortical cells. The fluxes across plasmalemma are governed by the nitrate concentration in MobP (Table 1). Dynamic interaction of compounds of the system is manifested by oscillations of net uptake rate and NO_3^- tissue concentration (Fig. 1).

Thus, in wheat seedlings adapted to temperature conditions the system of nitrate uptake regulation can be presented as a set of interrelated successive events involving functional and space nitrate multi compartmentation and a complex system of nitrate transport processes providing anion translocation into shoots Fig. 2(a). The limiting step in this system is the process of nitrate export from roots to shoots. The commonly considered site of ions translocated regulation is the release of ions from symplast to the xylem [5,9,22,29]. We believe that there is another step which may be sensitive to the low temperature and to restrict the translocation rate. The NO_3^- transport across the membrane of ER and forming of TP in cortex cells may be the second set responsible for translocation rate (Fig. 2(a)). Such location of controlling system allows to integrate the plasmalemma fluxes, translocation and compartmentation in the unified system of uptake regulation.

3.2. *Mathematical Model*

The mathematical model was developed on the base of the empirical and kinetic schemes to understand the regulation mechanism of nitrate utilization in detail and to explain its temperature dependence. In the mathematical model $\boldsymbol{X}, \boldsymbol{Y}, \boldsymbol{Z}, \boldsymbol{R}$ correspond to the compartments of root nitrate $\boldsymbol{MobP} = (\boldsymbol{NAbP} + \boldsymbol{TP}), \boldsymbol{StP}$ and $\boldsymbol{MP}$ (Figs. 2(a) and (b)). The $\boldsymbol{X}$ pool is responsible for the nitrate efflux from roots and the exchange of nitrate with of MP and StP and translocational pool $\boldsymbol{Y}$ determines the interaction with NabP and nitrate translocation rate to the shoots.

A set of experimental evidences and assumptions were used when constructing the kinetic model of the nitrate uptake. The nitrate influx in roots at a given temperature occurs at a constant rate V. This actually takes place because under test conditions the nitrate concentration in the medium solution was little affected by NO_3^- uptake by plants. The efflux from the root is proportional to the nitrate concentration in cytosol X(NAbP) and has a rate constant k_1. The fluxes of the exchange between X and vacuole nitrate of Z are of the first order. It is true because in accordance with experimental data the size of X is much less than the size of Z. This is also confirmed by the long half-life time of nitrate exchange in Z, half-life exchange for NO_3^- takes about 20 hours [7]. The transfer of nitrate from symplast into a xylem proceeds according to the Michaelis-Menten mechanism [22] via translocator F. The transfer of cytozole nitrate X into TP Y is regulated by a positive feedback control mechanism with respect to Y concentration. The nitrate concentration in Y provides positive feed back control for transfer of cytosol nitrate into Y (TP). Such type of positive autocatalytic regulation by the product is well known for some metabolic processes [13,26,27].

According to the kinetic scheme (Figs. 2(b)) and the above consumptions we have written at first a total system of ordinary differential equations (see Appendix). Taking into account the hierarchy of times we simplified the system of differential equations. In the limit transition the system of differential equations within dimensionless variables is rewritten in the form

$$\begin{aligned} dx/d\tau &= v - k \cdot x - m \cdot x \cdot y \\ dy/d\tau &= m \cdot x \cdot y - y/(1+y) \end{aligned} \tag{3.1}$$

where $v = (V + k_{+3} \cdot K_m \cdot z_c)/(F_0 \cdot k_{+6})$; $k = (k_1 \cdot K_m + k_{+3} \cdot K_m)/(F_0 \cdot k_{+6})$; $m = k_{+2} \cdot K_m/(F_0 \cdot k_{+6})$. The steady states concentrations for system (3.1) are

$$\begin{array}{lll} \textbf{1. } x_c = (v-1)/(k-m) & \text{and} & \textbf{2. } x_c = v/k \\ \quad y_c = (v - k/m)/(1-v) & & \quad y_c = 0 \end{array} \tag{3.2}$$

The second steady state corresponds to the absence of the ≪mobile pool≫ and is realized under special conditions of starvation. So we will consider only the first one.

Theoretical considerations were obtained by means of investigation of spectrum of the Jacobian matrix.

We now introduce the parameter $p = \sqrt{(1-v)^2 \cdot (v - \frac{k}{m})/m \cdot \left(1 - \frac{k}{m}\right)^3}$ for the analytical investigation of the system (3.1), which deals with the trace of Jacobian matrix -Sp in the following manner: $Sp = (p^2 - v)/x_c$ .The condition of unstability is $Sp > 0$ that is $v < p^2$ ($x_c > 0$ from biological sense of the problem). The condition of the oscillative regime is $(v + p^2)^2 < 4p^2$. The Hopf bifurcation takes place when both conditions $v = p^2$ (an eigenvalue pair with zero real part) and $v < 1$ (det (Jacobian) > 0) are fulfilled.

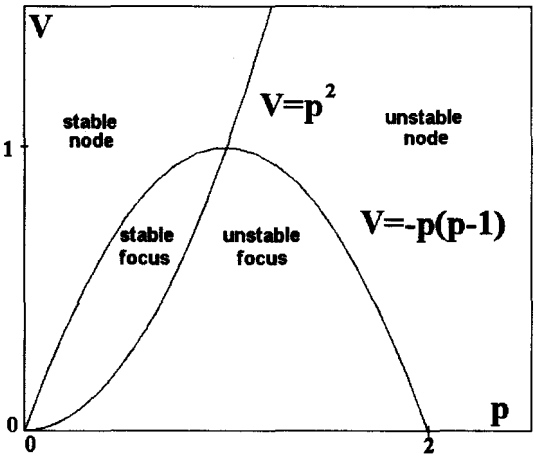


Fig. 3. Bifurcation diagram under steady-state conditions in the model system (3.1). 1. Unstable focus associates with the possibility of self-sustained oscillations appearing; 2. Stable focus associates with damped oscillations; 3. Stable node shows nonoscillative regime; and 4. Unstable node. Axes: ordinate — v (see Eq. 4), abscissa — p (complex parameter) $p = \sqrt{(1-v)^2 \cdot \left(v - \frac{k}{m}\right)/m \cdot \left(1 - \frac{k}{m}\right)^3}$. We used such complicated form of abscissa, because it gives the most simple form of bifurcation diagram and allows to define the region where self-sustained oscillations can arise.

The bifurcation diagram with the regions of stability and unstability, oscillative and nonoscillative regimes is presented in Fig. 3. This diagram will be used below to evaluate parameter values responsible for oscillations observed at two temperatures 12°C and 25°C in the unstable focus region.

To compare the results of numerical investigations with the experimental data we return to the dimensional system by applying the designations (4) (see appendix) to the system (3.1). Thus we have the following system:

$$\begin{aligned} dX/dt &= V_0 - k_0 \cdot X - k_{+2} \cdot X \cdot Y\,; \\ dY/dt &= k_{+2} \cdot X \cdot Y - V_x \cdot Y/(K_m + Y)\,, \end{aligned} \tag{3.3}$$

where

$V_0 = V + k_{-3} \cdot Z_c$ - is overall rate of nitrate fluxes from the media and vacuole into X;

$k_0 = k_1 + k_{+3}$ - is overall nitrate efflux rate constant from $NAbP$ to the media and vacuole;

$V_x = k_{+6} \cdot F_0$ - is the possible maximal rate of the flux into xyleme;

$K_m = (k_{-5} + k_{+6})/k_{+5}$ - is the analogue of apparent Michaelis-Menten constant.

4. Results

The reduction of this system (see appendix) allows to represent the nitrate transport flows from the medium to the xyleme in accordance with the empirical scheme

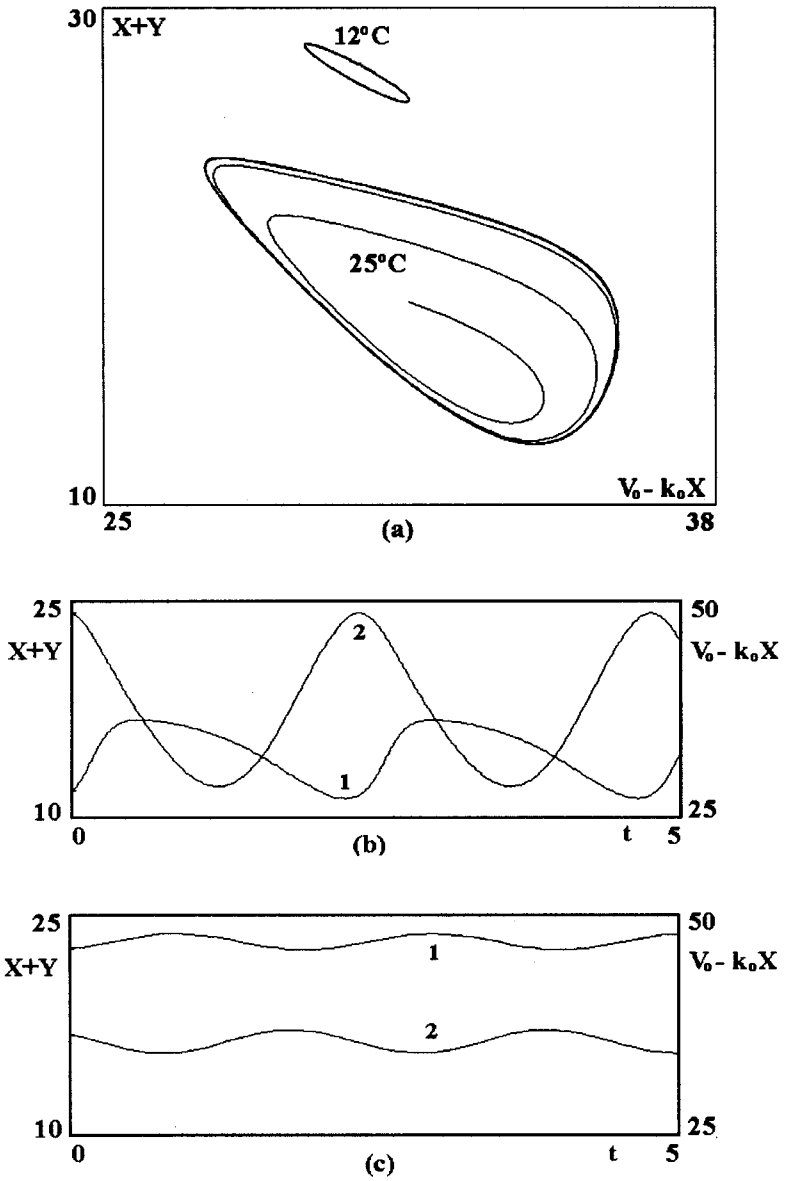


Fig. 4. The numerical solution of the model system (3.3) for two temperature regimes:
(1) 25°C with the parameters of the model: $V_0 = 38,\ k_0 = 0.5, k_{+2} = 0.7, V_x = 63, K_m = 3.8$
(2) 12°C with the parameters of the model: $V_0 = 50,\ k_0 = 0.8, k_{+2} = 0.43, V_x = 157, K_m = 12.$
(a) Phase diagram, abscissa is nitrate net-uptake $V_0 - k_0 \cdot X$, ordinate is overall endogenous nitrate concentration $X + Y$; (b) Simulation of kinetics of the net-uptake $V_0 - k_0 \cdot X$ (curve 1) and the changes of overall endogenic concentration of nitrate $X + Y$ (curve 2) at 25°C; (c) Simulation of kinetics of the net-uptake $V_0 - k_0 \cdot X$ (curve 1) and the changes of overall endogenic concentration of nitrate $X + Y$ (curve 2) at 12°C. Numerical calculations were obtained by means of software TRAX [21] for solving ordinary differential equations, in which Runge-Kutta method of the fourth order of approximation is used.

(Figs. 2(a) and (b)):

$$V_0 - k_0 x \qquad k_{+2} xy \qquad V_x y/(k_m + y) \qquad \text{(Scheme c)}$$
$$\Rightarrow \quad \boldsymbol{X} \quad \Rightarrow \quad \boldsymbol{Y} \quad \Rightarrow$$

We conducted further numerical investigation for parameter values from region of unstable focus. Numerical calculations were obtained by means of software TRAX [21] for solving ordinary differential equation, in which Runge-Kutta method of the fourth order of approximation is used. Because the obtained experimental data refer to the nitrate net-uptake and the dynamics of the overall nitrate endogenous concentration but not to the dynamics of nitrate in cytosole and mobile pool we have the following complex variables: $(\boldsymbol{V_0} - \boldsymbol{k_0}(\boldsymbol{X})$ which refers to the nitrate net-uptake and $(\boldsymbol{X} + \boldsymbol{Y})$ which refers to MobP.

The results of numerical calculations of the system of differential Eq. (3.1) are presented in Fig. 4. When conducting the numerical calculations we fitted the model parameters to obtain the qualitative coincidence with the experimental curves i.e. the decrease in the oscillation amplitudes should be accompanied by the increase in $(\boldsymbol{X} + \boldsymbol{Y})$ and decrease in $(\boldsymbol{V_0} - \boldsymbol{k_0 X})$ levels. The nitrate content was evaluated by the position of the fixed point in the system (3.3).

The behavior of the system at two different temperature in the model could be described with two sets of parameters $\boldsymbol{V_0}, \boldsymbol{k_0}, \boldsymbol{k_{+2}}, \boldsymbol{V_x}, \boldsymbol{K_m}$ (3.3). Oscillations of nitrate net-uptake $(\boldsymbol{V_0} - \boldsymbol{k_0} \cdot \boldsymbol{X})$ proceed nearly in the counterphase with respect to oscillations of the overall endogenous nitrate concentration $\boldsymbol{X} + \boldsymbol{Y}$ (Figs. 4(b) and (c)). Curves which corresponds to the experimental data at 25°C describe the oscillations with the large amplitude. The amplitude of the oscillations at 12°C decreased. At 12°C as compared to 25°C the limit cycle position on the phase plane changed (Fig. 4(a)). The limit cycle which corresponds to 12°C is shifted to the less values of $(\boldsymbol{V_0} - \boldsymbol{k_0} \cdot \boldsymbol{X})$ (net-uptake rate in experiment) and to the greatest values of $(\boldsymbol{X} + \boldsymbol{Y})$ (the nitrate concentration of MobP). It can be seen that the shapes and the period of oscillation of model and experimental curves are quite similar. The difference in the model and experiment concentration and uptake rate scales are actually due to the fact, that we do not possess the definite experimental information about the values of parameters of the model. That's why our model reflects only qualitative features of the system.

The mathematical model permits to explain the following main effects revealed by the investigation of nitrate utilization process in roots depending on temperature conditions: changes in the nitrate net uptake level, endogenous nitrate concentration and pool sizes; the occurrence of oscillations of nitrate uptake rate and nitrate tissue concentration; the variation of oscillation amplitudes in relation to environment temperature; the change in the nitrate translocation.

5. Discussion

Earlier limited number of models taking into account the nitrate compartmentation were developed to describe nitrate uptake by roots [23,25]. These models were based

mainly on the experimental determination and calculation of NO_3^- fluxes directed into and out of the roots and partially on evidences of anion transfer from roots to shoots. The main attention was focused on the regulation of influx/efflux system depending on exogenous NO_3^- concentration and endogenous NO_3^- and amino acid concentrations in root tissues. The models contain linear differential equations so that the analysis of these models gives the exponential form of time-courses describing the nitrate saturation processes in root tissues.

In our model we proceeded from the assumption that the feed-back interaction takes place between two parts of MobP: the newly absorbed pool $\boldsymbol{X}$ and the translocational pool $\boldsymbol{Y}$ (Figs. 2(a) and (b)). Since radial nitrate flow proceeds in the ER [5, 28] the barrier separating cytosol NAbP ($\boldsymbol{X}$) from TP ($\boldsymbol{Y}$) in MobP is ER membrane, and the site of regulation is transport systems of ER membrane. The absorbed nitrate is loaded into ER in the root cortex and the main part of anion appears to be in the ER in the epidermal cells [28,30]. Although there are no data concerning actual nitrate transport mechanism across the ER membrane obviously this process as well as nitrate transport across plasmalemma or tonoplast requires energy consumption. There is also good reason to think that nitrate transport system in ER membrane like the tonoplast one is coupled to ATPase and is controlled by NO_3^- cytosol concentration [4,6,12].

The analysis of the suggested model (Figs. 2(a), (b), Scheme c) shows that oscillations of the nitrate uptake rate and the endogenous concentration as well as their dependence on temperature can be simulated if only nitrate transport across ER membrane is described by the non linear terms in Eqs. (3.1) and (3.3). It implies the type of regulation similar to the positive feed-back mechanism [13,27]. Since temporal changes of main variables in the model are periodical, all fluxes (the net uptake, efflux to the media and the flow to xylem) change periodically as well. The experimental data confirm this for the nitrate net uptake (Fig. 1). As known the positive feed-back control is also responsible for the oscillation in other metabolic systems [10,13,26,27]. For example in the initial stages of glycolysis the oscillation of substrate phosphorilation rate is due to the positive adenilate feed-back control of phosphofructokinase activity [11,12]. Another example is the oscillation dynamics associated with the positive feed-back regulation of the Ca^{2+} release from intracellular structures triggered by the rise of cytosolic Ca^{2+} [10].

Thus, by means of mathematical model consideration the conclusion could be made that nitrate transport across ER membrane presents the type of process where nitrate Y (TP) activates anion transfer from cytosol X (NAbP) to ER. It is described by non-linear kinetic equation similar to those describing metabolic processes where regulation is performed through positive feed-back mechanism. In the real situation the size TP and the NO_3^- concentration in TP ($Y+Y'$) determine the rate of NO_3^- translocation from roots to shoots. Our data and their analysis in the mathematical model have suggested that the nitrate translocation from roots may be regulated at the stage of TP formation in cortex cells. Moreover the process of

TP formation (more appropriately the NO_3^- transport across ER membrane) is incorporated into regulation of NO_3^- uptake (Fig. 2).

6. Conclusion

The spatial and temporal organization of cell, tissue and organ metabolism is rested on principle of compartmentation of processes, pathways, compounds as well as transport of metabolites and substances across membranes. The control of metabolism by means of compartmentation and change of the rate and direction of fluxes operates along with other known types of regulation (metabolic, genetic, hormonal) [4–6,9,17,20]. The system of compartmentation and partition between different structures provides the effective utilization of absorbed mineral nutrition particularly nitrate nitrogen [1,7,16,18,20,28].

The presented multi-compartmental model (Fig. 2) of nitrate metabolism regulation in roots of wheat plants adapted to different temperature regimes and its mathematical description is rested on the following facts and considerations:

- occurrence in root tissues of MobP and its multi-partition in tissue structure of root;
- close interrelation between MobP and NO_3^- uptake reflected in the contraphase oscillations of the uptake rate and endogenous NO_3^- concentration;
- the appreciable modifications of NO_3^- translocation fluxes in roots depending on the environmental temperature;
- coupling of the NO_3^- uptake regulation to the translocation system.

The experimental data (Table) generalized in our models (Fig. 2) as well as additional data [4,16,17,20,28,30] suggested that in roots a set of functional, space divided pools have to exist in the symplast (StP, MP, NAbP and TP) and in the apoplast — TP. The apoplast TP is presented by nitrate of xylem solution and obviously by cortical free space pool. The mathematical model confirms that two compartmental pools (NAbP and TP), both comprising MobP are involved in NO_3^- uptake regulation. There are good reasons to believe that symplast TP formation takes place in cortex cells in which newly absorbed nitrate is transferred into ER. The model demonstrates how positive feedback control mechanism generates oscillations of nitrate concentrations in root tissue and uptake rate and causes changes of their amplitude with temperature (Figs. 1 and 4(a), (b)). The feedback link between two cytosole portion of MobP is responsible for the elevation of endogenous nitrate content and enhancement of its efflux from roots to the media at low temperature (Table 1). The oscillations in such systems as glycolysis [11,13,26,27], the release of secondary messenger (Ca^{2+}) in cytosol [10], nitrate uptake and tissue concentration in root (Fig. 1) illustrate the generality of these fundamental regulation mechanisms occurring in biological systems.

Acknowledgment

This work has been supported by a grant from the Russia Fond Fundamentals Research N 97-04-49477

Appendix

According to the kinetic scheme (Fig. 2(b)) and the consumptions of Sec. 3. we can write a system of ordinary differential equations:

$$dX/dt = V - k_1 \cdot X - k_{+3} \cdot X + k_{-3} \cdot Z - k_{+4} \cdot X \cdot (R_0 - R) - k_{+2} \cdot X \cdot Y \quad (1a)$$

$$dY/dt = k_{+2} \cdot X \cdot Y - k_{+5} \cdot Y \cdot F + k_{-5} \cdot W \quad (1b)$$

$$dZ/dt = k_{+3} \cdot X - k_{-3} \cdot Z \quad (1c)$$

$$dW/dt = k_{+5} \cdot Y \cdot F - k_{-5} \cdot W - k_{+6} \cdot W \quad (1d)$$

$$dR/dt = k_{+4} \cdot (R_0 - R) \cdot X \quad (1e)$$

$$F + W = F_0 \quad (1f)$$

R is the concentration of nitrate in MP; R_0 - is the bulk of nitrate which may be reduced by NR, F_0 in the Eq. (1f) is the overall concentration of the carrier.

There are three characteristic time scales involved in the scheme: slow process of nitrate exchange in Z-StP (half-life exchange for $NO_3^- \approx 20$ h) [7], fast reaction of nitrate reduction and exchange of R-MP ($t_{1/2} \approx 17$ min [7] or 7,5 min [20]) and process of intermediate time scale such as oscillations of uptake rate and nitrate concentration in MobP (period $\approx 1 - 2$ h) (Fig. 1). We pass over then to dimensionless variables:

$$x = X/K_m, \quad y = Y/K_m, \quad \text{where} \quad K_m = (k_{-5} + k_{+6})/k_{+5},$$

$$r = R/R_0, \quad w = W/F_0, \quad f = F/F_0, \quad z = Z \cdot K_3/K_m,$$

$$\text{where} \quad K_3 = k_{-3}/k_{+3}, \quad \text{and} \quad \tau = t \cdot F_0 \cdot k_{+6}/K_m \quad (2)$$

The value of the Michaelis-Menten constant K_m is of the same order of magnitude as nitrate concentrations X and Y. We can consider $k_{-3} \cdot K_m$ and $F_0 \cdot k_{+6}$ as the rates of the nitrate efflux from vacuole Z and the nitrate flux to xylem respectively. Since according to experimental evidence $k_{-3} \cdot K_m << F_0 \cdot k_{+6}$ it allows one to introduce a small parameter $\varepsilon_1 = k_{-3} \cdot K_m/(F_0 \cdot k_{+6})$. As a rule the carrier concentration F_0 is much less then the concentration of the substrate. So we can propose that $F_0 << K_m$ and introduce a second small parameter $\varepsilon_2 = F_0/K_m$.

With dimensionless variables the system has the following form, where we take into account the hierarchy of times:

$$\begin{aligned} \boldsymbol{dx/d\tau} &= \boldsymbol{V/(F_0 \cdot k_{+6})} - \boldsymbol{k_1/(F_0 \cdot k_{+6}) \cdot x} - \boldsymbol{k_{+3} \cdot K_m/(F_0 \cdot k_{+6}) \cdot (x - z)} \\ &\quad - \boldsymbol{k_{+4} \cdot R_0 \cdot K_m/(F_0 \cdot k_{+6}) \cdot x \cdot (1 - r)} \\ &\quad - \boldsymbol{k_{+2} \cdot K_m \cdot K_m/(F_0 \cdot k_{+6}) \cdot x \cdot y} \qquad (3a) \\ \boldsymbol{dy/d\tau} &= \boldsymbol{k_{+2} \cdot K_m \cdot K_m/(F_0 \cdot k_{+6}) \cdot x \cdot y - k_{+5} \cdot K_m/k_{+6} \cdot y \cdot f} \\ &\quad + \boldsymbol{k_{-5}/k_{+6} \cdot w} \qquad (3b) \\ \boldsymbol{dz/d\tau} &= \varepsilon_1 \cdot \boldsymbol{(x - z)} \qquad (3c) \\ \varepsilon_2 \cdot \boldsymbol{dw/d\tau} &= \boldsymbol{k_{+5} \cdot K_m/k_{+6} \cdot (y \cdot f - w)} \qquad (3d) \\ \varepsilon_2 \cdot \boldsymbol{dr/d\tau} &= \boldsymbol{k_{+4} \cdot K_m/k_{+6} \cdot (1 - r) \cdot x} \qquad (3e) \\ \boldsymbol{f + w} &= 1 \qquad (3f) \end{aligned}$$

Equation (3c) contains the small parameter ε_1 in the right side whereas Eqs. (3d) and (3e) contain the small parameter ε_2 in the left side. It means that Eq. (3c) describes the slowest processes and Eqs. (3d) and (3e) describe the fastest processes in the system.

Due to $\varepsilon_1 \to \mathbf{0}$ the equation $\boldsymbol{dz/d\tau} = 0$ is true for the slow variable $\boldsymbol{z}$. So $\boldsymbol{z} = \boldsymbol{z_c}$ is constant. Because of $\varepsilon_2 \to 0$ we can make the limit transition according to Tikhonov theorem to get algebraic equations and exclude fast variables by the method of quasi steady states concentrations.

Let us introduce dimensionless parameters:

$$\boldsymbol{v} = \boldsymbol{(V + k_{+3} \cdot K_m \cdot z_c)/(F_0 \cdot k_{+6})};$$

$$\boldsymbol{k} = \boldsymbol{(k_1 \cdot K_m + k_{+3} \cdot K_m)/(F_0 \cdot k_{+6})}; \qquad (4)$$

$$\boldsymbol{m} = \boldsymbol{k_{+2} \cdot K_m \cdot /(F_0 \cdot k_{+6})}. \qquad (5)$$

Taking into account the above proposals we simplify the system of differential equations. In the limit transition the system of differential equations within dimensionless variables is rewritten in the form

$$\begin{aligned} \boldsymbol{dx/d\tau} &= \boldsymbol{v - k \cdot x - m \cdot x \cdot y} \\ \boldsymbol{dy/d\tau} &= \boldsymbol{m \cdot x \cdot y - y/(1 + y)} \end{aligned} \qquad (6)$$

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