Critical Reviews in Plant Science, 28:368–374, 2009

ISSN: 0735-2689 print / 1549-7836 online
DOI: 10.1080/07352680903241246

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Populus Responses to Edaphic and Climatic Cues: Emerging Evidence from Systems Biology Research

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The emergence of *Populus* as a model system for tree biology continues to be driven by a community of scientists dedicated to developing the resources needed to undertake genetic and functional genomic studies in this genus. As a result, understanding the molecular processes that underpin the growth and development of cottonwood, aspen, and hybrid poplar has steadily increased over the last several decades. Recently, our ability to examine the basic mechanisms whereby trees respond to a changing climate and resource limitations has benefited greatly from the sequencing of the *P. trichocarpa* genome. This landmark event has laid a solid foundation upon which biologists can now quantify, in breathtaking and unprecedented detail, the diversity of genes, proteins, and metabolites that govern the growth and development of some of the longest living and tallest growing organisms on Earth. Although the challenges likely to be encountered by scientists who work with trees are many, recent literature provides a few examples where a systems approach, one that focuses on integrating transcriptomic, proteomic, and metabolomic analyses, is beginning to provide insights into the molecular-scale response of poplars to their climatic and edaphic environment. In this review, our objectives are to look at evidence from studies that examine the molecular response of poplar to edaphic and climatic cues and highlight instances where two or more omic-scale measurements confirm and hopefully expand our inferences about mechanisms contributing to observed patterns of response. Based on conclusions drawn from these studies, we propose that three requirements will be essential as systems biology in poplar moves to reveal unique insights. These include use of genetically-defined individuals (e.g., pedigrees or transgenics) in studies; incorporation of modeling as a complement to transcriptomic, proteomic and metabolomic data; and inclusion of whole-tree and stand-level phenotypes to place molecular-scale insights into a real-world context.

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Keywords Environmental stress, forestry, genomics, molecular biology, nutrients, trees

I. SETTING THE STAGE FOR SYSTEMS BIOLOGY IN *POPULUS*

Trees, like other multicellular plants, carry out the processes of growth, development, and reproduction in a constantly changing environment. They must possess and marshal a suite of physiological capabilities to cope with harsh climatic conditions and limited availability of nutrient resources, while at the same time ensuring their survival from one season to the next. The coordination of such events must be accomplished by orchestrating a series of complex molecular events in organisms that are distinguished from annuals by their perennial growth, complex crown architecture, dormancy, and juvenile-mature phase changes (Bradshaw *et al.*, 2000; Li *et al.*, 2006; Taylor, 2002; Wu *et al.*, 2000; Wullschleger *et al.*, 2002b). Many of these characteristics arise as a result of, or are facilitated by, long-distance signaling and distribution of water and nutrients and the storage and redistribution of resources, as modified by diurnal, seasonal, and intra-annual variation in climate (Lough and Lucas, 2006).

There have been many attempts to understand the molecular, physiological, and morphological processes by which trees

tackle these challenges, yet, to date, answers are few. A number of molecular studies on stress-responsive gene expression, protein, or metabolite profiling in trees, including *Populus*, have been undertaken. Much of this activity focused on single genes, proteins, or metabolite classes in the years pre-dating the new genetic and genomic resources for trees. While the utility of poplar as an experimental organism was widely recognized (Bradshaw *et al.*, 2000), the capacity to measure molecular-scale processes was a bottleneck. This has changed with the emergence of new technologies made possible by the draft sequencing of the *P. trichocarpa* genome (Tuskan *et al.*, 2006). In theory this should enable scientists to undertake a more comprehensive documentation of genes, proteins, and metabolites in poplar that are responsive to water and nutrient stress, variation in daily and seasonal cues, and encourage better interpretation and integration of such information.

In the wake of this landmark activity it is appropriate to examine how tree biologists have taken advantage of the opportunities created by sequencing the first tree genome. Jansson and Douglas (2007) have already shown that the number of citations for *Populus* has increased nearly 10-fold since the first EST data set was published for poplar (Sterky *et al.*, 1998). While this trend continues, it is additionally appropriate to ask what insights have been gained from our initial investments in sequencing the poplar genome and the increased availability of genomic and molecular tools for this model woody organism (Wullschleger *et al.*, 2002a; Tuskan *et al.*, 2004). Not surprisingly, the community has quickly embraced the opportunities at hand and research is currently being conducted to document the response of genes, proteins, and metabolites to several environmental and edaphic stresses (Azri *et al.*, 2009; Ferreira *et al.*, 2006; He *et al.*, 2008; Kieffer *et al.*, 2009; Lu *et al.*, 2008; Nanjo *et al.*, 2004; Xiao *et al.*, 2009). It is most encouraging that although the field is still in its infancy we are also beginning to see evidence of multiple omic-scale measurements in poplar, including the co-analysis of genes and metabolites, transcript and protein profiling, and metabolite and gene expression dynamics. It is particularly powerful to couple broad surveys of molecules with parallel observations of transgenic lines known to be perturbed in key aspects of the plant response, or with genome sequence-enabled dense marker coverage for QTL analysis. These studies mark the emergence of systems biology in poplar and are the focus of this article.

One often-noted promise of systems biology is that it will enhance our understanding of individual and collective plant functions and thereby provide a more integrated view of plant physiological responses to stress (Guy *et al.*, 2008; Hammer *et al.*, 2004). Some important clues regarding the way poplar copes with climatic stress and resource limitations are beginning to emerge and these are discussed here in relation to water and nutrient availability, and seasonal cues. Our focus is on the non-targeted analysis of genes, proteins, and metabolites, and on insights and understanding derived from global analysis of omic-scale responses in combination with other ecophysiological or genetic observations. Specifically, our objectives are to look at evidence from studies that examine the molecular response of poplar to edaphic and climatic cues and highlight instances where two or more omic-scale measurements either confirm or hopefully expand our inferences about mechanisms contributing to the observed patterns of response. We conclude with an assessment of where systems biology currently stands for poplar and make several recommendations on the future needs of this field.

II. RESPONSES TO WATER AND NUTRIENT AVAILABILITY

A lack of soil water constitutes a frequent limitation to plant growth and productivity, especially in poplar. Fast-growing trees in this genus produce maximum biomass when grown under irrigation (Coyle *et al.*, 2006; Samuelson *et al.*, 2007) or when they otherwise occupy riparian habitats in natural populations (Brunner *et al.*, 2004; DiFazio, 2005). As a result, productivity in poplar relies heavily on having readily available source of soil water (Dickmann *et al.*, 1992; Tschaplinski *et al.*, 1998). Previous research has shown that tolerance to water deficits varies widely among poplar genotypes, both inter- and intraspecifically, suggesting that the genus provides a good model in which to investigate the molecular and genetic basis of traits associated with drought tolerance (Monclus *et al.*, 2005; Tschaplinski *et al.*, 2006; Street *et al.*, 2006).

Although the biochemical, physiological and morphological behavior of important tree species, including poplar, to drought is well documented, only a handful of studies have characterized treatment or genotypic differences using a systems biology approach. Street *et al.* (2006) were among the first to combine classic ecophysiological measurements of plant water relations with quantitative trait loci (QTL) analysis and microarray experiments to examine adaptive strategies to drought in poplar. These authors used a full-sib F_2 mapping population that was previously developed from a cross between *P. trichocarpa* and *P. deltoides* (Bradshaw and Stettler, 1993) and subjected the grandparents and 167 of the F_2 progeny to a 14-d period of soil drying. Two microarray experiments using POP1 cDNA arrays (Andersson *et al.*, 2004) characterized the transcriptome in response to drought. One experiment focused on the two grandparents, while the second focused on a subset of extreme genotypes either sensitive or insensitive to drought on the basis of leaf abscission. In both of the experiments, drought stress resulted in a profound remodeling of the transcriptome. In particular, Street *et al.* (2006) showed that the divergent drought response of two poplar species exhibited segregation within an F_2 population, and that this results in the emergence of highly contrasting adaptive drought responses. Furthermore, comparing the transcriptional response of a set of high- and low-abscission genotypes revealed a striking and surprising degree of separation, suggesting that an expanded understanding of the molecular processes that contribute to leaf abscission could lead to improved biomass

productivity through conventional plant breeding or advanced genetic approaches targeting increased drought tolerance and/or length of growing season in water-limited environments.

While transcript profiling is a powerful and useful indicator of plant response to drought, it provides only one layer of information. Indeed, transcript profiling does not provide information related to protein turnover, sub-cellular localization of proteins or the complex interactions between proteins (Plomion *et al.*, 2006). To understand the major mechanisms that *P. trichocarpa* x *P. deltoides* cv. Beaupré evokes to tolerate drought stress, Plomion *et al.* (2006) investigated stress-induced gene regulation at transcript and protein levels. Both transcriptional and protein expression profiles revealed a general stress response that was consistent with the physiological data that was simultaneously collected. About 1300 and 1600 proteins (i.e., spots) from roots and leaves, respectively were resolved on Coomassie-stained 2D gels. However, only a handful of drought-induced proteins in the leaves and roots showed an increased level of their transcripts. This limited overlap between drought-regulated proteins and drought-induced transcripts likely reflects the different physical and chemical properties of the proteins investigated and the somewhat restricted set of genes (i.e., 2500) represented on the cDNA arrays used in this study. To their credit, what Plomion *et al.* (2006) did show was that a change in protein levels can occur with little or no detectable change in transcript abundance and vice versa. This demonstrates nicely the complementary nature of the transcriptomic and proteomic approaches, and the necessity to combine the two methods to reach full insights into the molecular plasticity response to drought or any other environmental cues.

Interesting and relevant research has sought to address the drought response of *P. euphratica*, a species that, unlike other members of the genus, grows in semiarid regions and is known to tolerate soils with high salinity (Chen *et al.*, 2003). In a series of studies that were conducted in natural (Brosché *et al.*, 2005) and controlled (Bogeat-Triboulot *et al.*, 2007) environments, the expression profiles of ca. 6,340 genes and of proteins and metabolites were recorded for roots and leaves. In the case of the controlled studies, in which young plants were subjected to increasing water deficits for 4 weeks, less than 1.5% of the genes on the arrays displayed significant changes in transcript levels; 70 genes in leaves and 40 genes in the roots. Moreover, the expression profile in roots was very different from that of leaves. Changes in the roots occurred earlier, at lower stress intensity, and predominately consisted of decreased, not increased, transcript abundances. In leaves and roots, most genes displaying altered expression during water deficit returned to control levels within a few days after the plants were re-watered and allowed to recover. Surprisingly, in contrast to the transcriptional response in leaves, the number of proteins whose abundance was modified by water deficit showed no correlation with water stress intensity. Bogeat-Triboulot *et al.* (2007) conclude that molecular response to water deficit in *P. euphratica* involves the regulation of different gene networks in roots and shoots.

Responses to carbon and nitrogen resource availability occur at multiple scales within trees and ecosystems (Millard *et al.*, 2007; Cooke and Weih, 2005). Novaes *et al.* (2009) used an integrated approach to test the hypothesis that responses to increased nitrogen resource availability are under genetic control in poplar. Novaes *et al.* (2009) grew clonal propagules from the pseudo-backcross family 52-124, generated from a [*P. trichocarpa* x *P. deltoides*] x *P. deltoides* cross, under conditions of low and high nitrogen availability. Cell wall metabolites reflecting abundance of syringyl and guiacyl lignin, C5 sugars and cellulose were quantified, as well as biomass traits. Strikingly, 45 of the 51 QTL identified in this study were specific to one condition of nitrogen resource availability. In particular, all of the genes regulating wood chemistry traits appeared to be highly responsive to nutrient availability, since no QTL were co-located under both nitrogen levels. The phenotypic plasticity of poplars in response to increased nitrogen resource availability had been described in a single clone (Cooke *et al.*, 2003) and a feed-forward conceptual model put forward that integrates nitrogen and carbon resource availability (Cooke *et al.*, 2005), and now the analysis of Novaes *et al.* (2009) has revealed the genomic regions governing these plastic responses to nitrogen availability on cell wall metabolites as well as growth traits.

III. RESPONSES TO SEASONAL CUES

Integrated, genome sequence-enabled approaches to dissect poplar storage and redistribution of resources induced by environmental cues are beginning to emerge. The seasonal recurrent transition to and from dormancy is a distinct feature of perennial plants and poplar has long been used to investigate such processes. Previous studies have shown that photoperiod and light quality (Howe *et al.*, 1996) as well as temperature, both heat (Wisniewski *et al.*, 1997) and cold (Rohde and Bhalerao, 2007), are critical for driving dormancy. Adversely cold temperatures ($<20^{\circ}$C) constrain the full genetic potential of plants by inhibiting metabolic reactions directly and indirectly through cold-induced drought resulting in reduced water uptake and cellular dehydration (Chinnusamy *et al.*, 2007). Although much work has been conducted on cold stress signaling and regulation in herbaceous species such as *Arabidopsis* (Zhu *et al.*, 2007), relatively few studies have investigated such processes in poplar. Benedict *et al.* (2006) report one of the few examples of such an approach and investigate the role of C-repeat binding factor (CBF) on acquiring freezing tolerance. Previous research in herbaceous annuals shows that CBF plays an important role in binding to the cis-elements of cold responsive gene promoters and orchestrating transcriptional cascades leading to increased freezing tolerance (Jaglo *et al.*, 2001; Lee *et al.*, 2005). Benedict *et al.* (2006) used ectopic expression of *Arabidopsis At*CBF1 and the POP1 cDNA microarray platform (Andersson *et al.*, 2004) to investigate CBF-mediated low temperature signaling. The authors found that ecotopic expression of *At*CBF1 increases freezing tolerance of nonacclimated

Populus and that comparative transcript profiles between *Populus* and *Arabidopsis* showed strong conservation in CBF regulation. However, there are some distinct differences. In contrast to herbaceous plants, for example, there was differential expression of CBF paralogs between perennial stem tissue and ephemeral leaf tissue. Functional analysis on differential genes between stem and leaf tissues were also evident leading the authors to suggest that perennial driven evolution may have led to specific roles for annual and perennial tissues.

Some recent studies have integrated transcriptome and metabolome profiling to generate new insights into shifts in resource allocation associated with seasonal cues. Such insights were not discernible prior to the availability of the poplar genome sequence. Most notably are the works of Ruttink *et al.* (2007) and Druart *et al.* (2007) and the multi-omics approach that led them to propose multistep-models for dormancy induction in poplar based on the systems integration of transcriptomic and metabolomic data sets.

Ruttink *et al.* (2007) examined poplar trees grown under contrasting long-day and short-day (SD) photoperiods, an inducing treatment known from previous studies to promote bud formation (Goffinet and Larson, 1981) and storage compound accumulation (Nelson and Dickson, 1981; Dickson and Nelson, 1982; Coleman *et al.*, 1992). Ruttink *et al.* (2007) evaluated apical shoot developmental stages using electron microscopy, which identified the cell types involved and the relative timing of storage reserve deposition. They monitored over one-third of the transcriptome at weekly intervals using the POP2 cDNA array (www.populus.db.umu.se), which contains 24,735 probes for 16,494 genes, or 34.6% of the 45,550 genes predicted in the poplar genome. In their transcriptome monitoring experiments, Ruttink *et al.* (2007) included transgenic lines known from previous studies to be perturbed in bud formation (Rohde *et al.* 2002). Specifically a line overexpressing and a line underexpressing *ABSCISIC ACID-INSENSITIVE3* (a transcription factor whose Arabidopsis and maize homologs also play dormancy-associated roles) were included to define mechanisms of *ABI3* action. The experimental design was an interconnected, balanced loop design of the three lines (two transgenic and one nontransgenic) and seven time points, which generated a great deal of statistical power to detect significantly regulated transcripts and metabolites. They evaluated transcripts and metabolites that varied at least four-fold with FDR <0.0001 (1091 genes) and FDR <0.01 (162 compounds) and found progressive shifts in anatomy, transcriptome and metabolome. Analysis of sequential time points suggested two major coordinated phases of shifts in transcript and metabolite abundance, one within the first week of SD treatments and another after 3 weeks of SD when budset normally occurs. The authors took advantage of additional insights gained previously from studies of poplar lines expressing a mutant version of *ETHYLENE TRIPLE RESPONSE1* (in which bud formation is disrupted; Ruonala *et al.*, 2006), which they correlated with shifts in ethylene-associated transcript abundance. The ethylene-associated effects were detected as a third stage, between the two major shifts in metabolite abundance. Thus the authors proposed three partially overlapping stages in bud development, specifically autumn bud formation, acquisition of desiccation and cold tolerance, and dormancy development. Dormancy development is distinct from bud formation since all of the transgenic lines were perturbed in bud formation but were dormant after six weeks of SD. Perhaps most importantly for future research, genes and metabolites were identified that are markers for discrete stages of this complex developmental process. It is now feasible to identify naturally occurring alleles in poplar populations that are genetically differentiated with respect to dormancy induction, and evaluate the robustness of the multistage model in the context of these naturally-occurring alleles in field environments.

Druart *et al.* (2007) also proposed a multistep model for dormancy induction in the vascular cambium based on time course experiments in which transcript abundance shifts and metabolite shifts were correlated in field-grown trees. The specific targeting of cambial cells is a particular strength of the approach, which employed thin sectioning to recover specific cell types prior to transcript (using POP1 cDNA arrays) and metabolite profiling (using GC-MS after extraction and derivatization). A role for ABA was hypothesized for coordination of late-stage acquisition of cold hardiness based on its peak abundance after the induction of transcripts associated with early-stage cold hardiness. Furthermore, gibberellin (GA) biosynthesis and action were proposed to coordinate transitions out of, and into cambial dormancy, respectively. This was based primarily on the observed accumulation of *REPRESSOR OF GA1-3* transcripts during growth cessation, and the transient induction of *GA-20 OXIDASE* during cambial reactivation. An interesting potential role for chromatin remodeling in dormancy transitions was inferred based on observed expression patterns of poplar homologs of *FERTILISATION INDEPENDENT ENDOSPERM* and *ENHANCER OF ZESTE*, known components of transcriptional repression complexes in *Drosophila*.

IV. CONCLUSIONS AND RECOMMENDATIONS

The *Populus* genome sequence is a landmark in the establishment of a genomics toolkit for forest trees and, as we have hopefully shown in this review, a gateway to systems biology research opportunities. The genome sequence has been leveraged in multiple ways to generate the tools required for integrated, systems-level research. For example, in a short period of time, the assembly of the genome sequence obtained from a single reference genotype created the template required for identifying allelic variants in other genotypes, enabling the construction and use of high-density maps for QTL mapping purposes (Woolbright *et al.*, 2008; Wullschleger *et al.*, 2005; Yin *et al.*, 2009). Furthermore, the identification of gene models obtained by annotation of the reference sequence enabled substantial portions of the transcriptome to be assayed in poplar trees. In this review we describe what may be the genesis of

systems biology research in poplar, manifest by the integration of information from two or more categories of traits (transcriptomic, proteomic, metabolomic) most of which was collected within a genetic framework (a genetically defined population, or transgenic contrasts).

It will be exciting in the next several years to witness the explosion of research and the new syntheses that are likely to emerge from integrative approaches. As mentioned above, the sequencing of the poplar genome has enabled high-density QTLs, which greatly reduces the chromosomal regions explaining the percentage of trait variation. The challenge now is how to explain the underlying processes by which allelic polymorphisms affect such QTLs (Benfey and Mitchell-Olds, 2008), and how best to scale those mechanisms through biological and ecological complexity. Network approaches developed in the biomedical arena have proven to be powerful ways to identify relationships among pathway and process components that are not obvious when transcripts or metabolites are analyzed separately. This systems-oriented approach groups individual genes into functionally relevant modules, which reduces the dimensionality of the omics-data from tens of thousands of genes to a few modules in a biologically meaningful way (Zhang and Horvath, 2005). Modules are then associated with QTL regions and markers, such as single-nucleotide polymorphisms (SNPs), or directly to traits of interest (Ghazalpour *et al.*, 2006; Weston *et al.*, 2008). Such an approach allows the identification of the chromosomal regions or markers influencing module expression and possible mechanisms associated with QTL regions.

Finally, having now reviewed studies in which integrated approaches were taken to dissect poplar responses to the environment, we envision that three features will be essential in development of new insights: 1) explicit inclusion of genetically defined individuals in the experimental framework. The inclusion of genotype information effectively "anchors" the phenotypes collected to allelic or gene expression variation. The ability to clonally propagate poplars allows these genotypes to be immortalized, shared and re-measured in the future; 2) explicit modeling of genetic polymorphisms (allelic variation) as a variable along with transcriptomic, proteomic and metabolomic data. Network approaches developed in the biomedical arena have proven to be powerful ways to identify relationships among pathway and process components that are not obvious when transcripts or metabolites are analyzed separately; and 3) explicit inclusion of whole-tree and stand-level phenotypes to place the molecular-level information in a real-world context. Systems biology can be an organizational framework for understanding tree processes at multiple temporal and spatial scales, but this will require that higher-level growth and yield data are meaningfully integrated into experimental analyses.

ACKNOWLEDGEMENTS

Support provided by the U.S. Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER). Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the DOE under contract DE-AC05-00OR22725.

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