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Parameter Identification and Assessment of Nutrient Transporters in AM Symbiosis through Stochastic Simulations*

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Abstract

This paper presents a methodology for identifying the kinetic parameters of a biological system and their relationships with the concentration variations of the species by means of in silico experiments. A nonlinear goal programming technique is employed to identify the kinetic parameters that best satisfy the biological hypothesis of the system behaviour. The simulations are performed on a stochastic simulator for the Calculus of Wrapped Compartments. The extraction of parameters with optimization techniques is usually applied to systems modeled by means of differential equations. Its application in a stochastic framework is rather new. The methodology may open a new path for the investigation of a broader class of biochemical systems (including those that cannot be easily modeled by differential equations) when in vitro experimental measurements are unavailable. The methodology is applied to model the nutrient exchanges in arbuscular mycorrhiza, the most wide-spread plant/fungus symbiosis focusing in particular to a newly discovered ammonium transporter: LjAMT2;2. This case study of ammonia and phosphate plant uptake from the fungus has been dissected in known and hypothetical mechanisms. The results of the analysis provide setting of recent experimental results of carrier LjAMT2;2 and support new insights into biological hypotheses to be put in place.

Keywords: Systems Biology, Formal Calculi, CWC, Mycorrhizal Symbiosis, Optimization, Parameter Identification

1 Introduction

The modelling of biological systems must tackle problems such as incomplete or uncertain data and qualitative information about the relationships among the various elements of the system [17]. Optimisation techniques are often used for parameter identification in the context of biochemical systems: given a set of experimental

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data, calibrate the model in order to reproduce the experimental results at best [25,43,31] or attempting to use constraints based modeling to achieve desired performance as it has been done using linear programming [42,40] or non-linear downhill methods [26]. The parameter identification problem, however, is usually applied to models in which the biological system are described essentially by differential equations.

This paper presents a methodology to coordinate the data resulting from the analysis of a sequence of stochastic simulations into an optimization flow in order to identify unknown parameters related to metabolic activities. Only recently, this problem has been addressed in the context of stochastic simulations [33]. The aim is to provide biologists with a tool to assess unknown quantities by means of in-silico experiments.

A useful and flexible tool of mathematical programming to solve this kind of problems is the *goal programming* [22,34]. It uses a special formulation of the problems that can easily be "translated" in terms of constrained minimization that allows to phrase the search of a good parametrization to control the system through several objectives. Moreover, unlike the multi-objective stochastic search methods based on evolutionary paradigms [24], the goal programming represents the problem as a set of constraints and develops solutions to satisfy them with less computational effort. The formulation of goal programming allows to define the parametrization of a biological model in terms of performance metrics that must be satisfied. Each of these performance measure is then translated into a goal. The overall objectives, together with constraints that the biological system must satisfy, analyse the model parametrization in the search space. This approach allows to deal with biological systems for which information derived from experimental data are poor and which rely mainly on biological hypotheses or assumptions. In goal programming approach for multiobjective optimization [29], the relations among targets are analysed in order to describe the model behaviours. Thus the designer is able to assess incrementally, goal by goal, the consistency of the model to experimental data, required constraints, and quantitative assumptions.

The proposed methodology is applied to model a recently discovered ammonium transporter [19] which acquires minerals for plants in a plant-fungus symbiosis. In this kind of systems, flux measurements capturing all metabolic processes are very poor due to the difficulty of in vivo experiments. The information obtained from the model parameter identification provides an indirect measure of the chemical energy (provided by H^+-ATPases pumps) necessary for plants and fungi to sustain the symbiotic exchange of nutrients. This measure enables a biologist to do an assessment of the difference between the two LjAMT in their efficiency in ammonium traslocation in terms of the source of energy that they use, and the consequent operation of other transporters involved in nutrients exchange.

The underlying biological system was represented in the Calculus of Wrapped Compartments (CWC) [10,8,9] which allows a natural description of a large class of biological systems by providing a direct representation of the notions of membrane and compartment. The CWC, moreover, is provided with an efficient stochastic

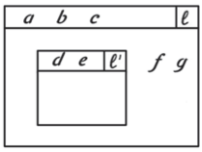

Fig. 1. A graphical representation of $(a\ b\ c \rfloor (d\ e \rfloor \bullet)^{\ell'}\ f\ g)^{\ell}$

simulator which exploits in an efficient way multi-core architectures [3,11] and with a tools supporting on-line parallel statistical analysis of results to extract the behavioural features of biological systems [2].

The paper is organized as follow. Section 2 recalls the CWC framework. Section 3 describes the goal programming methodology. Section 4 illustrates the nutrient transport model in AM symbiosis. Section 5 presents the results of this analysis expounding the methodology. Section 6 briefly discusses related work and Section 7 concludes the paper.

2 The Calculus of Wrapped Compartments Framework

The Calculus of Wrapped Compartments (CWC) [10,8,9] has been designed to describe biological entities by means of a nested structure of ambients delimited by membranes.

The terms of the calculus are built on a set of *atoms* (representing species e.g. molecules, proteins or DNA strands) , ranged over by a, b, ..., and on a set of *labels* (representing compartment types e.g. cells or tissues), ranged over by ℓ,.... A *term* is a multiset $\overline{t}$ of *simple terms* where a simple term is either an atom a or a compartment $(\overline{a} \rfloor \overline{t'})^{\ell}$ consisting of a *wrap* (a multiset of atoms $\overline{a}$), a *content* (a term $\overline{t'}$) and a *type* (represented as a label ℓ). For uniformity we assume that the term representing the whole system is always a single compartment labelled by the distinguished label $\top$ with an empty wrap. Multisets are denoted by listing the elements separated by a space. The empty multiset is denoted by $\bullet$. As usual, the notation $n{*}t$ to denotes n occurrences of the simple term t. For instance, the term $2{*}a\ \ (b\ \ c \rfloor a\ \ b)^{\ell}$ represents a multiset containing two occurrences of the atom a and an ℓ-type compartment $(b\ \ c \rfloor a\ \ b)^{\ell}$ which, in turn, consists of a wrap with two atoms b and c on its surface, and containing the atoms a and b. See Figure 1 for a pictorial representation of a term.

Interaction between biological entities are described by rewriting rules written as $\ell : \bar{p} \to \bar{o}$ where $\bar{p}$ (the *pattern*) and $\bar{o}$ are terms built on an extended set of atomic elements which includes *term* variables (ranged over by X, Y,...) and *wrap* variables (ranged over by x, y,...). The label ℓ represents the compartment type to which the rule can be applied. An example of rewrite rule is $\ell :\ a\ b\ X \to c\ X$ that is often written as $\ell :\ a\ b \to c$ giving X for understood to simplify notations. [3] The application of a rule $\ell : \bar{p} \to \bar{o}$ to a term $\overline{t}$ consists in finding (if it exists) a subterm $\overline{u}$ in a compartment of type ℓ such that $\overline{u} = \sigma(\bar{p})$ for a ground substitution σ and

[3] We force *exactly* one variable to occur in each compartment content and wrap of patterns. This prevents ambiguities in the instantiations needed to match a given compartment.

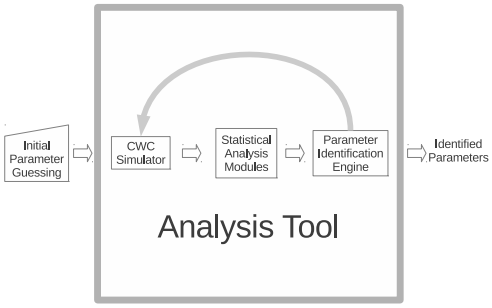

Fig. 2. Parameter Identification Flow incorporating the CWC Simulator

replacing it with $\sigma(\bar{o})$ in $\bar{t}$ obtaining a new term $\overline{t'}$. We write $\bar{t} \to \overline{t'}$ to mean that $\overline{t'}$ can be obtained from $\bar{t}$ by applying a rewrite rule.

The leading algorithm in the stochastic simulation of well-mixed systems is that presented by Gillespie [14]. In Gillespie's algorithm each considered chemical reaction is associated with a *rate* which is used as the parameter of an exponential distribution modeling the probability that the reaction takes place. In CWC this rate is obtained by applying a function, associated to each rule (notation $\ell : \bar{p} \to^f \bar{o}$), to the contents of the compartment in which the rule is applied. To model the standard mass action law this function is evaluated by multiplying the kinetic constant of the reaction by the number of possible combinations of reactants that may occur in the compartment. In this case a stochastic rule is written as $\ell : \bar{p} \to^k \bar{o}$ where k represents the kinetic constant of the corresponding reaction [4]. The CWC simulator [3,2,11] implements Gillespie's algorithm on CWC terms. It can handle CWC models with different rating semantics (mass action law, Michaelis-Menten kinetics, Hill equation) and it can run independent stochastic simulations, featuring deep parallel optimizations for multi-core platforms exploiting the FastFlow [1] technology.

3 The Goal Programming Methodology

Mathematical programming techniques, such as multiobjective optimization [36,39], can help in the definition and in the parametrization of biomodels. Model-based optimization is a key methodology system design and analysis. In some cases there is a need to optimize more than one system performance in order to set up a kinetic model and no single performance dominates all the others [13]. With the multiobjective approach, a system performance is described by a vector-valued measure in which the components are the individual performance functions. By using a performance vector, the condition characterizing a compromise solution can be expressed in an analytic form so that trade-off alternatives can be determined directly with conventional optimization techniques [34]. This condition, which follows the ordinary meaning of compromise, is simply that: "No improvement in any component

[4] The reaction rate in this case is obtained, as usual, by multiplying the kinetic constant by the number of possible combinations of the reagents

can be achieved except at the expense of a degradation in at least one of the other components". A vector for which this condition holds is called a *non-inferior*, and is a pivot point where a compromise or trade-off would be made. Furthermore, the set of all such vectors describes the complete range of compromise solutions available. Expressed analytically, this condition becomes an optimality criterion for optimization with a vector (*multiobjective optimization*).

The methodology developed in this study concerns the integration of the CWC simulator in a simulation flow in order to identify parameters related to the model rules. Figure 2 describes this integration where an initial guessing of parameters is processed in a loop to improve the target matching until a consistent biological behaviour is achieved. The simulator is used to compute a vector of functions $\mathbf{f}$ of the parameter set under consideration (e.g. the rate of some rules). Each function f_i of the vector computes a statistical quantity of the biological system (for instance the mean value of a population) on a set of simulations. A vector of numerical values $\mathbf{g}^*$, representing the goals to be satisfied, is assigned to the statistics $\mathbf{f}$. The goals model the biological behaviours under investigation.

In the goal attainment method, non inferior index vector are found by solving the following parametrised problem:

$$\min_{\lambda\in\mathbf{R}^+,\mathbf{x}\in\mathbf{X}}(\lambda) \quad \text{such that} \quad f_1(\mathbf{x}) - w_1\lambda \leq g_1^* \ \cdots \ f_n(\mathbf{x}) - w_n\lambda \leq g_n^*$$

where $\mathbf{x} \in \mathbf{X}$ is the vector of parameters under consideration and $f_i(\mathbf{x})$ are the statistical values computed by the simulator, $\lambda \in \mathbf{R}^+$ is a slack variable and the w_i represents the weights assigned to the various goals. The minimization of the scalar λ leads to the finding of a nondominated solution which under or overattains the specified goals to a degree represented by the quantities $w_i\lambda$. The terms $w_i\lambda$ introduce an element of (relative) slackness into the problem, which otherwise imposes that the goals $\mathbf{g}^*$ be rigidly met. The values assigned to each *goal* g_i^* can be interpreted as the desired level of the corresponding i-th performance. It is assumed that the decision maker has a sufficient intuitive understanding of the problem at hand to know what values he/she would like to attain for each of the performances. In many cases, goal values already exist in the form of specifications (such as biochemical ratios and concentrations). Suppose, for example, that the goal vector selected, $\mathbf{g}^*$, is not attainable. If w_i is small in relation to the other weighting coefficients, the under-attainment $f_i(x) - g_i^*$ will also be small. That is, the relative magnitude of w_i will determine how near $f_i(\mathbf{x})$ will be to g_i^*. By using different w_i weighting coefficient values, alternative non-inferior solutions with varying degrees of under-attainment can be found. Using this approach in the goal attainment method, a trade-off is made by sacrificing the attainment of one goal for that of another.

The goal programming is represented geometrically in Figure 3 in the case of a vector of two goals, $\mathbf{g}^* = (g_1^*, g_2^*)$, that we consider equally important (i.e. $w_1 = w_2$), where $m(\lambda) = \mathbf{w}\lambda$ denotes a point of the search line with the direction given by $\mathbf{w}$ into a feaseble region F, and $\mathbf{g^s} = (g_1^s, g_2^s)$ is the convergence point to the best solution.

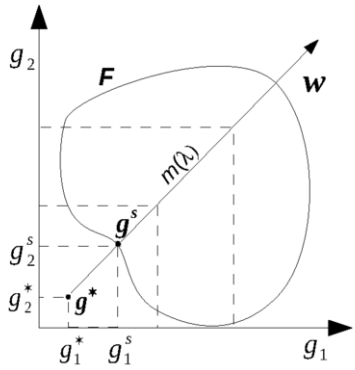

Fig. 3. Geometrical Representation of the Goal Programming

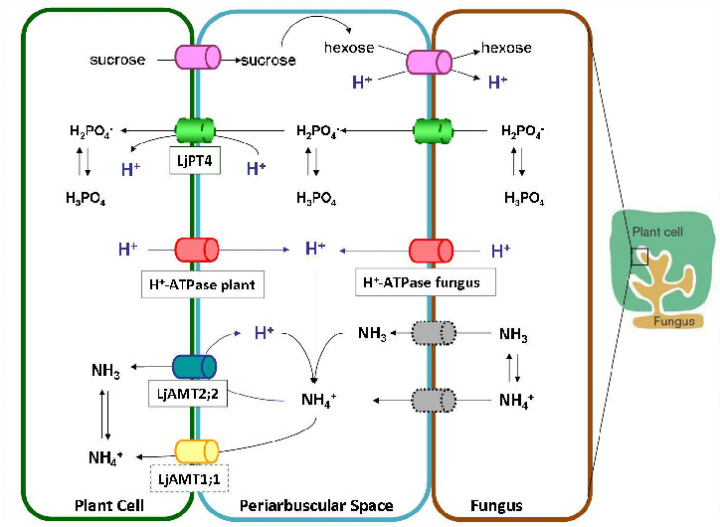

Fig. 4. The scheme illustrates nitrogen, phosphorus and sucrose exchanges at the mycorrhizal interface according to [19].

4 Nutrient Transport Model in AM Symbiosis

Arbuscular Mycorrhizal (AM) fungi are particularly important in organic farming systems which rely on biological processes, rather than agrochemicals, to supply nutrients and to control weeds, pests and diseases. The mutualistic nature of their interactions with plants is based on nutritional exchanges: AM fungi depend on a carbon flux from plants, in return, they supply plants with nutrients, such as nitrogen and phosphate. The scheme in Figure 4 illustrates nitrogen and phosphorus exchanges at the mycorrhizal interface according to previous works and the results of [19,18] on *Lotus japonicus* roots colonized with the fungus *Gigaspora margarita*.

Our study focuses on a balance of acidity of the periarbuscular space (PAS) that supports the exchange of nutrients between the plant and the fungus.

Nitrogen Uptake. Nitrogen (N) is one of the nutrient for which mycorrhizal fungi play a crucial role: plants are in fact completely dependent on N availability in the

soil for their growth and productivity [15]. The way in which N is transferred from the AM fungi to their host is still under debate. $NH_3/NH_4{}^+$ is thought to be the preferential form of N released by the fungus [7]. Five high affinity ammonium transporters (AMT) have been described so far in *Lotus japonicus*, three of which belong to the AMT1 and two to the AMT2 family. Gene expression anlysis on *Lotus japonicus* roots colonized by *Gigaspora Margarita* have revealed that LjAMT2;2 was the highest up-regulated gene [19] in the arbusculated cells which are the root cells containing the mychorrizal interface (Periarbuscular Space) between plants and AM fungi. According to [19] the LjAMT2;2 is a functional transporter that recruits from the PAS the $NH_4{}^+$ ion that is deprotonated prior to its transport across the membrane and then releases it in its uncharged NH_3 form into the plant cytoplasm in a pH dependent manner. The structural features of the transporter and the transport experiments suggested that the transporter LjAMT2;2 makes up to 99.998% of the total $NH_3/NH_4{}^+$ in the periarbuscular space, which has been demonstrated to have an acid pH of approximately 4.5. Together with the protons, which are actively transported into the PAS by the plant H^+-ATPases, the protons coming from the $NH_4{}^+$ deprotonation process remain in the interface space and maintain or even reinforce the gradient for H^+-dependent transport processes. This indirect coupling of proton-dependent and proton-independent transport processes reduces the transport costs that are paid by the plant.

Phosphate Uptake. Phosphorous is one of the mineral nutrients essential for plant growth and development. In symbiotic association with AM fungi, plants profit from phosphate acquired by the extensive network of extra radical hyphae. The primary source of phosphorus for plants is inorganic phosphate (Pi) [32,23]. Pi exists in several different forms in solution and the concentration of each form varies as a function of pH. The pK values for the dissociation of H_3PO_4 into $H_2PO_4^-$ is 2.1. Therefore, below pH 6.0 most Pi will be present as the monovalent $H_2PO_4^-$ species, while the other two species, HPO_4^{--} and PO_4^{---}, will be only present in minor proportions and are neglected from the model. Several studies on the pH dependency of Pi uptake into plant cells have demonstrated that uptake rates are highest between pH 4.5 and 6.0, where the $H_2PO_4^-$ species is predominant. Thus, it has been suggested that Pi is taken up as $H_2PO_4^-$ across the plasma membrane into the plant cell. Root cells absorb Pi not only against the steep concentration gradient between the soil solution and the cytosol, as the monovalent Pi anion additionally has to overcome the negative membrane potential that is characteristic for plant cells. The combination of both factors emphasises the requirement for energised transport of Pi across the plasma membrane making use of H^+-ATPase pumps. In *Lotus japonicus* the uptake of phosphate is mediated by a mycorrhiza-specific Pi transporter (LjPT4) which transport $H_2PO_4^-$ [18]. Members of this family are $H_2PO_4^-/H^+$ symporters. $H_2PO_4^-$ ions and protons move from the outside of the plant membrane to the inside through the inner pore of the transporter protein structure. Analogously to the process of nitrogen uptake, plant H^+-ATPase must pump protons to the outer surface of the membrane inside the PAS in order to maintain an electrochemical potential gradient and to energize the process.

Sucrose Uptake. Plant-derived carbon is released into the periarbuscular space probably as Sucrose and then cleaved into hexoses which are acquired by AM fungi [37,38]. Hexoses are transported over their membrane by so far unknown hexose transporters. It is likely that these transporters are proton cotransporters as the GpMST1 described for the glomeromycotan fungus *Geosiphon pyriformis* [35].

Reactions in the Model. Kinetic models for various metabolic pathways and regulatory circuits have been developed. However, only a few modelling analyses have been reported for the ammonia/ammonium assimilation pathways. A recent work [28] presented a detailed kinetic model for the complex ammonium assimilation regulation system of *Escherichia coli* but no relevant works are found in AM symbiosis systems. According to [19] NH_3/NH_4^+ is released from the fungus in the periarbuscular space from arginine, which is transported from the extra- to the intra-radical fungal structures. It is hypothesized that NH_4^+ is released from the fungus into the periarbuscular space by so far unknown mechanisms (transporter, diffusion, or vesicle mediated), where, due to the acidic environment, its ratio shifts toward NH_4^+ (99.99%). The NH_4^+ ion is deprotonated prior to its transport across the plant membrane via the LjAMT2;2 protein and released in its uncharged NH_3 form into the plant cytoplasm. The NH_3/NH_4^+ acquired by the plant is then incorporated into amino acids. AM fungi might control the net Pi release by their own Pi transporters, which may reacquire phosphate from the periarbuscular space [4]. Phosphate is then released from the fungus to the PAS by so far unknown mechanisms into the interfacial apoplast while LjPT4 is the responsible for the plant uptake of $H_2PO_4^-/H^+$. The biochemical reactions occurring in the symbiosis are the following.

(1) $P : NH_4^+ \rightarrow^{ni} \bullet$

(2) $P : H_2PO_4^- \rightarrow^{pi} \bullet$

(3) $P : \bullet \rightarrow^{ss} C_{12}H_{22}O_{11}$

(4) $F : \bullet \rightarrow^{ns} NH_4^+$

(5) $F : \bullet \rightarrow^{ps} H_2PO_4^-$

(6) $F : C_6H_{12}O_6 \rightarrow^{si} \bullet$

(7) $F, P : NH_3\ H^+ \rightarrow^{k_{N_1}} NH_4^+$

(8) $F, P : NH_4^+ \rightarrow^{k_{N_2}} NH_3\ H^+$

(9) $F, P : H_2PO_4^-\ H^+ \rightarrow^{k_{P_1}} H_3PO_4$

(10) $F, P : H_3PO_4 \rightarrow^{k_{P_2}} H_2PO_4^- H^+$

(11) $\top : (x \rfloor X\ NH_4^+)^F \rightarrow^{nt_F} NH_4^+ (x \rfloor X)^F$

(12) $\top : (x \rfloor X\ H_2PO_4^-)^F \rightarrow^{pt_F} H_2PO_4^- (x \rfloor X)^F$

(13) $\top : (x \rfloor X\ C_{12}H_{22}O_{11}\ H^+)^P \rightarrow^{st} C_{12}H_{22}O_{11}\ H^+ (x \rfloor X)^P$

(14) $\top : C_6H_{12}O_6\ H^+\ (x \rfloor X)^F \rightarrow^{mst} (x \rfloor X\ C_6H_{12}O_6\ H^+)^F$

(15) $\top : C_{12}H_{22}O_{11} \rightarrow^{Inv} 2 * C_6H_{12}O_6$

(16) $\top : NH_4^+\ (x\ LjAMT2;2 \rfloor X)^P \rightarrow^{k_{LjAMT2;2}} H^+\ (x\ LjAMT2;2 \rfloor X\ NH_3)^P$

(17) $\top : H^+\ H_2PO_4^-\ (x\ LjPT4 \rfloor X)^P \rightarrow^{k_{LjPT4}} (x\ LjPT4 \rfloor X\ H^+\ H_2PO_4^-)^P$

(18) $\top : (x\ ATPase \rfloor X\ H^+)^P \rightarrow^{k_{ATP_P} \cdot \{H^+_{ph} \dot{-} occ(H^+)\}} H^+ (x\ ATPase \rfloor X)^P$

(19) $\top : (x\ ATPase \rfloor X\ H^+)^F \rightarrow^{k_{ATP_F} \cdot \{H^+_{ph} \dot{-} occ(H^+)\}} H^+(x\ ATPase \rfloor X)^F$

where $occ(H^+)$ denotes the number of H^+ atoms occurring in the P or F compartment. To avoid the modeling of the process of water molecules dissociation only hydrogen ions H^+ is considered in the model. The set of kinetic reactions adopted for the model is shown in CWC rules (1-19). Labels: F, $\top$ and P specify the biological compartments where reactions take place, respectively: fungus, periarbuscular space, and plant roots.

Reactions (1-6) describe the flux of nutrients furnished and internalized by the plant and the fungus. Reactions (7-10) represent the chemical conversion of nitrogen (7-8) and phosphate (9-10) in the different compartments. Reactions (11-12) describe nutrient transport from the fungus to the PAS. Reactions (13-14) models the transport of sucrose from the plant to the PAS and then from the PAS to the fungus, respectively. In the PAS, sucrose is cleaved into hexose by means of reaction (15). The reaction (16) models the transport of ammonia through LjAMT2;2, while reaction (17) model the transport of phosphate through LjPT4.

The acidity of the periarbuscular space is established by the plant/fungal H^+-ATPases and other mechanisms [21,4] which provide the energy for H^+-dependent transport processes. In order to model correctly chemical reactions at different pH it was important to add a formulation for H^+ that was capable of reaching and keeping the right concentration in the PAS. The plant and fungus H^+-ATPase pumps, transporting the protons into the interfacial apoplast, are modelled by reactions (18) and (19) which are the only rules where a special function is used to determine the rates. Simply, they increase the ions into the PAS when their concentrations is under the equilibrium level H^+_{ph}. The parameters k_{ATP_P} and k_{ATP_F} can be interpreted as the power provided by plant and fungus to sustain the nutrient exchanges.

5 Results

The CWC simulation of the biological model was linked to the goal programming technique coded in Octave [12] in a processing flow (see Figure 2). The goal programming approach is based on a general nonlinear minimization using a sequential quadratic programming (SQP) algorithm of the Octave's optimization package. Since SQP is a local optimization algorithm, an initial random search points was generated to globally explore the parameter domain. To evaluate a set of rates for the target transport equilibrium, a mean of 30 simulation at the end of time interval was computed.

Model Analysis. The model (cf. end of Sect. 4) was tested in a equilibrium transport state represented by the CWC term showed in Figure 5. The value of the quantities n_i, given in Table 1, hold the equilibrium of the different pH of each compartment during the exchanges modelled by the value of rates in rules (1-6), given in Table 2. The total amount of ammonium and phosphate is inspired by biological hypothesis based on previous experiments ([27,41] for ammonium transport and [23,32,20] for phosphate transport). Let $\mathbf{x}$ be the vector of simulation kinetics in the rules (7)-(19), the two parameters under investigation k_{ATP_P} and k_{ATP_F} are

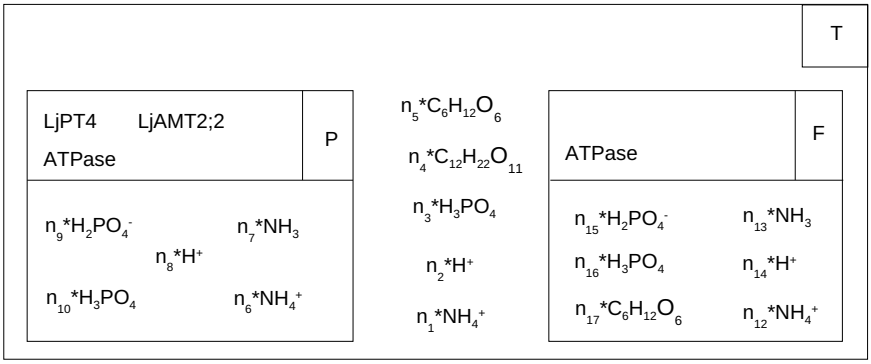

Fig. 5. Graphical representation of the CWC term encoding the biological system under investigation: $n_1 * NH_4^+ \quad n_2 * H^+ \quad n_3 * H_2PO_4^+ \quad n_4 * C_{12}H_{22}O_{11} \quad n_5 * C_6H_{12}O_6 \; (ATPase \; LjAMT2;2 \; LjPT4 \rfloor n_6 * NH_4 \; n_7 * NH_3 \; n_8 * H \; n_9 * H_2PO_4^+ n_{10} * H_3PO_4 \; n_{11} * C_{12}H_{22}O_{11})^P \; (ATPase \rfloor n_{12} * NH_4 \; n_{13} * NH_3 \; n_{14} * H \; n_{15} * H_2PO_4^+ \; n_{16} * H_3PO_4 \; n_{17} * C_6H_{12}O_6)^F$

	PAS	Plant	Fungus
H^+	100 (n_2)	20 (n_8)	20 (n_{14})
NH_4^+	5000 (n_1)	10000 (n_6)	10000 (n_{12})
NH_3	0	100 (n_7)	100 (n_{13})
$H_2PO_4^-$	16000 (n_3)	15000 (n_9)	15000 (n_{15})
H_3PO_4	0	50 (n_{10})	50 (n_{16})
$C_{12}H_{22}O_{11}$	20000 (n_4)	20000 (n_{11})	0
$C_6H_{12}O_6$	40000 (n_5)	0	5000 (n_{17})

Table 1
Values n_i at the equilibrium

the kinetics of the rules (18) and (19), respectively. The vector of functions $\mathbf{f}$ has 17 elements $f_i(\mathbf{x}) = |n_i' - n_i|$, where the values n_i are the initial quantities listed above and the value n_i' are the average final quantities computed over 30 parallel runs of the simulator. Each goal g_i is set to 0 (reflecting the fact that we are looking for the values of $\mathbf{x}$ that keep the equilibrium). As described in Section 3, the weights $\mathbf{w}$ can be changed in subsequent iterations of the parameter identification in order to emphasize the search for specific targets. The strategy used in this case has been to increase the weights proportionally to the distance of the objective with respect to the initial guessing.

LjAMT2;2 and LjAMT1;1 Models Comparison. The biological question answered by this study concerns the efficiency of two ammonium transporters in terms of energy spent by plant/fugus system to maintain acceptable transfer rates for the transporters that exploits the acidity of the periarbuscolar space. The plant/fungus ATPase pumps balance the H^+ ions involved in nutrients exchanges using metabolic energy. The aim of this comparison was to investigate whether the over-expression of LjAMT2;2 in the arbusculated cells is functional and related to its peculiar transport process that does not "consume" the periarbuscular H^+ and therefore does not compete with other transporters, such as those for phosphates. The level of the work done by the plant ATPase pumps to maintain the periarbuscular acidity was taken

ni	pi	ss	ns	ps	si
2	3	1	2	3	2

Table 2
Fixed fluxes of nutrient furnished and internalized by the plant and the fungus

	LjAMT1;1	LjAMT2;2
kN_1 (dissociation rate)	$1.5218 \cdot 10^{-2}$	$1.5177 \cdot 10^{-2}$
kN_2 (dissociation rate)	$3.3769 \cdot 10^{-3}$	$1.0356 \cdot 10^{-3}$
kP_1 (dissociation rate)	$1.2366 \cdot 10^{-4}$	$8.4505 \cdot 10^{-5}$
kP_2 (dissociation rate)	8.7536	8.0705
nt_F	$3.0436 \cdot 10^{-3}$	$3.0354 \cdot 10^{-3}$
pt_F	$2.4735 \cdot 10^{-3}$	$2.0236 \cdot 10^{-3}$
st	$1.8551 \cdot 10^{-3}$	$1.5177 \cdot 10^{-3}$
mst	$6.7758 \cdot 10^{-4}$	$7.5884 \cdot 10^{-4}$
k_{LjAMT}	$8.0870 \cdot 10^{-3}$	$5.0708 \cdot 10^{-3}$
k_{LjPT4}	$7.9225 \cdot 10^{-5}$	$6.7842 \cdot 10^{-5}$
Inv	$2.0218 \cdot 10^{-3}$	$1.5177 \cdot 10^{-3}$
$kATP_P$ (Plant ATPase)	2.8797	1.9428
$kATP_F$ (Fungus ATPase)	$4.2254 \cdot 10^2$	$6.3232 \cdot 10^2$

Table 3
Comparison of the estimated values for the fungus H^+-ATPase pumps flux for the two different transporters LjAMT2;2 and LjAMT1;1

as a measure of the LjAMT2;2 contribution to the symbiotic equilibrium.

The comparison was performed with respect to a well known ammonium transporter in *Lotus japonicus*: LjAMT1;1. This transporter recruits from the periarbuscular space the $NH_4{}^+$ ion that is deprotonated after its transport across the plant membrane and is released in its uncharged NH_3 form into the plant cytoplasm. This biological model is the same as the one described in Section 4 except that the reaction (16) has been replaced by the following one.

$$(20)\quad \top : NH_4^+\ (x\ LjAMT1;1 \rfloor X)^P \rightarrow^{k_{LjAMT1;1}} (x\ LjAMT1;1 \rfloor X\ NH_3\ H^+)^P$$

The simulation of the biological model with LjAMT2;2 transporter was coupled with the biological model with LjAMT1;1 transporter considering equal values of model parameters except the one modelling the plant ATPase pumps. 30 runs of the optimization process were performed in order to find different equilibrium states comparing the different obtained values for the ATPase pumps for the model including the two different transporters. The last two rows of Table 3 show the estimated mean value for the plant and fungus H^+ flux given by the ATPase pumps to balance the H^+ inside the PAS. The results confirm the efficiency of the LjAMT2;2 transporter compared to LjAMT1;1 from the plant side. In this model the fungal partner gives the main contribution to the symbiosis in terms of spent of energy through ATPase pumps activity.

6 Related Work

Optimization methods have been widely applied in both metabolic control analysis and biochemical systems theory [6]. In particular, the problem of parameter identification in biochemical pathways, formulated as a nonlinear programming problem subject to the pathway model acting as constraints, has received great attention.

Since these problems are frequently multimodal, global optimization methods are needed in order to avoid local solutions. A local solution can be very misleading when calibrating models: it would indicate a bad fit even for a model which could potentially match perfectly a set of experimental data. In this context, evolutionary computation methods are a class of stochastic methods which have shown good performance in systems biology applications [30]. Hybrid methods, combining global and local techniques, have also shown great potential with difficult problems like parameter estimation [5]. Much more work is needed to further enhance the efficiency and robustness of these approaches in order to make them applicable to large scale models.

Most previous studies of parameter identification in regulatory networks have dealt only with deterministic models. For the simulation of stochastic biochemical systems, parameter values are set to biologically plausible levels or estimated from in vitro experiments on the macroscopic level. For the inverse problem of obtaining parameters, rate constants are normally estimated by fitting mass law action dynamics to experimentally measured concentration curves. Some recent studies have attempted to estimate stochastic rate constants from exact molecule count data, assuming that the counts follow a Markov chain (as in the Gillespie algorithm) employing hidden Markov models [33], or a stochastic differential equation approximation [16].

7 Conclusions

A methodology to analyse biological models has been presented. The general schema illustrated relies on the goal programming approach which allows to search parameters through the definition of goals. The flexibility of goals to phrase biological issues related to biological interactions was tested. The mycorrhizal symbiosis between *Lotus japonicus* and *Gigaspora Margarita* fungus was studied and its exchange was modelled to get an indirect measure of ATPase energy involved in the processes. This analysis supported interesting insights on the transport efficiency of two different AMT proteins of the plant root cell. Our study suggested that LjAMT2;2 high level of expression in the arbusculated cells could be synergistic with the transporter for other substances: LjAMT2;2 could rely on the NH_4^+ gradient created by the fungi as its energy source and does not need to use the H^+ gradient, thus leaving more energy for other transporters; while LjAMT1;1 is not highly expressed here as long as it would represent a "waste" of energy for the plant.

Many other questions about the plant nutrient uptake and competitive fungal reimport process might be answered. Based on the transport properties of transporters from different fungal and/or plant species, theories could be developed which explain different mycorrhiza responsiveness of host plants; meaning why certain plant-AM fungus combinations have a rather disadvantageous than beneficial effect for the plant.

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