Systems-level modeling—A new approach for engineering efficient photosynthetic machinery

Xin-Guang Zhu

Plant Systems Biology Group, Partner Institute of Computational Biology, Chinese Academy of Sciences/Max Planck Society, YueYang Road, Shanghai 200031, China

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ABSTRACT

Photosynthesis produces the basic building block for crop biomass and yields; however, improving photosynthesis has not been effectively used as a breeding goal. More and more evidences suggested that improving photosynthesis can substantially increase crop yields. The complexity of photosynthesis however makes experimentally identifying new ways to engineer higher photosynthesis inherently time-consuming and costly. Combining systems modeling with evolutionary algorithm makes it possible to identify optimal engineering options for future global climate change scenarios and simultaneously consider environmental constraints, such as with constant or even decreasing nitrogen fertilizer application in the field. This method enables in silico examination of a large number of engineering options which natural selection has not explored, for higher photosynthetic energy conversion efficiency. The new approach comes particularly timely for now when our society is facing serious challenges in food security and global climate change. The traditional reductionist’s approach will continue generating critical knowledge required to support this systems biology method to engineering higher photosynthesis.

1. Introduction

The success of the green revolution in the 1960s and 1970s dramatically increased world food production. However, the major cereal crops, with the exception of maize, have minor, if not completely zero, improvement in yield per unit area over the past 10 years. This, together with the increasing world population, global climate change and increasing energy costs, is endangering our global food security. In 2008, the stock to use ratios for wheat and rice were at their lowest levels for the past 50 and 30 years, respectively (FAOReport, 2008). In fact, despite the recent recession of the global economy, the prices for rice, wheat and soybean remained at near their historic highs (von Braun, 2008). To ensure that agricultural systems meet future production needs, it is required to overcome the physiological barriers to crop yields and improve the resiliency of crops to adverse conditions such as climate change (Dawe, 2007). Theoretical analysis suggested that increasing photosynthetic energy conversion efficiency ($\varepsilon_c$) is one feasible approach to substantially increase crop yields (Long et al., 2006b; Zhu et al., 2008).

2. The demand of developing systems models of photosynthesis

Photosynthesis is one of the most researched processes in plants. Now, the detailed biophysical and biochemical processes in photosynthesis are largely known (Ben-Shem et al., 2003; Eberhard et al., 2008; Nelson and Yocum, 2006). Furthermore, modern biotechnologies make it possible to engineer nearly every protein or gene involved in photosynthesis. However, up to now, the advances in our understanding of photosynthesis has not resulted in increased crop yields (Raines, 2006). More and more evidences from field experiments clearly indicated that increase in photosynthesis can improve crop yields. For example, elevated atmospheric CO2 concentrations, which increases leaf photosynthetic rate, have resulted in increased crop yields in soybean, wheat, rice, and maize (Long et al., 2006a). Factors influencing photosynthesis such as high or low temperatures (Sage and Kubien, 2007) and drought (Atkin and Macherel, 2009) led to decreased crop productivity (Boyer, 1982). Increase in photosynthesis and biomass have shown high correlation with increase in rice yields in the last decade (Hubbart et al., 2007). So, given that the current average photosynthetic efficiency achieved in the modern agricultural system is usually less than 25% of the maximal efficiency (Zhu et al., 2008), identifying new options to increase photosynthesis holds great potential to increase crop yields.

Identifying the potential targets to engineer for higher photosynthesis is technically challenging. One potential way to solve this problem is to take a systems approach, i.e. developing mathematical models, which can faithfully simulate all the biophysical and biochemical reactions involved in photosynthesis, and then, to conduct numerical experiments to identify targets to engineer higher productivity. Over the years, a number of systems models of photosynthesis with different levels of mechanistic details...
have been developed (Gross et al., 1991; Hahn, 1984, 1991; Laisk et al., 1989, 1997, 2006; Laisky and Walker, 1986; Pearcy et al., 1997; Pettersson and Ryde-Pettersson, 1988; Poolman et al., 2000; Rovers and Giersch, 1995; Woodrow, 1986). These models were developed mostly to examine a specific part of photosynthesis, but not the whole, one exception is the model of Laisk et al. (2006). On the other hand, a complete model of photosynthesis is required to accurately simulate the detailed dynamics of photosynthesis in field conditions (Niinemets, 2007; Schurr et al., 2006) where CO$_2$, light, humidity are all changing simultaneously. Leaves experiencing different light histories alter their responses of photosynthetic CO$_2$ uptake rate (A) to light levels (Q) (Zhu et al., 2004a). A complete dynamic model of photosynthesis is required to accurately predict this change of the responses of A to Q, because this change is associated with an altered capacity to quench absorbed light energy (Niyogi, 1999; Niyogi et al., 2005), which is dependent on the light histories the leaves experienced.

Given that photosynthesis includes more than 100 proteins involved in various biophysical and biochemical processes (Eberhard et al., 2008; Geiger and Servaites, 1994; Nelson and Yocum, 2006), is it possible for us to develop a complete systems model to simulate all these processes simultaneously? A number of factors now make this task feasible. First, photosynthesis is one of best-characterized biological processes on earth. Its major biochemical reactions, processes and regulatory mechanisms are largely known (Eberhard et al., 2008; Raines, 2003, 2006). Second, modern computational power and numerical algorithms enable simulations of complex systems on the latest generation of PCs. Thirdly, proteomics and metabolomics promise to deliver the concentrations of proteins and metabolites which can be used as input into such systems models (Hammer et al., 2004; Raikhel and Coruzzi, 2003).

Although with great application potential, systems models of photosynthesis are only used effectively by a limited number of labs (Farquhar et al., 2001; Laisk et al., 2006; Zhu et al., 2007). This is partly because biologist usually are not exposed to the required mathematics and computational background to enable effective development and utilization of such systems models. Furthermore, most of the current models cannot be easily extended to fit specific needs for hypothesis testing. In this review, the basic principles and procedures to develop systems models are described; in addition, an optimization algorithm, i.e. evolutionary algorithm, is introduced to combine with systems models to identify new engineering targets (Zhu et al., 2007). Finally, the insights gained through such modeling approach and caveats of using such an approach are briefly discussed.

3. Basic principles and procedures to develop systems models

First, the principle of divide-and-conquer. Here I use the analogy of building a machine to illustrate the point. When a complex machine is built, each functional module of the machine needs to be built separately first; then, these functional modules are combined to build the final complete machine. Similarly, to build a model of a complex biological system, this system needs to be first divided into a number of relatively small subsystems; then, a submodel is developed and validated for each subsystem; finally these individual submodels are combined to build the complete model for the whole system.

Secondly, an appropriate division of the whole system should ensure high connectivity inside the same submodel while minimize the connectivity between submodels. Minimizing the interdependence between submodels can greatly help model development and validation. When developing kinetic models of metabolic systems, the connectivity of a reaction is defined as the number of reactions that this reaction shares a substrate with. In the case of photosynthesis model, we divided the whole photosynthesis process into three submodels, i.e. one for the light absorption, transfer and electron transfer processes around photosystem II, the second is for the electron transfer processes after plastoquinon formation until generation of ADP and NADPH, and the third is for photosynthetic carbon metabolism (Zhu et al., 2005, 2007; Zhu, 2004).

Thirdly, each submodel should have at least one measurable signal which can be used to judge the validity of simulation results. For example, the submodel of photosynthetic carbon metabolism was tested using the CO$_2$ uptake rate under different CO$_2$, O$_2$ and light conditions (Zhu et al., 2007). The predictions from the photosynthetic light reactions were tested using chlorophyll fluorescence (Zhu et al., 2005), O$_2$ evolution, transmembrane pH gradient and transmembrane electric potential (Zhu, 2004).

Fourthly, a model building process is a process of gradual improvement of model completeness and predictive capacities. Several models of the photosynthetic carbon metabolism have been developed historically (Giersch, 2000; Hahn, 1984, 1986, 1987; Pettersson, 1997; Pettersson and Ryde-Pettersson, 1988; Poolman et al., 2004). Each one of these models has contributed substantially to our understanding of photosynthesis, such as the role of phosphate in the generation of photosynthetic oscillation (Laisk et al., 1991; Laisk and Walker, 1986), quenching of absorbed light energy (Laisk et al., 1997) and the role of Rubisco activase in limiting non-steady-state photosynthesis (Mott and Woodrow, 2000). Due to the different requirements for hypothesis testing, these models differ by their mechanistic basis, for example, the reversibility of reactions (Hahn, 1984, 1986, 1987; Pettersson and Ryde-Pettersson, 1988; Poolman et al., 2000), inclusion of photorespiration (Hahn, 1984, 1986, 1987; Pettersson, 1997), and starch degradation (Hahn, 1984, 1986, 1987; Poolman et al., 2000). Because these models are typically developed to test specific hypotheses, they usually simplified part of the photosynthetic processes. For example, Pearcy et al. (1997) developed a relatively simplified model of photosynthetic carbon metabolism to describes photosynthetic performance under varying light regimes, which was further extended by including rate-limiting factors, especially the time constants for gain and loss of RuBP regeneration capacity (Kirschbaum et al., 1998). Zhu et al. (2007) developed a complete model of the photosynthetic carbon metabolism, which includes the detailed reactions of the Calvin cycle, photorespiratory pathway, starch and sucrose synthesis pathways. This model has been extended to include the detailed energy and electron transfer processes of the light reactions to form a complete model of C3 leaf photosynthesis (Zhu, 2004), which can be combined with an evolutionary algorithm to identify new targets to engineer for higher photosynthetic energy conversion efficiency.

4. A general procedure to develop kinetic models of metabolism

The first step to develop a kinetic model of metabolism is to compile all reactions closely involved in the metabolism to form a reaction diagram (Fig. 1). For each reaction involved, the following basic information needs to be collected: the Michaelis–Menten constants, the activators and their activation constants, the inhibitors (both competitive and non-competitive inhibitors) and their inhibition constants, the pH and temperature dependences of enzymes, catalytic number, molecular weight, and in vivo activity of each enzyme. These parameters are usually obtained based on literature, databases, and biochemistry textbooks.

After the basic information is collected, the rate equation for each reaction, usually in the form of Michaelis–Menten equation, needs to be developed (Zhu et al., 2007). For a general reversible
reaction of the form:
A + B ⇌ C + D

The rate equation used is:
\[ v = \frac{V_m[A][B] - ([C][D]/k_c)}{K_{mA}K_{mB}(1 + ([A]/K_{mA}) + ([B]/K_{mB}) + ([C]/K_{mC}) + ([D]/K_{mD}) + ([A]/K_{mA}K_{B}/K_{mB}) + ([C]/K_{mC})([D]/K_{mD}))} \] (1)

following the standard kinetic equation for a reversible reaction with two substrates and two products (Cleland, 1963), where \([A], [B], [C]\) and \([D]\) represent the concentrations of the metabolites A, B, C and D, respectively. \(K_{mA}, K_{mB}, K_{mC}\) and \(K_{mD}\) are the Michaelis–Menten constants for A, B, C and D, respectively. \(k_c\) is the equilibrium constant of this reaction; \(V_m\) is the maximum rate of this reaction. If a reaction does not follow Michaelis–Menten kinetics, the best rate equation from literature describing the particular reaction needs to be used. For example, the concentration of active sites of Rubisco in the chloroplast stroma is of the same order of magnitude as the concentration of the enzyme’s substrate, RuBP (Bassham and Krause, 1969; Dietz and Heber, 1984; Schimkat et al., 1990; Woodrow and Mott, 1993). This makes Rubisco catalyzed reaction does not follow Michaelis–Menten kinetics. To solve this issue, Farquhar (1979) developed an equation relating the rates of carboxylation and oxygenation to total RuBP concentration \((R)\). The solution of this equation was used in Zhu et al. (2007) instead of the typical Michaelis–Menten rate equation.

In addition to the rate equations, there are also algebraic equations that need to be developed. These algebraic equations usually represent relationships between substrates or reactions involved in the system under study. For example, during photosynthesis, the total concentration of certain compounds does not change. In the model of the photosynthetic carbon metabolism (Zhu et al., 2007), the adenylate nucleotides ([CA]) in the chloroplast stroma, i.e. the sum of [ATP] and [ADP], is constant. Similarly, the sum of [NADPH] and [NADP] in the chloroplast stroma ([CN]) is constant. These constants were represented as algebraic equations in the model.

After rate equations and the algebraic equations are developed, a systems model representing this metabolic process is constructed by developing a system of linked ordinary differential equations. Each ordinary differential equation (ODE) describes the rate of change of the metabolite concentration with time, formed by the sum of the rates of formation minus the sum of the rates of consumption of this metabolite. For example, in the photosynthetic carbon metabolism, RuBP (Ribulose-1,5-bisphosphate) is formed through Ru5P (Ribulose-5-phosphate) phosphorylation via the reaction:

\[ \text{Ru5P} + \text{ATP} \rightarrow \text{ADP} + \text{RuBP} \] (3)

It is consumed via both RuBP carboxylation (Eq. (4)) and RuBP oxygenation (Eq. (5)):

\[ \text{RuBP} + \text{CO}_2 \rightarrow \text{2PGA} \] (4)
\[ \text{RuBP} + \text{O}_2 \rightarrow \text{PGA} + \text{PGCA} \] (5)

where PGA is 3-phosphoglycerate and PGCA is 2-phosphoglycollate. Assuming that the rate of RuBP phosphorylation is \(v_{\text{rt}}\) and the rates of RuBP carboxylation (Eq. (4)) and oxygenation (Eq. (5)) are \(v_i\) and \(v_o\), respectively, the rate of RuBP concentration change with time is described as:

\[ \frac{d[\text{RuBP}]}{dt} = v_{\text{rt}} - v_i - v_o \] (6)

The complete set of linked differential equations forms a system of ordinary differential equations (ODEs). This system of ODEs, together with the rate equations and algebraic equations forms a metabolism model. Generally, analytical solutions are not available for ODEs representing any realistic metabolic system. Therefore, efficient numerical integration algorithms are needed to solve the systems of ODEs. Various numerical integration algorithms are available from both commercial and public domains. The ode15s procedure of MATLAB (v6, Mathworks Inc., Natick, MA, USA) proved to be effective to solve the system of ODEs. Compared to other routines available in MATLAB, this ode15s routine is computational efficient dealing with a “stiff” set of equations, i.e. where rates of change differ significantly between components. The solution of the ODEs provided by ode15s is the time evolution of the concentrations of metabolites. The initial concentrations of metabolites need to be parameterized based on either measurements or literature survey.

Once a system of ordinary differential equations has been constructed, a series of numerical experiments are required to test and validate the model. Normally, a systems model has to show its ability to gain steady states, robustness against external perturbations, and the ability to predict commonly observed phenomena. The model of photosynthetic carbon metabolism (Zhu et al., 2007)
reached a steady state within about 200 s; further it accurately simulated the commonly measured $A$–$c_i$ curve, i.e. the CO$_2$ uptake versus intercellular CO$_2$ concentration ($c_i$) curve, for both normal (21%) and low (2%) oxygen levels. Furthermore, after a drastic perturbation of the intercellular O$_2$ concentration, the model quickly regained a new steady state with a realistic photosynthetic CO$_2$ uptake rate. Finally, the model faithfully simulated the phosphate limited photosynthesis under conditions of low rate of triose phosphate utilization (Fig. 2, from Zhu et al., 2007). Only after a model is thoroughly validated using different data, can it be used to generate new insights regarding the dynamics, control properties, and engineering potentials of the system.

5. Systems models combined with an evolutionary algorithm to identify new targets for higher photosynthesis

Improving photosynthetic efficiency is often associated with increasing photosynthetic resource (e.g. light, nitrogen and H$_2$O) use efficiency, in particular, nitrogen use efficiency. Given that production of nitrogen fertilizer requires substantial energy consumption, in addition, increasing nitrogen fertilizer application potentially cause eutrification of water system (Tilman et al., 2002), engineering leaves with higher nitrogen use efficiencies is highly desirable. Experimentally, it has been shown that when plants are grown under different conditions, allocation of nitrogen into different proteins of photosynthetic apparatus changes. For example, when under high light conditions, more nitrogen is allocated into enzymes of the Calvin cycle and proteins involved in photosynthetic electron transfer chain, which led to higher light-saturated rate of leaf photosynthesis (Evans, 1989; Hikosaka and Terashima, 1995). Is the current allocation of nitrogen in different proteins or enzymes of photosynthetic apparatus optimal for photosynthetic CO$_2$ uptake rate? Or in another word, how can we identify the optimal nitrogen distribution to gain a higher photosynthetic rate?

Experimentally identifying the best targets to engineer for higher photosynthetic nitrogen use efficiency through over-expressing and anti-sensing selected genes would be time-consuming and costly. To show this point, we assume that 30 key proteins or enzymes are closely related to photosynthetic CO$_2$ uptake rate, identifying the best combination of proteins to engineer for the optimal CO$_2$ uptake rate would require $2^{30}$ transgenic experiments assuming that each protein only needs to be either doubled or halved. Though with the advances of our understanding of roles of different genes in controlling photosynthesis, we can substantially decrease the number of enzymes or combinations that need to be tested for higher photosynthetic efficiency, using purely a transgenic approach to identify the optimal engineer targets might still be too costly. The optimal changes in each protein or enzyme will not be exactly twice or half of the current protein concentration.

The inefficiency of using current experimental approaches to identify options to engineer higher photosynthesis partially explains the lack of success of engineering higher photosynthetic rates so far. Systems biology and modeling however provide a feasible approach to tackle this problem, as illustrated in Zhu et al. (2007). This approach is to some degree analogous to the traditional crop breeding. In traditional crop breeding, crossing different plants creates variations in certain traits, such as crop yields. Seeds from those individuals with superior traits are selected and used to generate the next generation. After many generations, the selected
traits are gradually improved. Evolutionary algorithm essentially mimics the selection process in traditional breeding. Here the selection pressure is to have a higher photosynthetic CO₂ uptake rate. Furthermore, to identify targets to increase nitrogen use efficiency, we keep the nitrogen invested in the enzymes of the photosynthetic carbon metabolism to be constant. In this algorithm, each generation has many individuals, with each individual representing one set of enzyme concentrations required to run the model of photosynthetic carbon metabolism. For each individual, or set of enzyme concentrations, there is a corresponding steady-state photosynthetic CO₂ uptake rate (A). In each generation, the individual with a higher A will be selected to “seed” the next generation. To do this, the selected individual will first be duplicated to generate a population of enzyme sets, then, each set of enzyme concentrations in this population will be “mutated” via adding random variations to the enzyme concentrations. This process of selection and mutation iterates and the photosynthetic CO₂ uptake rate gradually increases over generations. This process terminates when the rate of photosynthesis cannot be increased with more generations (Fig. 3).

This approach suggested that RuBisco, sedoheptulose-1,7-bisphosphatase (SBPase) and fructose-2,6-bisphosphatase (FBPase) need to be increased for a higher A (Zhu et al., 2007). Furthermore, an increase in sink capacity (through the increase of ADP glucose pyrophosphorylase, i.e. ADPGPP the key enzyme in starch synthesis) is also required to gain increased photosynthetic rates. Using the evolutionary algorithm, we can identify not only the enzymes whose concentrations need to be increased, but also those enzymes whose concentrations need to be decreased to free nitrogen out to be allocated to those enzymes whose concentrations need to be increased (Fig. 3). Would these predicted changes in enzyme activities actually increase photosynthesis in plants? When SBPase, one of the identified enzymes was over-expressed in tobacco, a higher photosynthesis and higher biomass production were observed (Lefebvre et al., 2005). This demonstrated that combining evolutionary algorithm with systems models can identify potential engineering targets for higher photosynthesis.

6. Systems approach provides a timely method to explore options for higher photosynthesis in the context of global climate change

Most breeding programs focus on breeding high-yield crops for the current environmental conditions. Different factors in global change, i.e. elevated CO₂ and temperature, elevated O₃ concentrations, all substantially influence photosynthesis and correspondingly the crop yields (Heagle, 1989; Long et al., 2006a). This inevitably requires current breeding programs to design crops not only for the current but also for the future environments. Systems model will greatly facilitate identification of targets to engineer for higher photosynthesis and yields for future climates.

One of the features of global climate change is that these different factors change simultaneously. Over the past few decades, the impacts of temperature, CO₂ and ozone on photosynthesis and productivity have been extensively studied (Ainsworth et al., 2002; Clifton-Brown et al., 2002; Flexas et al., 2004; Stitt, 1996). However, these studies usually examined influences of single factors on photosynthesis and productivity, but not all these factors simultaneously. In the field, these different factors usually change simultaneously. For example, drought often concurs with high temperature in arid areas (Easterling and Apps, 2005; Wang et al., 2003); low temperature often concurs with high light in the early spring in high latitudes (Farage et al., 2006); ozone is a factor that exposes a severe stress on various crop species (Long et al., 2005; Morgan et al., 2006) and can occur simultaneously with many other stress factor (Long et al., 2005; Schmidhuber and Tubiello, 2007). All these factors influence many closely linked metabolic processes, e.g. photosynthesis, respiration, and nitrogen metabolism (Long and Naidu, 2002; Sage and Kubien, 2007; Stitt and Krapp, 1999). Due to the close interactions between these different metabolic processes, the responses of metabolism to the combined stresses are highly non-linear and cannot be predicted based on knowledge about the responses of plants to single factors. One well-known example is that elevated CO₂ alters both the magnitude of the response of leaf and canopy carbon gain to rising temperature, and
sometimes, even the direction of the responses (Long, 1991). Some new research initiatives are begin to explore impacts of the combinations of different factors on photosynthesis and productivity in the field, such as the Free Air CO2 Enrichment facility in Illinois (Bernacchi, Leaky personal communication). However, given the large number of potential combinations of these environmental factors, systems models being able to predict the photosynthesis and crop potential yields are highly desirable. Combining such models with evolutionary algorithm provide a unique way to design ideal plant types (not only metabolic and regulatory processes, but also plant architectures) in future climate conditions. Such models can help design crops that can simultaneously satisfy additional breeding targets, such as higher nitrogen use efficiency (Zhu et al., 2007).

Similar to evolutionary algorithm, another method to identify engineering targets is metabolic control analysis (MCA) through the calculation of flux control coefficient (Fell, 1997). The flux control coefficient of an enzyme is calculated as the ratio of the relative change in the metabolic flux, here the CO2 uptake rate, to the relative change in the activity of this enzyme, while keeping activities of all other enzymes fixed. Compared to the evolutionary algorithm, flux control coefficient represents a local view of the control of this enzyme over the system flux, while the evolutionary algorithm identifies an optimal distribution of all enzymes in the metabolism by simultaneously changing concentrations of many enzymes. Therefore, theoretically, evolutionary algorithm can identify targets for higher photosynthesis from a more global perspective. Though evolution and selection of higher photosynthesis in the field might not necessarily reflect optimal nitrogen allocation due to limited number of possible mutations experienced, evolutionary algorithm combined with systems model however can explore all potential engineering options efficiently. Compared to the method of calculating flux control coefficient, evolutionary algorithm also has the added benefit of identifying not only those proteins or enzymes whose concentrations need to be increased, but also enzymes or proteins whose concentrations need to be decreased. This will be critical for us to engineer plants to gain higher photosynthetic nitrogen use efficiency, which will help develop sustainable agricultural systems and preserve our natural ecosystems (Cloern, 2001; Smith et al., 1999; Tilman, 1999).

7. Reductionist’s approach will continue play enabling roles in providing critical knowledge supporting systems approach to identify new engineering targets

Though systems models combined with evolutionary algorithm can help design crops for higher photosynthesis and crop potential yields, the application of this approach is still limited by our incomplete understanding of metabolism itself, not by the mathematical techniques required. First, even in photosynthesis, one of the best-characterized biological systems, there are many fundamental issues to be resolved. For example, what is the relationship between photosynthesis and plant growth? Does photosynthesis determine plant growth and set the upper limit of photosynthetic capacity or vice versa? Though there has been much research on this topic (Fischer, 2007; Reynolds et al., 2007), the exact mechanism setting the source and sink capacities is still not well understood. A better understanding of this mechanism will enable us to model and design balanced source and sink capacities, which has been suggested to be a promising approach to increase photosynthesis and productivity, at least in wheat (Fischer, 2007; Reynolds et al., 2007). Second, photosynthesis is closely coupled with other metabolic processes in plants, such as respiration, photorespiration, and nitrogen assimilation, as reviewed (Lawlor and Tezara, 2009). Though the basic biochemistry of these pathways has been thoroughly studied, we still lack complete understanding of the mechanisms behind the interaction between these different metabolisms, e.g. between photosynthesis and respiration under light (Nunes-Nesi et al., 2005, 2007), and between photorespiration and nitrogen assimilation (Foyer et al., 2009). Understanding how these different processes interact to reach and maintain metabolic homeostasis will help better predict photosynthesis under different conditions. Thirdly, identifying optimal photosynthetic apparatus in the field needs to consider the high heterogeneity of environmental conditions, such as the spatial and diurnal variations of light, temperature, humidity and even CO2 concentrations in the canopy (Niinemets, 2007; Pearcy, 1990). These different factors influence many enzymes and proteins involved in photosynthesis. Unfortunately, our ability to predict the responses of photosynthesis to these different factors is still far from being satisfying due to lack of systematic characterization of impacts of these different factors on photosynthesis. For example, though detailed characterization of responses to temperature has been done for Rubisco (Bernacchi et al., 2001), but responses of most of other enzymes involved in photosynthesis to temperature have not been well characterized. Furthermore, plants show acclimation to prolonged changes in environmental conditions, such as CO2 levels and temperature (Long et al., 2004; Sage and Kubien, 2007), the mechanisms of which are also poorly understood. All these inevitably limit our capacity to predict photosynthetic performance under changing environmental conditions regularly occurring in the field. Lastly, the current systems models suffer from severe under-parameterization. So far, a consistent set of enzyme activities for all photosynthetic enzymes in one sample is still not available. Therefore, the traditional reductionist’s approach will continue play an important enabling role to support systems biology approach.

8. Why after millions of years of evolution, plants have not evolved an optimal photosynthesis?

Multiple options have been identified to increase photosynthesis and correspondingly crop productivity (Hubbart et al., 2007; Long et al., 2006b). One philosophical question often asked is, after millions of years of evolution, why plants have not evolved an optimal photosynthesis? There are a few reasons behind this phenomenon. First, evolution selects survival and fecundity, not necessarily higher photosynthesis or productivity. To ensure survival under stress conditions, a certain amount of resources, such as nitrogen, must be invested in protective mechanisms, such as photorespiratory pathways, at the cost of a lower photosynthetic efficiency. For example, in a typical photosynthetic apparatus, nitrogen is over-invested to the photorespiratory pathway to ensure survival of plants under extremely hot and drought conditions (Zhu et al., 2007). Though such extreme stress conditions are rare, its frequency might still be high enough to completely eliminate plants without a substantial photorespiratory flux (Zhu et al., 2007). Secondly, certain metabolic pathways have not evolved in higher plants. For example, Kebeish et al. (2007) showed that photorespiratory bypass can dramatically increase photosynthesis. By extending the model of photosynthetic carbon metabolism to include the photorespiratory bypass pathway, we showed that the increase in photosynthesis is mostly attributed to the increased CO2 concentration inside chloroplast stroma, while the decreased ATP consumption plays only a minor role in increasing photosynthetic CO2 uptake rate in the mutants (unpublished results). One intriguing question to ask is why such mechanisms have not been adopted during evolution. This is possible due to the inefficiency or lack of efficient mechanisms to transfer genes from prokaryotes to high plants, another possibility is that plants with this bypass might be less stress tolerant in hot and drought conditions. Thirdly, chang-
ing global climate might be another factor contributing to the less than optimal photosynthetic apparatus. The ambient atmospheric CO₂ concentration has been around 280 ppm for the past 25 million years (Barnola et al., 1999) before the rapid increase in the CO₂ concentration after the Industrial Revolution. The current nitrogen investment pattern in photosynthetic carbon metabolism, e.g. higher investment in the photorespiratory pathway, might represent an optimum nitrogen allocation for the pre-industrial CO₂ levels (Zhu et al., 2007). We have demonstrated that one of the critical enzymes in photosynthesis, Rubisco, is indeed optimized for higher CO₂ uptake for an atmospheric CO₂ concentration of 280 ppm (Zhu et al., 2004b). There are also other more subtle reasons contributing to the non-optimal nitrogen allocation in photosynthetic carbon metabolism. For example, the sub-optimal investment of SBPase might be related to the role of SBPase as a controlling point in photosynthetic carbon metabolism. The over-investment of enzymes in the photorespiratory pathway might also be due to the strict ratio between RuBP carboxylation and oxygenation (Zhu et al., 2007). The non-optimal enzyme distribution might also come from the particular parameterization of models used in the optimization. In Zhu et al. (2007), the evolutionary predicted a dramatic increase in the key enzyme of starch synthesis, ADP glucose pyrophosphorylase (ADPGPase) (Zhu et al., 2007). This apparent under-investment of ADPGPase in current plants however might not necessarily represent a result of natural selection. This is because the maximal rate of triose phosphate export was set to be a constant in the simulation (Zhu et al., 2007). As a result, when the photosynthetic CO₂ uptake rate gradually “evolved” to beyond the capacity of triose phosphate export rate, increase in ADPGPase activity becomes the only option to store the extra carbohydrate generated.

In summary, increasing photosynthesis is a new frontier to dramatically increase crop productivity. Because natural selection does not necessarily select for high photosynthesis while in agricultural context the primary goal is to increase productivity, identifying new ways to increase photosynthesis hold great promises to increase crop yields. Given that the evolutionary algorithm can effectively explore millions of permutations and identify the optimal design of metabolism for different defined environments, it provides a unique opportunity to explore ideal plant properties, ranging from properties at canopy structure level, plant architecture level, leaf physiology level, until cellular metabolism or regulatory levels. It is foreseeable that the evolutionary algorithm combining with systems model of photosynthesis will play an important role in our search for solutions to ensure food security for years to come. Given this, the traditional reductionist approach will continue play a critical enabling role in generating knowledge to support systems modeling and optimization research.

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