

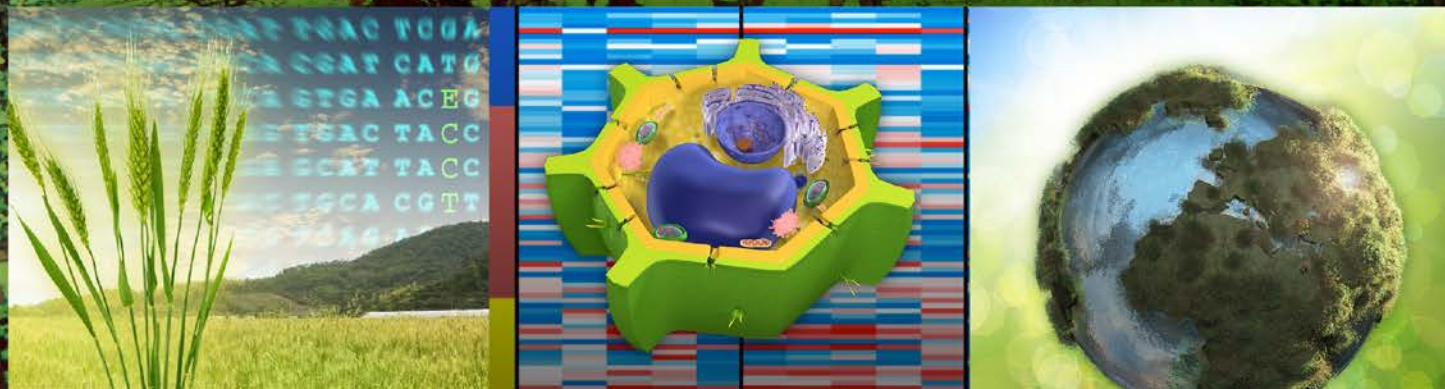
ENVIRONMENTAL
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Workshop Report

March 12-13, 2015

PhytoPhenomics



Prepared for the U.S. Department of Energy's Office of Biological
and Environmental Research under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99352



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C Jansson

September 2015

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Executive Summary

The PhytoPhenomics Workshop was held at EMSL, the Environmental Molecular Sciences Laboratory, on March 12-13, 2015. Nine external experts in plant phenomics attended along with several staff members from Pacific Northwest National Laboratory (PNNL) (Appendix A). The agenda (Appendix B) for the workshop included a presentation by Christer Jansson on a proposed PNNL Plant Phenotyping Platform and associated research projects; a presentation by Allison Campbell on EMSL as a research and user facility; a presentation by David Koppenaal on the process and logistics in initiating new capabilities at EMSL, and presentations by Galya Orr, Ljiljana (Lili) Paša-Tolić, Errol (Robby) Robinson, Kim Hixson, Nancy Hess, and Alex Guenther on existing EMSL capabilities. Nancy Washton gave a guided tour to the external participants. The external experts gave presentations both days that covered a wide range of plant phenotyping research projects and strategies. Additionally, the participants discussed the merits and specifics of the proposed phytoPhenomics efforts.

The general consensus at the workshop was to strongly encourage the proposed development of a Plant Phenotyping Pipeline at EMSL and PNNL. The rationale for building the EMSL plant phenomics capability can be summarized as follows:

- The vast and growing numbers of plant genomic sequences represent a virtually untapped resource for discovery of novel bioenergy-relevant genes, pathways, and traits.
- There is an urgent need to develop computational models that connect plant genotypes (G) to phenotypes (P) via environmental (E) influences and perturbations, i.e., develop G-E-P maps for predictive plant biology.
- Because of limited access to existing plant phenomics platforms, there is a pent-up demand for plant phenotyping approaches in the scientific community, including the EMSL user community, to provide an empirical foundation for any modeling efforts.
- The unique omics, imaging, spectroscopy, and computing capabilities at EMSL will significantly advance deep, high-precision (DHP) plant phenotyping and model development.

It was concluded that the proposal will be further socialized with experts in the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), and others after the workshop report is produced.

Acronyms and Abbreviations

BER	Biological and Environmental Research
BVOC	biogenic volatile organic compounds
CAAPP	Center for Advanced Algal and Plant Phenotyping
CEC	Controlled-environment conditions
DHP	Deep, high-precision
DOE	Department of Energy
EMSL	Environmental Molecular Sciences Laboratory
FCSD	Fundamental and Computational Science Directorate
GEP	Genotype-Environment-Phenotype
HTP	high throughput
LEAP	Land Ecosystems - Atmosphere Processes
MSU	Michigan State University
PEPC	Plant Ecosystems Phenomics Center
PNNL	Pacific Northwest National Laboratory
WSU	Washington State University

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1.0 Background

As costs for genome sequencing keep spiraling downward and with improved technologies for long reads, re-sequencing a large number of plants for assessing allelic variation is becoming routine. Furthermore, large collections of mutants, accessions, and recombinant lines now allow high-definition genotyping in a robotized way. To harness the wealth of genomic information available in such extensive catalogues of plant genetic diversity, we need first to understand the link between genotype and phenotype, and how this link is affected by the interaction between the plant and the environment. In other words, we need to establish computational Genotype-Environment-Phenotype (G-E-P) models. These G-E-P models can subsequently be applied to existing genetic resources such as germplasm collections. Plant phenotyping is an emerging science that links genomics with plant ecophysiology and biochemistry. The functional plant body (phenotype) is formed during plant growth and development from the dynamic interaction between the genetic background (genotype) and the physical world in which plants develop (environment). These interactions determine plant performance and productivity measured as accumulated biomass, commercial yield and resource use efficiency.

The PhytoPhenomics Workshop was held to discuss the launch of a Plant Phenomics Initiative at EMSL-PNNL and was centered on four key questions:

- What are the scientific drivers for BER and others at DOE to support a plant phenotyping initiative?
- Since phenomics is at the forefront of research needs in plant biology, why should BER fund a plant phenotyping platform at EMSL-PNNL, as opposed to someplace else?
- How should a plant phenotyping platform at EMSL be configured and what science should be emphasized and offered to the user community?
- What would be the expected demand from the user community for a plant phenotyping platform at EMSL?

2.0 A PNNL Plant Phenotyping Platform

2.1 Recommendations

Generally, the development of a Plant Phenotyping Pipeline at EMSL and PNNL was strongly supported by workshop participants. The following recommendations were made:

- Develop methodologies and models that allow prediction of field-relevant phenotypes from data obtained under controlled-environment conditions (CEC), e.g., to identify field-relevant genes that have escaped previous high-throughput (HTP) efforts based on CEC.
- Vigorously employ molecular phenotyping to obtain suites of early biomarkers for bioenergy-relevant traits such as drought tolerance and biomass yield.
- Direct DHP phenotyping to obtain detailed molecular and imaging data of selected specific cell types, such as bundle-sheath (BS) cells.
- Increase efforts relating to root and rhizosphere phenomics, including the development of HTP, non-invasive root phenotyping for CEC.
- Involve the KBase and iPlant platforms for modeling and data storage/sharing.

2.1.1 Field environment

The CEC versus field dichotomy, i.e., difficulty in translating information obtained under indoor CEC to field-relevant conditions, was discussed at some length. Growing and analyzing plants under indoor CEC is invaluablely useful in isolating specific phenomena; e.g., regulatory and metabolic pathways in acclimation to drought. However, it poorly represents the complex outdoor field environment and the fluctuating microenvironments at different depths of the plant canopy. It was stressed that it is important to complement plant growth under CEC with outdoor field sites, but CEC for plant growth should be furnished to better mimic outdoor field conditions. It was generally felt that plant phenomics chambers and equipment from commercial suppliers like Lemnatech [1] and PSI [2] offer a wide range of phenotyping measurements; however, these suppliers leave much to be desired in terms of delivering field-relevant data. The plant phenotyping facility developed by David Kramer at Michigan State University (MSU)-DOE Plant Research Laboratory illustrates an alternative solution. Kramer presented the MSU Center for Advanced Algal and Plant Phenotyping (CAAPP) [3], partly supported by DOE-BES. Kramer's group studies dynamic regulation of photosynthesis and growth in plants and algae using an approach referred to as Dynamic Photosynthetic Phenometrics. In this method, plants are grown in multiple specially designed chambers simulating different kinds of fluctuations seen in nature, with measurements taken continuously and non-invasively to capture transient events. By imaging fluorescence under continuous white light they obtain information about photosynthetic efficiency, photoprotection, redox states, and stomatal aperture.

Besides, it was suggested that the DHP phenotyping proposed in the PhytoPhenomics Initiative could be directed to develop models that allow prediction of field-relevant phenotypes from data obtained under CEC, e.g., to identify field-relevant genes or molecular profiles that have escaped previous HTP efforts based on CEC.

2.1.2 Root phenotyping

The workshop participants agreed that root phenotyping is a major obstacle in HTP plant phenomics particularly as it pertains to BER relevance. It was suggested that the proposed research on root phenomics be expanded and that developing technologies and instruments for HTP root phenotyping for CEC and the field would be a task worthy of EMSL.

2.1.3 Untargeted versus hypothesis-driven approaches

The virtues of untargeted versus hypothesis-driven approaches in phenotyping were discussed. Several external participants argued that the phytophenomics research as much as possible should strive to be hypothesis driven and focus on specific pathways, tissues, or cell types.

In a similar vein, it was proposed that obtaining high-granularity molecular profiles and imaging data at the tissue or cellular level is something EMSL is uniquely positioned for. Single-cell resolution is a perspective that is lacking in current virtual plant models. Different tissue or cell types could be selected for DHP phenotyping depending on the biological question studied, e.g., bundle-sheath cells in C₄ grasses and guard cells are prime candidates when exploring genotypic diversity in photosynthetic performance or water-use efficiency.

It was recognized that molecular phenotyping, such as metabolite or protein profiling, has the potential to generate biomarkers for agronomic traits like drought tolerance and terminal biomass yield. Intense research in this area would have the potential to significantly accelerate breeding for improved bioenergy crops. In the same direction, efforts to increase the throughput in molecular phenotyping were discussed. In this context, Richard Smith from PNNL's former Fundamental and Computational Science Directorate (FCSD) described the HTP MS pipeline his group is developing.

In the discussions on metabolite profiling, the importance of measuring metabolic fluxes came up. Bill Cannon from PNNL's former FCSD suggested that code developed at PNNL allowed metabolite profiles to be used to simulate and reconstruct metabolic networks through statistical thermodynamics without the use of rate constants.

2.1.4 Volatile organic compounds (VOC)

Alex Guenther presented the Atmospheric Aerosol Systems Program and gave a brief summary on the Land Ecosystems - Atmosphere Processes (LEAP) Workshop that he organized the week before the PhytoPhenomics Workshop. The Plant Science and the Atmospheric Aerosol Systems Program intersects around the importance of biogenic volatile organic compounds (BVOC) emitted from plants, to a large extent as part of stress responses, that enter the atmosphere to form aerosol particles. The prospects for the Plant Sciences initiative and the Atmospheric Aerosol Systems Program to coordinate efforts in setting up environments for controlled plant growth and BVOC analyses was highlighted as a unique opportunity for EMSL and PNNL in plant phenotyping and atmospheric sciences.

2.1.5 Virtual plant model

The development of a virtual plant model, which is a cornerstone in the proposed Plant Phenomics Initiative, was discussed at length. The value of virtual plant models in plant biology are indisputable; however, virtual plants can mean different things, from genome-wide association studies for correlating genotypic data with specific agronomic traits, to more elaborate network or mechanistic models. It was clear from the discussions throughout the workshop that the virtual plant as a concept needs to be further developed and assigned a predefined scope.

2.2 Conclusions and perspectives

Based on recommendations from the participants in the PhytoPhenomics Workshop, the synchronous LEAP Workshop, and ensuing meetings and discussions at EMSL and across PNNL, we propose the establishment of a Plant Phenomics Platform as a novel key capability at PNNL and as an integrated part of the EMSL user facility. In a longer 5-year time frame, we see the Plant Phenomics Platform as an integral part of a PNNL Plant Ecosystems Phenomics Center (PEPC). The goal of PEPC would be to further our understanding of plant ecosystem performance and responses in the context of integrated system behavior, information that is critical for our ability to enhance crop yield and stress tolerance, estimate terrestrial carbon fluxes from vegetation change and ecosystem dynamics, and predict the effects of climate change on terrestrial ecosystems.

3.0 BER Relevance

The consensus at the workshop was that bridging the G-E-P gap represents a Grand Challenge in plant biology, and that increased efforts in plant phenotyping and developing virtual plant systems align very well with BER's mission statement and strategy[4-7]. Thus the scientific drivers for BER and others at DOE to support plant phenomics research were considered not to be an issue. As to why BER would be interested in supporting a plant phenomics facility, it was noted that since existing plant phenotyping labs offer no or limited access to outside groups, there exists a pent-up demand for plant phenotyping in the scientific community. Brad Fabbri (Monsanto), Doug Allen (Donald Danforth Plant Science Center), and Dave Kramer (MSU-DOE Plant Research Laboratory) mentioned that the plant phenotyping platforms at their sites are booked for years to come. Furthermore, incorporating a Plant Phenomics Platform in the portfolio of EMSL user capabilities would require an on-site facility.

4.0 Why EMSL?

The consensus at the workshop was that bridging the G-E-P gap represents a Grand Challenge in plant biology, and that increased efforts in plant phenotyping and developing virtual plant systems align very well with BER's mission statement and strategy[4-7]. Thus the scientific drivers for BER/DOE to support plant phenomics research were considered not to be an issue. As to why BER would be interested in supporting a plant phenomics facility, it was noted that since existing plant phenotyping labs offer no or limited access to outside groups, there exists a pent-up demand for plant phenotyping in the scientific community. Brad Fabbri (Monsanto), Doug Allen (Donald Danforth Plant Science Center), and Dave Kramer (MSU-DOE Plant Research Laboratory) mentioned that the plant phenotyping platforms at their sites are fully booked for years to come. Furthermore, incorporating a Plant Phenomics Platform in the portfolio of EMSL user capabilities would require an on-site facility.

5.0 Summary

The workshop provided valuable content and feedback for moving forward with the Plant Sciences Program and the Plant Phenomics Initiative. The external participants were selected for their wide range of aspects and strategies in plant phenotyping and for being leading experts in their respective field. Their input will continue to be important in post-workshop meetings and deliberations, and in setting up facilities for plant growth and phenotyping. The interaction between the external panel and EMSL-PNNL participants allowed dynamic discussions about the potential for EMSL's place in future plant phenomics.

Appendix A
Workshop Participants

Appendix A

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Additional EMSL and PNNL Participants

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Tim Scheibe, Senior Scientist and Lead Scientist, for Multiscale
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Martina Köberl, Visiting Postdoctor, FCSD

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Richard Smith, Battelle Fellow, FCSD

Scott Tingey, Chief Operating Officer, EMSL

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Appendix B

Agenda

Appendix B

PhytoPhenomics Workshop

March 12-13, 2015

Location: EMSL 1077

Agenda

Thursday, March 12, 2015

Time	Topic/Participant(s)
7:30 a.m.	Morning Refreshments
8:00 a.m.	Welcome and Introductions – <i>Christer Jansson, EMSL</i>
8:15 a.m.	Presentation of EMSL and the User Program - <i>Allison Campbell or Karl Mueller, EMSL</i>
8:45 a.m.	EMSL's Infrastructure and Equipment – future plans – <i>Dave Koppelaar, EMSL</i>
9:15 a.m.	PNNL's Plant Science Program: Vision and Strategy - <i>Christer Jansson, EMSL</i>
9:45 a.m.	EMSL's omics and imaging capabilities for molecular plant phenotyping - <i>Robby Robinson, Galya Orr, Kim Hixson, EMSL</i>
10:15 a.m.	Break
10:25 a.m.	EMSL's omics and imaging capabilities for molecular plant phenotyping, cont. – <i>Lili Pasa-Tolic, Nancy Hess, EMSL</i>
11:15 a.m.	Interactions between the EMSL Plant Sciences & Aerosol Programs – <i>Alex Guenther & Christer Jansson, EMSL</i>
11:30 a.m.	Lunch, Discussion, Writing of Report
12:30 p.m.	Tour of EMSL – <i>Nancy Washton</i>

Presentations of Plant Phenotyping Research

1:30 p.m.	Using isotopic labeling and metabolic flux analysis to assess environmental or genotypical variation in phenotypes - <i>Doug Allen, USDA-ARS/Donald Danforth Plant Science Center</i>
2:00 p.m.	Leaf-level to Field Phenomics: Advancements and Challenges - <i>Sindhua Sankaran, Washington State University</i>
2:30 p.m.	Developing and using <i>Brachypodium</i> spp. as functional genomic models for the grasses – <i>John Vogel, JGI & Lawrence Berkeley National Laboratory</i>
3:00 p.m.	Five years Plant Phenotyping at WSU-Pullman – A Retrospective – <i>Helmut Kirchhoff, Washington State University</i>
3:30 p.m.	Break

- 3:40 p.m.** Implementing root traits for tolerance to abiotic stress – *Kathleen Brown, Penn State*
- 4:10 p.m.** Summary and Discussion; Writing of Report
- 5:40 p.m.** Adjourn
- 6:30 p.m.** Dinner (pay your own way) for external participants - *Anthony's, 550 Columbia Point Dr, Richland*

Friday, March 13, 2015

Time	Topic/Participant(s)
7:30 a.m.	Morning Refreshments
8:00 a.m.	Using phenomics in reverse genetics approaches to characterize orphaned DNA – <i>Thomas Bureau, McGill University Canada</i>
8:30 a.m.	Plant phenotyping at Monsanto – <i>Bradon Fabbri, Monsanto</i>
9:00 a.m.	Dynamic Plant Phenometrics: From Field to Lab and Lab to Field – <i>David Kramer, PRL, Michigan State University</i>
9:30 a.m.	Multiscale characterization of phenotypes in microbes and metazoans: A model for network modeling of plant phenotypes – <i>Nitin Baliga, Institute for Systems Biology</i>
10:00 a.m.	Break
10:10 a.m.	Plant Phenotyping Centers and Networks in Europe, Australia and Canada – Experiences and impressions – <i>External participants</i>
10:40 a.m.	How can a Plant Phenotyping Platform best leverage EMSL's unique position as a Research Facility and as a User Facility? - <i>All</i>
12:00 p.m.	Lunch, Summary and Discussion, Writing of Report
1:00 p.m.	Summary and Discussion, Writing of Report, cont.
2:00 p.m.	Wrap-up Discussion, Recommendations, Writing of Report
3:00 p.m.	Adjourn

Appendix C

References

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