FINAL REPORT

A novel poplar biomass germplasm resource for functional genomics and breeding

2014-2018

ACCOMPLISHMENTS

1. What are the major goals of the project?

Interspecific hybridization is the most effective method for producing poplar clones for commercial applications, including biomass production. Previous research has shown that commercial F1 hybrids frequently exhibit dosage variation, i.e. variation in the number of pieces or entire chromosomes, and that these variants can have novel or more extreme phenotypes compared to their parents, that can be desirable for biomass production. Poplar is a convenient system for studying and exploiting gene dosage because germplasm can very easily be propagated vegetatively, via cuttings, for research and production, and advanced genome-enabled tools can be directly employed on the actual feedstock. In the previous funding cycle, we had produced and characterized a population of interspecific hybrids carrying insertions and deletions, which together, tile the entire genome with dosage variation. In this funding cycle, we completed the development of this unique resource for the research community and started using the germplasm to investigate the role of gene dosage in poplar hybrid performance, and to identify chromosomal regions and genes that contribute to poplar bioenergy traits.

Our specific goals were to:

i) maintain and finish the characterization of this resource
ii) investigate the phenotypic effects of dosage change
iii) exploit the indel germplasm for functional genomics.

2. What was accomplished under these goals?

During the funding period, we made excellent progress on all goals and are in the process of completing final analyses and writing publications. Specifically, we made the following progress on the three main objectives:

i) Maintain and finish the characterization of the resource

Expanding on the existing resource, we have included an additional ~300 individuals for a total of > 800 interspecific hybrids coming from two crosses, both using gamma-irradiated pollen from the same *P. nigra* parent. Each tree has been characterized for the presence of indels using genome sequencing. We have characterized > 800 genotypes and found ~ 1,200 indel mutations. Approximately 8% of our F1 hybrids are triploids, which adds the ability to characterize gene-dosage effects using a series of dosage variants. We have verified the information by repeating the analysis for a random set of individuals and we now consider the dataset complete.

Additionally, we have identified a few genotypes with dosage variation consistent with catastrophic genome restructuring, a process called chromothripsis and observed initially in cancer cells and later in arabidopsis plants that have undergone haploid induction. We have sequenced these individuals at increased depth and are in the process of investigating the mechanism underlying these genomic changes.

ii) Investigate the phenotypic effects of dosage change

The F1 hybrid seedlings have been clonally replicated and ~600 of them (along with ~ 55 non-irradiated controls) have been entered into phenotypic field trials. Tree clones from each original F1 tree were

randomly distributed in the field, and the field was maintained for several growing seasons. We have phenotyped the trees for a number of growth and morphological traits relevant to biomass and bioenergy production, including leaf size and shape, height and diameter growth, leaf stomata, crown architecture, branch angle and phenology. An additional cohort of clones has been subjected to a drought tolerance trial and a tension wood formation trial. All trees were coppiced at the same time in the Fall of 2017, to characterize variation in wood properties, and growing behavior after coppicing, both important characteristics for the poplar industry as well.

Many of the dosage variants display transgressive variation in specific traits. At the same time, replicate clones of the same F1 hybrid genotype generally behave very similarly to each other, confirming that this system provide a robust platform for functional genomics.

iii) Exploit the indel germplasm for functional genomics.

For some of the traits, we have identified chromosomal regions associated with these traits, regions that we call dosage qtl (dQTL). For example, we have characterized how dosage variation can modulate biomass and phenology, two important traits for bioenergy crops. We are in the process of identifying and characterizing candidate genes. We are using a similar approach to investigate the mechanisms regulating leaf size and shape, traits that can be important to biomass formation, and adaptation to environmental conditions, respectively. The population is still present and maintained in the field in Placerville, and available to the community for any other phenotypic characterization.

We have harvested leaf material from a large subset of the trees present in the field, to characterize variation in gene expression, associated with the observed variation in growth traits. We have started analyzing this extensive RNAseq dataset to understand the molecular basis of gene-dosage variation across entire poplar chromosomes, and relating that to phenotypic variation. Our final goals are to describe how large numbers of genes interact to influence complex quantitative bioenergy traits, and ultimately identify individual candidate genes underlying QTL.

3. What opportunities for training and professional development has the project provided?

The following individuals have been trained in the context of this project:

Weier Guo, Masters student, Comai laboratory – Detailed characterization of poplar chromothriptic variants.

Fresia Rodriguez-Zaccaro, rotating graduate student, Comai Laboratory and later graduate student, Groover laboratory – Variation and genetic characterization of the factors regulating wood vessel formation in poplar tree.

Courtney Canning, Masters student, Groover laboratory – using indel variants to understand tree architecture traits relevant to bioenergy.

Zach Lipman, rotating graduate student, Comai Laboratory – Pilot study on the effect of indels variation on the microbiome associated with poplar leaves.

Jian Gao, visiting graduate student from China, Comai Laboratory – Genomic characterization of poplar indel variants.

Matthew Zinkgraf, post-doctoral researcher, Groover laboratory – Creation and maintenance of the indel variant population, phenotyping and associated analyses.

Heloise Bastiaanse, post-doctoral researcher, Groover laboratory – Phenotyping and associated analyses.

Clara Williams, junior specialist (now a graduate student in Ghent, Belgium), Comai laboratory – Genomic characterization of the indel variants

Txai Gomez, undergraduate student, Comai and Groover laboratories – Training in molecular biology

Jeremy Tran, undergraduate student, Comai laboratory – Genomic characterization of the indel variants.

Meric C. Lieberman, master student, Comai laboratory – Identification and characterization of sex-specific sequences in Populus nigra.

Ruth Lee, junior specialist, Comai Laboratory – Genomic characterizaion of indel variants, sex determination using RFLP markers.

4. How have the results been disseminated to communities of interest?

In addition to publications listed below, we have disseminated our results and resources in the following ways:

1) All sequencing reads have been deposited in the NCBI SRA database.

2) Lists of single nucleotide polymorphisms (SNPs) between the various populus species caracterized has also been made available.

3) Data about all trees in our collection is available as part of the PopGenIE resource, at http://popgenie.org/popindels.

5. What do you plan to do during the next reporting period to accomplish the goals?

The first two aims have been wrapped up and we do not anticipate any further work on these objectives. For the third objective, we are in the process of completing all of the final tasks and analyses for overlaying gene expression data, and integrating all datasets. These final steps will provide a detailed view of the genetic regulation of the multiple bioenergy traits being examined in this research project. A first paper describing phenotypic trait dissection has been submitted to PNAS (Feb 2019), and we anticipate more publications on traits to be submitted in the near future. Our analysis of the chromothriptic individuals is also well on its way and we anticipate submission in 2019. Finally, our analysis of the effects of dosage variation on gene regulation will follow shortly as well. We are hoping to combine data from our leaf transcriptome analysis with data from wood samples collected in the context of a different project, in order to obtain a more complete view of the regulatory networks in polar.

PRODUCTS

Peer-reviewed publications directly related to this project

Héloïse Bastiaanse, Matthew Zinkgraf, Courtney Canning, Helen Tsai, Meric Lieberman, Luca Comai, Isabelle Henry, and Andrew Groover (**2019**) "Effect of gene dosage variation on phenology and biomass in *Populus* trees", **submitted** to PNAS

Henry, IM, Comai L and Tan EK (**2018**). Detection of Chromothripsis in Plants. Chromothripsis: Methods and Protocols, Met Mol Biol, vol. 1769. Franck Pellestor (ed).

Tan EK, Comai C and Henry IM (**2016**). Chromosome Dosage Analysis in Plants Using Whole Genome Sequencing. *Bioprotocols 6(13):* e1854. http://www.bio-protocol.org/e1854.

Groover A (**2016**). Reaction woods and gravitropisms of forest trees. New Phytologist 10.1111/nph.13968

He X and Groover A (**2016**). The genomics of wood formation in angiosperm trees. In: Evolutionary and comparative genomics of angiosperm trees. Groover and Cronk (Eds.) Springer.

Matthew Zinkgraf, Kathy Haiby, Meric Lieberman, Luca Comai, Isabelle Henry and Andrew Groover (**2016**). Creation and analysis of irradiation hybrids in *Populus*. - Invited manuscript, accepted at Current Protocols in Plant Biology.

Henry IM, Zinkgraf MS, Groover AT, and Comai L. (**2015**) A System for Dosage-Based Functional

Genomics in Poplar. Plant Cell**,** 27:2370-2383.

Student work

Meric Chase Lieberman "Identification and characterization of male sex-specific sequences in Populus nigra", Master's thesis, Integrative Genetics and Genomics program, UC Davis, **May 2016**.

Oral/ Poster Presentations

"Embracing the complexity of nature: Genomic approaches for understanding the development and evolution of forest trees." A.T. Groover. Arnold Arboretum of Harvard University. December **2018**.

"Functional genomics for quantitative trait dissection in forest trees." A. Groover, H. Bastiaanse, M. Zinkgraf, I. Henry, L. Comai. Agriculture Bioscience International Conference. Weifang, China. September **2018**.

"Discovery and characterization of disease resistance loci using a unique gene copy number variant population", H. Bastiaanse, M. Marlin ,I.M. Henry , R. Rousseau ,A. T. Groover G. Newcombe, L. Comai , International Conference on Plant Pathology, Boston (July 29- August 3 **2018**)

"Widespread chromosome instability in regenerated potato plants" Isabelle M Henry, Michelle Fossi, Weier Guo, Kirk Amundsen, Benny Ordonez, Sundaram Kuppu, Anne Britt and Luca Comai, Plant & Animal Genomes XXVII Conference, January **2018**, San Diego, CA.

"Accelerated plant breeding through chromosome manipulation", Isabelle M. Henry, August 9, **2017**. Invited Speaker, 2017 Annual meeting of the National Association of Plant Breeders (NAPB), UC Davis, CA.

"Genomic approaches for understanding and harnessing dosage variation", Isabelle M Henry, Invited Speaker for Seminar Series, School of Agriculture, Kyoto University, April 8, **2016**, Kyoto, Japan

"Genomic-enabled insights into growth and wood formation in *Populus*." A. Groover. Umeå Plant Science Centre, Umeå University, Sweden, March 3 **2016**.

"Genomic approaches for functional discovery in polyploid species", Isabelle M Henry, Invited Speaker at the 2016 University of Nebraska Plant Breeding Symposium, March 29 **2016**, Lincoln, NE.

"Towards Understanding and Harnessing Dosage Variation in Populus" Isabelle M Henry, Matthew Zinkgraf, Heloise Bastiaanse, Andrew Groover and Luca Comai. Plant & Animal Genomes XXIV Conference, January **2016**, San Diego, CA.

"Genomic Discovery through Chromosome Manipulation**"** Isabelle M Henry**,** Ek Han Tan and Luca Comai. Plant & Animal Genomes XXIV Conference, January **2016**, San Diego, CA.

"Transcriptional and hormonal control of gravitropism in forest trees." Andrew Groover, Suzanne Gerttula, Mathew Zinkgraf, Harry Brumer, Shawn Mansfield, Vladimir Filkov. Hohhot, China, August **2015**.

"Transcriptional and hormonal control of gravitropism in forest trees." Andrew Groover, Suzanne Gerttula, Mathew Zinkgraf, Harry Brumer, Shawn Mansfield, Vladimir Filkov. Society of Developmental Biology 74th Annual Meeting, Snowbird Utah, July 10 **2015**.

"Strategies for Mutation Discovery and Characterization for Plant Functional Genomics" Isabelle M Henry, Andrew Groover, Matthew Zinkgraf, Thomas Tai and Luca Comai. Plant & Animal Genomes XXIII Conference, January **2015**, San Diego, CA.

"Genomic approaches for understanding biomass production and wood formation in trees using *Populus*." Andrew Groover. Symposium, From Plant Growth to Biomass Products. Huazhong Agricultural University, Wuhan, China. Sept **2014**.

"A Populus gene dosage resource for functional genomics and breeding." A. Groover. L. Comai, I. Henry, and M. Zinkgraf. International Poplar Symposium, University of British Columbia, Vancouver, Canada, July 21 **2014**.

INTELLECTUAL PROPERTIES DETAIL:

None

TECHNOLOGIES AND TECHNIQUES DETAIL:

None

OTHER PRODUCTS DETAIL:

None

PARTICIPANTS

Name	Role	Person month worked	Intl. Collaboration	Intl. travel
Luca Comai	PI	1	Yes (Sweden)	No
Isabelle M Henry	Co-PI	6	Yes (Sweden)	No
Weier Guo	Graduate student	3	No	No
Helen Tsai	Staff Research Associate	2	No	No
Meric Lieberman	Bioinformatician	3	No	No
Sergio Silva	Junior Specialist	3	No	No

Contribution to the project

Luca Comai, Professor. Dr Comai participated in project design, data interpretation and monthly discussions about future directions and potential problems. He was also an active participant in manuscript writing and public presentations of the results.

Isabelle M. Henry, project scientist. IMH was responsible for coordinating and carrying out the sequencing and bioinformatics efforts. She is also involved in manuscript writing, supervising technical personnel, and participating in monthly meetings with collaborators to discuss data interpretation, potential problems and future directions.

Weier Guo, graduate student. WG is working towards her Ph.D. in Integrative Genetics and Genomics. Specifically, she is investigating the consequences and mechanisms underlying chromothripsis in our poplar gamma-mutant population.

Helen Tsai, staff research assistant. Helen Tsai was responsible for technical procedures associated with the production of the RNA-Seq libraries and associated work.

Meric Lieberman, graduate student and bioinformatics programmer. Meric Lieberman was responsible for sequence data handling and processing, server maintenance and data back-up. He and IMH are also responsible for the sequence data analysis and initial interpretation. Meric Lieberman also worked towards a Master degree in Integrative Genetics and Genomics, including a project on the identification and characterization of male-specific sequences in our population of poplar trees.

Ruth Lee, junior specialist. Ruth Lee worked under the supervision of Isabelle Henry and was responsible for the technical procedures such as DNA extractions, sequencing library preparations, PCR etc.

Other collaborating organizations

None

IMPACT

1. What is the impact on the development of the principal discipline(s) of the project?

Our efforts add new, useful and unique tools that have both functional and practical applications for poplar breeding. This unique functional genomics resource can be used to investigate the role of gene dosage in poplar hybrid performance, and to identify genes that contribute to poplar bioenergy traits.

2. What is the impact on other disciplines?

The tools and methods developed in the context of this project are widely applicable to other biomass crops such as switchgrass, alfalfa, willow, and miscanthus.

3. What is the impact on the development of human resources?

None

4. What is the impact on physical, institutional, and information resources that form infrastructure?

The germplasm and associated data are all being made available to the poplar research community. We have also published several papers describing the methods used for creating and analyzing this type of resource, as an aid to other researchers and breeders.

5. What is the impact on technology transfer?

Because the approach does not involve the use of transgenics, it is potentially suitable for development of new germplasm for forest industry

6. What is the impact on society beyond science and technology?

Our germplasm collection includes trees with a new type of variation, that is not normally present in traditionally bred populations. This could include cultivars directly valuable for bioenergy applications.

7. Foreign Spending

None

CHANGES - PROBLEMS

1. Changes in approach and reasons for change

None

2. Actual or anticipated problems or delays and actions or plans to resolve them

None

3. Changes that have a significant impact on expenditures

None

4. Significant changes in use or care of human subjects, vertebrate animals, and/or biohazards.

None

5. Change of primary performance site location from that originally proposed

None

6. Carryover Amount

$0