

## CHAPTER 6

### CROP SYSTEMS BIOLOGY

*An approach to connect functional genomics with crop modelling*

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**Abstract.** The response of the whole crop to environmental conditions is a critical factor in agriculture. It can only be understood if the organization of the crop system is taken into account. A popular view in modern science is that genomics (and other ‘omics’) will provide knowledge and tools to allow the characteristics of the crop to be altered for improved actual and potential crop yields, increased resource use efficiency and enhanced crop system health. As a result of this view, (molecular) plant systems biology has been considered as an approach to assist crop improvement for increased production, via modelling ‘how things work’ in (sub-)cellular units. However, phenotypes at the crop level, for example, as expressed in grain yields, are extremely complex, and not only achieved by molecular pathways but also through multiple intermediate metabolic and physiological processes. These processes are controlled by numerous genes whose effects and expression are highly dependent on environmental perturbations. Current prevailing initiatives for (molecular) plant systems biology so far have put little emphasis on bringing the ‘omics’ information to the crop level. Here, crop systems biology is presented as a complementary modelling approach to assist plant-breeding programmes to improve the yield and related resource use efficiencies of major crops. This crop systems biology approach honours the combined role of modern functional genomics and traditional sciences (such as crop physiology and biochemistry) in understanding and manipulating crop phenotypes relevant to agriculture. A stepwise routine for the development of crop systems biology models is proposed. Ultimately, these models should enable *in silico* assessment of crop response to genetic fine-tuning under defined environmental scenarios.

#### INTRODUCTION

The word ‘systems’ is not new in biological science. Ludwig Von Bertalanffy – a theoretical biologist – published a book, called *General Systems Theory* (Von Bertalanffy 1969), a compilation of his writings, some of which date back to the 1930s. In contrast to the reductionistic approach, he recognized the importance of ‘wholeness’ – the ‘systems’ of various orders not understandable by investigating

their respective parts in isolation. This systems concept remains valid in biology as practised today.

Recently, a new scientific discipline – systems biology – was proposed (cf. Kitano 2002), in the wake of the need for instruments to summarize large amounts of experimental data from modern high-throughput technologies in functional genomics. Although the meaning of systems biology is still under debate (Kirschner 2005), systems biology generally aims to synthesize complex datasets from various genomic hierarchies (genome, transcriptome, proteome, metabolome) into useful mathematical models. It seeks to explain biological functioning in terms of ‘how things work’ in (sub-)cellular units. Similarly, plant systems biology was defined, in the 22<sup>nd</sup> Symposium on Plant Biology (summarized by Minorsky 2003), using computational modelling approaches to predict a plant cell(ome) from underlying genomic understanding.

However, (plant) systems biology should not be considered an entirely new field, or even a research paradigm shift (Bothwell 2006). Crop scientists have used systems analysis to investigate whole-crop physiology and crop ecology for decades. Dynamic crop growth models emerged in the mid-1960s with the pioneering work of De Wit and colleagues (e.g., De Wit 1965; De Wit et al. 1978), who introduced Von Bertalanffy’s systems theory and the dynamic simulation method of Forrester (1961) into crop science. Crop systems modelling differs from empirical statistical analysis, just as systems biology differs from bioinformatics. In these crop models, constituting elements and processes are put together in mathematical equations. The rules by which the elements or processes interact give rise to systems behaviour and emerging properties illustrated by simulation, which may well be unexpected and even counterintuitive. This is model heuristics, which in turn enhances the understanding of individual processes and improves the next-round modelling of the crop. Dynamic crop systems models have been used to support theoretical research and applied activities.

There is no doubt that systems biology, as currently defined, is scientifically challenging. It will facilitate the development of functional genomics as a scientific discipline since, arguably, ‘omics’ has been driven more by emerging experimental technologies than by novel hypotheses. However, Hammer et al. (2004) argued that the current definition of plant systems biology not only largely overlooks the rich history of crop systems modelling, it also is probably not the best approach to solve the real-world problems towards crop improvement for increased production – the ultimate goal plant systems biology (Minorsky 2003) wants to achieve. We argue that new initiatives for plant-based systems research should first draw on the conventional science-based crop-modelling developments. At the same time, one should make use of modern genomics by parameterizing and redesigning some subroutines of crop systems models. To this end, the concept ‘crop systems biology’ is proposed; this concept is potentially more promising to fulfil real-world challenges in dealing with complex traits at the crop level, such as grain yield and resource use efficiencies. Concepts, rationales, methodology and future prospects of crop systems biology to resolve gene-to-phenotype relationships will be discussed.

## THREE WAYS TO TACKLE COMPLEX TRAITS

Many crop traits related to agricultural production are quantitative and complex in nature, regulated by multiple interactive genes whose effects and expression may be highly dependent on environmental conditions and developmental times. To manipulate these complex traits, understanding their genetic basis is crucial. In terms of the linear causal framework: DNA → mRNA → protein → metabolite → phenotype, Miflin (2000) considered three basic types of approaches to understanding the genetic basis of crop performance: (i) starting at the beginning – genomics; (ii) starting at the end – trait analysis; and (iii) starting in the middle – metabolic analysis. In accordance with this classification, there have been three strategies or approaches for biological modelling: (i) bottom-up; (ii) top-down; and (iii) some combination of bottom-up and top-down as the ‘middle-out’ approach (Noble 2002; see also Struik et al. in press).

The first approach is relevant given that major efforts are undertaken to sequence genomes of a range of plant species, whereas the entire genomes of *Arabidopsis*, rice and poplar have already been sequenced. The next challenge is to analyse these sequences to infer functions for the genes. Functional genomics aims to discover the function of all genes, typically through high-throughput experimental studies in genome-wide transcriptomics, proteomics or metabolomics combined with bioinformatics tools for data analysis, but also by functional analysis using ‘loss of function’ mutant genotypes. Systems biology was proposed in the wake of the need for instruments to synthesize complex datasets from the ‘omics’ studies into explanatory mathematical models that help to elucidate ‘how things work’ in (sub-) cellular units. These ‘bottom-up’ studies will undoubtedly yield valuable information for gene functions, gene interactions and genetic regulatory networks. For example, Hirai et al. (2004) showed progress by combining genome-wide transcriptomics and metabolomics under deficiency of sulphur and nitrogen in the model plant *Arabidopsis*, an important step in linking genomic data with the function of metabolites in plants. Discovery of gene functions is a basic task in functional genomics; however, it is not sufficient for crop improvement and probably of little use for enhancing selection for quantitative traits such as crop yields (Bernardo 2001). The bottom-up approach also pays little attention to the modulation by (multiple) varying environments, as perceived by the whole crop and, therefore, is a long way from helping to explain the connections between multiple genes and complex phenotypes such as grain yield and quality traits in crop plants that are crucial for agriculture.

The second (top-down) approach has been an important tool to study the genetic basis of complex crop traits with the discovery of DNA-based molecular markers in the early 1980s. These markers are based on small base changes (deletions or additions of bases and base pair substitutions) or on variation in the number of repeats of short sequences, most likely in non-coding genome regions. These markers are naturally occurring, abundant in most species, and simply inherit as monogenic Mendelian factors. These markers are therefore suitable to construct a saturated marker map, which is essential for localizing quantitative-trait loci (QTL; genome regions conferring the variation of a quantitative trait). Typically, QTL

analysis depends on making a cross between two individual genotypes that differ in the trait under study, and then deriving a segregating population consisting of a large number (mostly >100) of individual lines. Individual lines are both genotyped using a marker technology and phenotyped through experiments for the trait(s) under study, and then any close association between markers and a phenotype gives evidence of a QTL for the trait. This trait analysis is a 'shortcut' approach in connecting genome regions to phenotypes without needing any information for intermediate steps at transcript, protein or metabolite levels. It can be performed relatively independently of the pre-conception of the researchers, for example by physiologists (Prioul et al. 1997). Yin et al. (2000) introduced this approach to crop modelling, considering model-input parameters as a special type of quantitative traits, an approach which Yin et al. (2004a) later called 'QTL-based crop modelling'. Several subsequent studies (e.g., Reymond et al. 2003; Yin et al. 2005; Quilot et al. 2005) have indicated the added values of this combined QTL-model approach in resolving 'genotype-by-environment interactions', at least for the relatively simply quantitative crop traits studied so far. One drawback of QTL analysis using a bi-parental cross is the experimental setup in which only two parents are involved; therefore, it is unlikely that the total genetic variation present in the complete germplasm pool will be found. Linkage disequilibrium (LD) mapping, in which association between genotypes and phenotypes is scrutinized over a large germplasm collection, is increasingly becoming valuable for identifying loci contributing to quantitative traits (Remington et al. 2001). Especially when based on new, high-throughput, allele (haplotype)-specific markers, notably single-nucleotide polymorphisms, LD mapping allows whole-genome molecular-diversity characterization to localize favourable genes and alleles or haplotypes. Together with QTL mapping, this new development in association analysis may renew opportunities for gene-based crop modelling – the concept, as first put forward and empirically practised by White and Hoogenboom (1996), that aims to predict crop yield traits via linking underlying component traits with relevant known candidate genes.

Although breeders can make direct use of the results of the QTL and LD analysis (e.g., marker-assisted selection), the second approach treats the intermediate steps from genomes to phenotypes as a 'black box'. It could therefore be valuable to investigate some underlying mechanisms for the phenotype-marker association, for example by studying plant metabolisms and identifying genes involved in the metabolic pathways and their associated physiology – the 'starting in the middle' approach. Mifflin (2000) has given examples for several aspects of this approach. From a broader perspective, this third approach has links with the second approach – targeting the identification of QTL by determining their contribution to biochemical or physiological components of macroscopic traits. Several studies have demonstrated that the combined QTL and biochemical or physiological analysis generated new insights into causal relationships between physiological traits that would have been difficult to obtain by conventional physiological approaches (e.g., Lebreton et al. 1995). The QTL approach has been applied to activities of metabolic enzymes (Prioul et al. 1999); apparent co-locations between QTLs for an activity and known enzyme structural-gene loci were found. Such a candidate-gene approach

can facilitate the search for genes underlying QTL. Damerval et al. (1994) showed that the QTL approach could be applied to the variable proteins, and protein quantity loci were identified for 42 polypeptides. This may help in analysing metabolic and regulatory bases of the variation of general quantitative traits, thus providing a tool for characterizing QTL (De Vienne et al. 1999). Similarly, the LD-based association analysis could be applied to biochemical/physiological traits. A big advantage of this approach is that it allows the use of ample existing knowledge of conventional sciences such as biochemistry and physiology, which have already had a major impact on phenotypic characterization and thereby are very likely to impact on crop improvement programmes. To enhance the realization of this potential, combined examination of the crop-level phenotype with its associated underlying physiological components, biochemical traits and enzyme activities (cf. Hirel et al. 2001; Ashikari et al. 2005) is especially insightful.

#### NEED FOR A HOLISTIC APPROACH TO TACKLE THE COMPLEX TRAITS AT THE CROP LEVEL

The ultimate goal of crop scientists is to achieve increased crop production with given resource inputs to meet the demands of growing populations for sufficient food, feed, raw material and energy. Tremendous increases in crop yields have been achieved in some parts of the world over the last decades, especially since the first 'Green Revolution'. For most cereal crops, major improvements in yield potentials have been attained from the introduction of a few genes (such as dwarfing genes and photoperiod-insensitivity genes) that lead to changes in mass partitioning in favour of harvested organs of the crop. However, empirical breeding (even when using the marker-assisted-selection approach) to manipulate partitioning further seems to approach its theoretical limits (Reynolds et al. 2000; Long et al. 2006). The possibility for improved incident-light use efficiency (LUE) via breeding should be explored; and selecting for super-high-yielding hybrid rice in China has already shown promises (cf. Normile 1999). To improve LUE and associated yield potential, a thorough understanding of the whole system of photosynthesis, assimilation and respiration based on insights from genes via metabolisms and physiology to crop yield is required to achieve the future long-term objectives of empirical breeding or genetic engineering (Lawlor 2002).

Systems simulation modelling has long been suggested as a powerful tool to understand crop yield formation and to assist crop improvement programmes. However, the lack of truly 'mechanistic' crop simulation models (which make use of biochemical information) is a major constraint to advance the understanding of crop yield traits (Lawlor 2002). Also the already proposed 'plant systems biology' modelling approach (Minorsky 2003) is probably not the best approach to increased global crop supply (Hammer et al. 2004). The approach (which is better specified as 'molecular plant systems biology') does not (yet) put emphasis on bringing the information from 'omics' to the crop level for understanding the complex traits such as grain yields, relevant to the real-world challenge for global food security. While the third approach mentioned in the previous section is well recognized in human

biology (Noble 2002), especially in terms of connecting the various organizational levels, the biological modelling along the line with this approach is scarce in plant science (Giersch 2000). Yet, the need for such an approach is even larger in plant science than in human biology, given the specific nature of the interactions between organization levels in plants and the large influence of variable environmental conditions on primary production processes and phenology.

Nevertheless, there are studies using quantitative modelling to assess options of increasing LUE and yield potentials derived from qualitative knowledge of biochemistry and physiology. For example, actual measured photosynthetic quantum efficiency and light-saturated photosynthetic rate in C<sub>3</sub> crop species (including the world's major food crops: rice and wheat) under favourable environmental conditions are about only 50% and 25-35%, respectively, of their theoretical maximum values. Many biochemical and physiological factors (cf. Long et al. 2006) contribute to the gaps. An important factor is the loss due to photorespiration, as a result of O<sub>2</sub> competing with CO<sub>2</sub> for the catalytic sites of Rubisco. The C<sub>4</sub> metabolism is more efficient as it has a CO<sub>2</sub>-concentrating mechanism via the integrated development of the Kranz leaf anatomy, the localization of C<sub>3</sub> and C<sub>4</sub> enzymes, and necessary membrane transporters. Modifying C<sub>3</sub> plants towards C<sub>4</sub> metabolism has been a long-held ambition of plant biochemistry. However, the success of the transformation for this entire mechanism seems an unlikely prospect for the near future (Leegood 2002). A simpler routine, viz., expression of C<sub>4</sub> enzymes in single mesophyll cells of C<sub>3</sub> crops, is not adequate in obtaining the full advantage of C<sub>4</sub> photosynthesis (Von Caemmerer 2003). An alternative approach is to express, in C<sub>3</sub> species, improved forms of Rubisco, notably those from non-green algae in which the relative specificity (Sc/o) for CO<sub>2</sub> compared to O<sub>2</sub> is higher than that of higher-plant Rubisco. Zhu et al. (2004) examined this opportunity, using the widely used biochemical model of C<sub>3</sub> photosynthesis (Farquhar et al. 1980) coupled with a canopy model, and concluded that a substantial increase (> 25%) in crop carbon gain could result if that specific Rubisco were successfully expressed in C<sub>3</sub> plants. Long et al. (2006) extended the analysis and suggested more potential routines of increasing LUE, including increased Rubisco Sc/o, improved canopy structure, increased rate of recovery from photoprotection of photosynthesis, increased Rubisco catalytic rate, and increased capacity for regeneration of RuBP. Collectively, these increases could bring potential crop yield to increase by up to 50%.

However, simulation studies of Zhu et al. (2004) and Long et al. (2006) were performed for one single day and for a crop canopy of a particular size (leaf area index = 3). Besides the neglect of the importance of sink capacity for yield determination, they did not consider the crop growth cycle, nor did they consider growth influencing factors other than light, CO<sub>2</sub> concentration ([CO<sub>2</sub>]) and temperature, such as nitrogen availability. In fact, their hypothesis that increased photosynthesis results in increased yield stemmed from some experimental results that showed an increased growth of plants grown under elevated-[CO<sub>2</sub>] environments in comparison with those grown under the current atmospheric [CO<sub>2</sub>]. Many experiments with a season-long [CO<sub>2</sub>] enrichment in C<sub>3</sub> plants showed photosynthetic acclimation to elevated [CO<sub>2</sub>] – the phenomenon that the early effect

of increased photosynthesis in response to elevated  $[\text{CO}_2]$  will decline or even disappear when development progresses (Arp 1991). As a result, there is probably little grain yield advantage for plants grown continuously under elevated  $[\text{CO}_2]$  (e.g., Van Oijen et al. 1999), since carbon accumulated in the grain comes mostly from post-flowering photosynthesis. This acclimation can be ameliorated by nitrogen supply (Geiger et al. 1999). Sinclair et al. (2004) reasoned from their theory that a 33% increase in leaf photosynthesis may translate into an 18% increase in biomass, and only a 5% increase in grain yield or even a 6% decline in grain yield in the absence of additional nitrogen. Makino et al. (2000) also showed experimentally that, for a given leaf nitrogen content, a higher photosynthesis did not necessarily show greater biomass accumulation. Understanding the mechanisms of both carbon and nitrogen assimilation is essential to increase LUE (Lawlor 2002). Indeed, nitrogen assimilation in many species takes place simultaneously with  $\text{CO}_2$  fixation in photosynthetic cells. There is a close interaction between nitrogen and carbon metabolism, both utilizing light energy, with some 10% of the chloroplast electron flux in photosynthesizing leaves used in nitrate assimilation (Foyer et al. 2001; Yin et al. 2006). Quantitative, integrated information on cellular production and utilization of ATP and reductants with regard to carbon and nitrogen metabolisms (cf. Noctor and Foyer 1998) may provide a first step to formulate a summary model for truly mechanistic approaches of yield trait analysis and prediction.

#### CROP SYSTEMS BIOLOGY AS AN APPROACH TO CONNECT FUNCTIONAL GENOMICS WITH CROP MODELLING

The modelling studies at the crop level using some knowledge of biochemistry are currently sporadic, modelling results published so far to analyse yield traits are inconsistent, and some models are based on untested hypotheses. We propose a more systematic modelling approach – ‘crop systems biology’ – to analyse complex traits at the crop level, not only with the aim of establishing close links with understanding at the gene or genome level but also in terms of its comprehensive reliance on the whole-metabolism biochemistry and physiology. Therefore, the proposed crop systems biology is a crop-level approach to modelling complex crop traits relevant to global food production and energy supply, via establishing the links between ‘omics’-level information, underlying biochemical understanding and physiological component processes.

Development of crop systems biology models certainly depends on what trait a research wants to target. Crop yield is a complex trait that most existing crop simulation models have attempted to predict. It may not be surprising that simulation of yield formation should be a first major trait in crop systems biology. In addition, if crop yield can be modelled accurately, underlying mechanisms for traits of resource use efficiencies (such as water use efficiency or nitrogen use efficiency) can be analysed accordingly. The question is how to achieve a crop systems biology model for the yield trait.

We propose a two-step strategy, largely following the earlier mentioned ‘middle-out’ approach. First, a widely used framework of many existing crop simulation

models including processes such as photosynthesis, respiration and assimilate partitioning to calculate LUE implicitly can still be used, especially as a starting point. At the level of these processes, there are rich biological (physiological and biochemical) data and therefore the understandings are of the highest confidence. For this first step, crop systems biology models may not be necessarily more complex than existing crop simulation models in structure, nor is their computational requirement. However, model algorithms for individual processes are supposed to be more mechanistic. In many cases, a summary of a detailed biochemical model – e.g., the photosynthesis model of Farquhar et al. (1980) or its generalized form (Yin et al. 2004b) in order to deal better with stress conditions – can be incorporated as a sub-model. In other cases, direct results or stoichiometries from biochemical studies (e.g., examination of the biochemical pathways for production of protein, carbohydrates and lipids from glucose by Penning de Vries et al. (1974)) can be utilized. A prototype of crop systems biology models needs to be made available from this first step, in which physiological and biochemical information at the process level is assembled and then scaled up to the crop level in a way similar to temporal and spatial integrations as practised in conventional crop simulation models. In relation to crop improvement, a key element of the first step would be to identify the parts of mechanisms that are conservative in energy and water transfer and carbon and nitrogen metabolisms, and the parts of mechanisms that show genetic variation and are potentially amenable to selection and engineering. In case of grain yield, the prototype models should allow identification and quantitative assessment of specific parts of metabolic pathways and processes, which could be altered to achieve improvement of yield potential. The parts showing genetic variation can be identified by genetic analysis or the ‘omics’ approach in terms of the expressions of specific genes, proteins or enzyme activities.

After the first step is achieved, crop systems biology modelling could move to the second step as further progresses in understanding at the ‘omics’ level are being made, towards reaching down to lower organizational levels. For this, it is necessary to map the organization levels and the communication systems between these levels for the different key processes. Modelling for reaching down to the lower levels is most likely to be done in a manner of one-process-at-a-time; and in this respect, a modular design of the model is important to ensure that changes of a sub-model will not affect other parts of the model. Welch et al. (2003) have already developed a neural network model of *Arabidopsis* flowering-time control, based on studies on qualitative, genetic characterization of major flowering-time genes in this model plant species. Similar modelling studies could be performed for phenology of crop species. Further, existing modelling of metabolisms, such as the Benson-Calvin cycle of photosynthesis and the photorespiratory cycle (cf. Giersch 2000) and nitrogen assimilation in relation to the activity of key enzymes (e.g., nitrate reductase and glutamine synthetase), could also be added. With the rapid development of functional genomics in the wake of high-throughput technologies, combined studies of physiological components with gene expression profiles should illustrate the function of genes, biochemical pathways and cellular processes that are affected in a coordinated manner (Stitt and Fernie 2003). Such studies should lay the groundwork for elucidating regulatory networks and causal linkages among gene

products, biochemistry and whole-plant physiology. Integrated molecular systems biology models for a particular metabolism or process are expected to become increasingly available. Such models can potentially be embedded into crop systems biology models. Clearly, different temporal, spatial and structural scales are required for different components, pathways and processes of the model; and ultimately, crop systems biology will develop into a highly computer-intensive discipline. Such coupled models should enable *in silico* assessment of crop response to genetic fine-tuning under defined environmental scenarios, thereby being powerful tools in supporting breeding for complex crop traits.

#### CONCLUDING REMARKS

It is understandable that people have different interpretations when a new science emerges. Systems biology is not an exception. Although a vast majority of existing systems biology reports are up to the level of a 'cell', systems biology should have a larger and more ambitious scope, and its definition should be anchored in the general systems theory put forth by Von Bertalanffy. For the case of plants, models of systems biology, if aimed to solve the real-world problems towards crop improvement for increased production (Minorsky 2003), should not be used in a predictive mode merely up to 'cell(s)'; instead, ultimately they should be applied to improving traits associated with agriculture (Gutiérrez et al. 2005). In this context, crop systems biology seems to be a more promising term and approach to fulfil real-world challenges in improving complex traits, to narrow gene-to-phenotype gaps, and to promote interaction and cooperation between modern and traditional disciplines. The authors believe that we, crop physiologists, should actively explore and involve modern plant-science research to find a niche that we deserve to help to establish the complete knowledge chain in plant biology. On the other hand, those working on modern plant sciences should not consider such a joint effort to be altruism from their side, as this effort has recently been argued essential for further success in crop improvement towards a second 'Green Revolution' (Wollenweber et al. 2005). To make genomics work, joint effort across the entire plant biology and crop science is probably the best way to develop a 'systems biology'. The most difficult part of the joint activity is the first step; but at least, plant and crop scientists share a common view that systems biology is a community effort.

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