Tree responses to environmental cues

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NSERC CRSNG
Ontario
DFG Deutsche Forschungsgemeinschaft
FVA Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg
Doug AdapT


3


19

Response to environmental cues reveals plant phenotypic plasticity

Environment

Signal (e.g. light) → Receptor (e.g. photoreceptor) → Signal cascade (e.g. induction of transcription factors) → Phenotype (e.g. synthesis of anthocyanin)

Response to environmental cues reveals plant phenotypic plasticity

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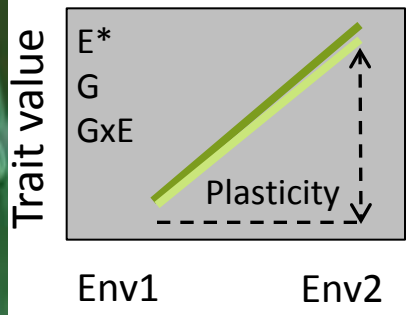

Response to environmental cues reveals plant phenotypic plasticity

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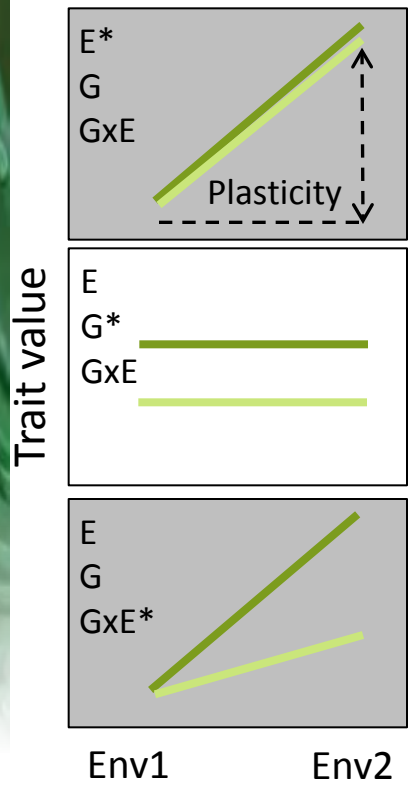
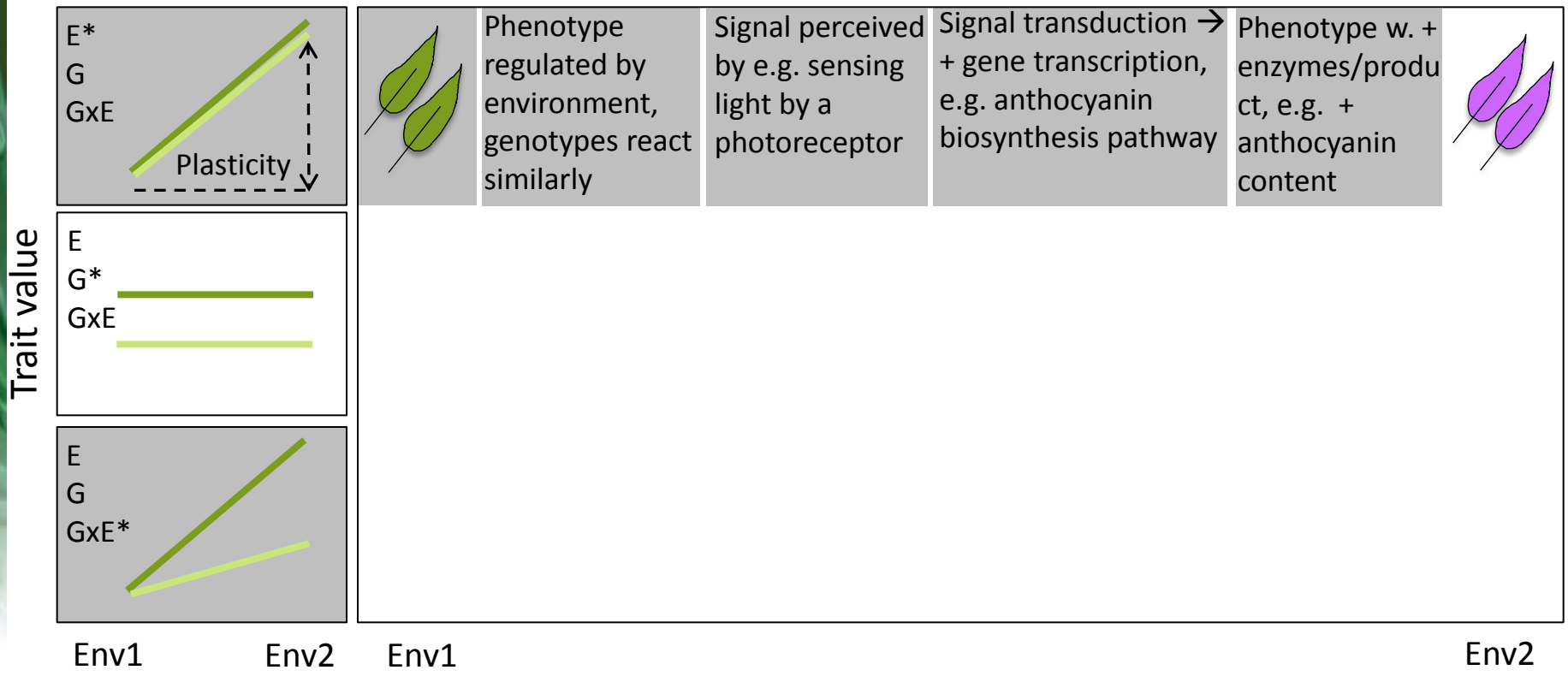


Response to environmental cues reveals plant phenotypic plasticity

Environment

Epigenetic, transcriptional or post-translational regulation

Signal (e.g. light) → Receptor (e.g. photoreceptor) → Signal cascade (e.g. induction of transcription factors) → Phenotype (e.g. synthesis of anthocyanin)



After Nicotra et al. (2010) TIPS

Response to environmental cues reveals plant phenotypic plasticity

Environment

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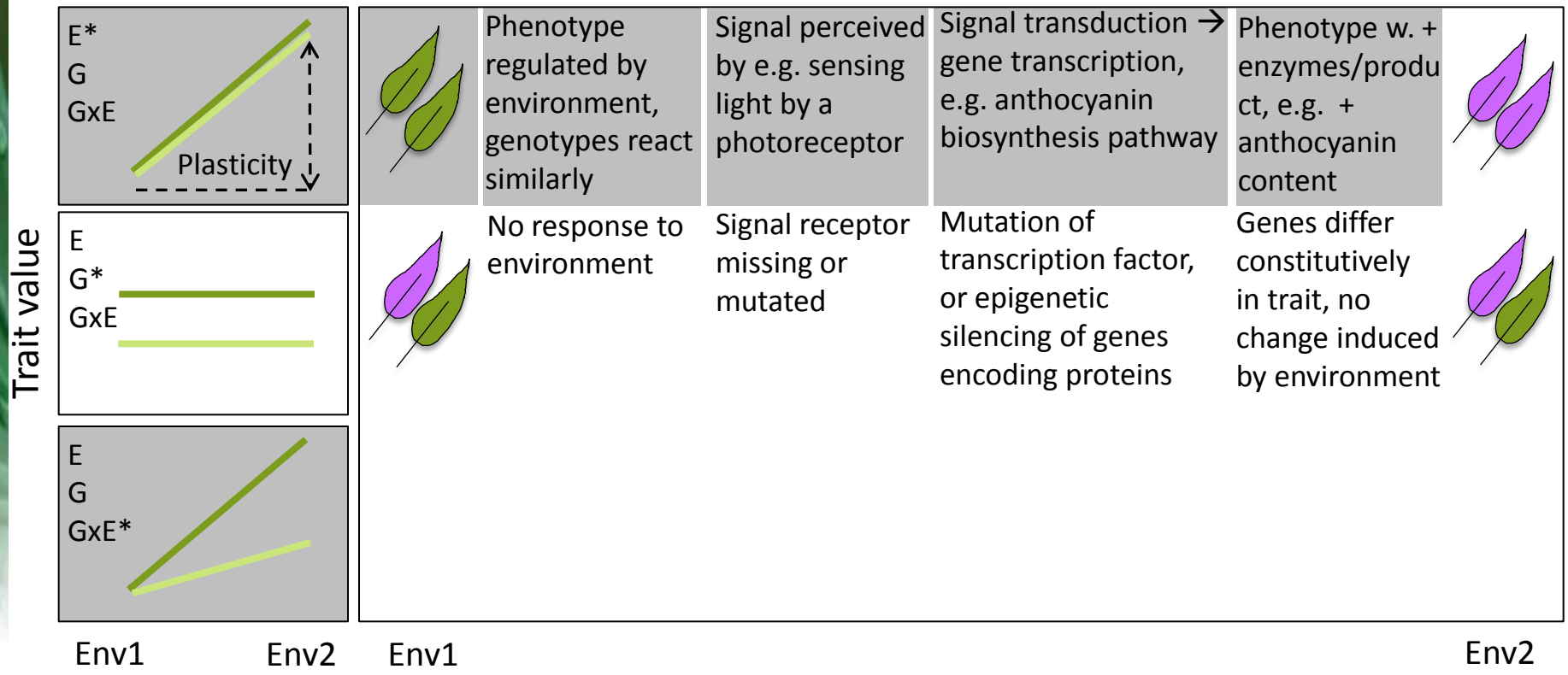

After Nicotra et al. (2010) TIPS

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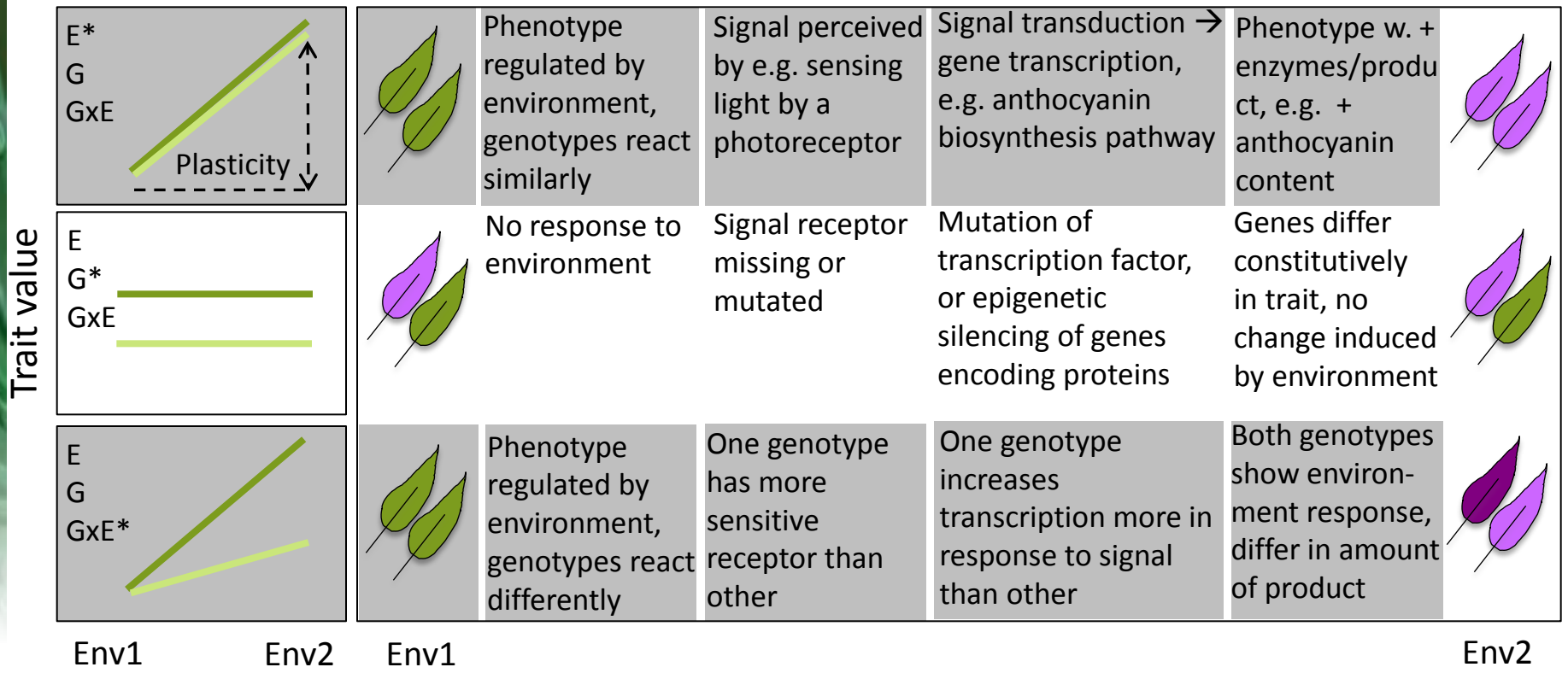
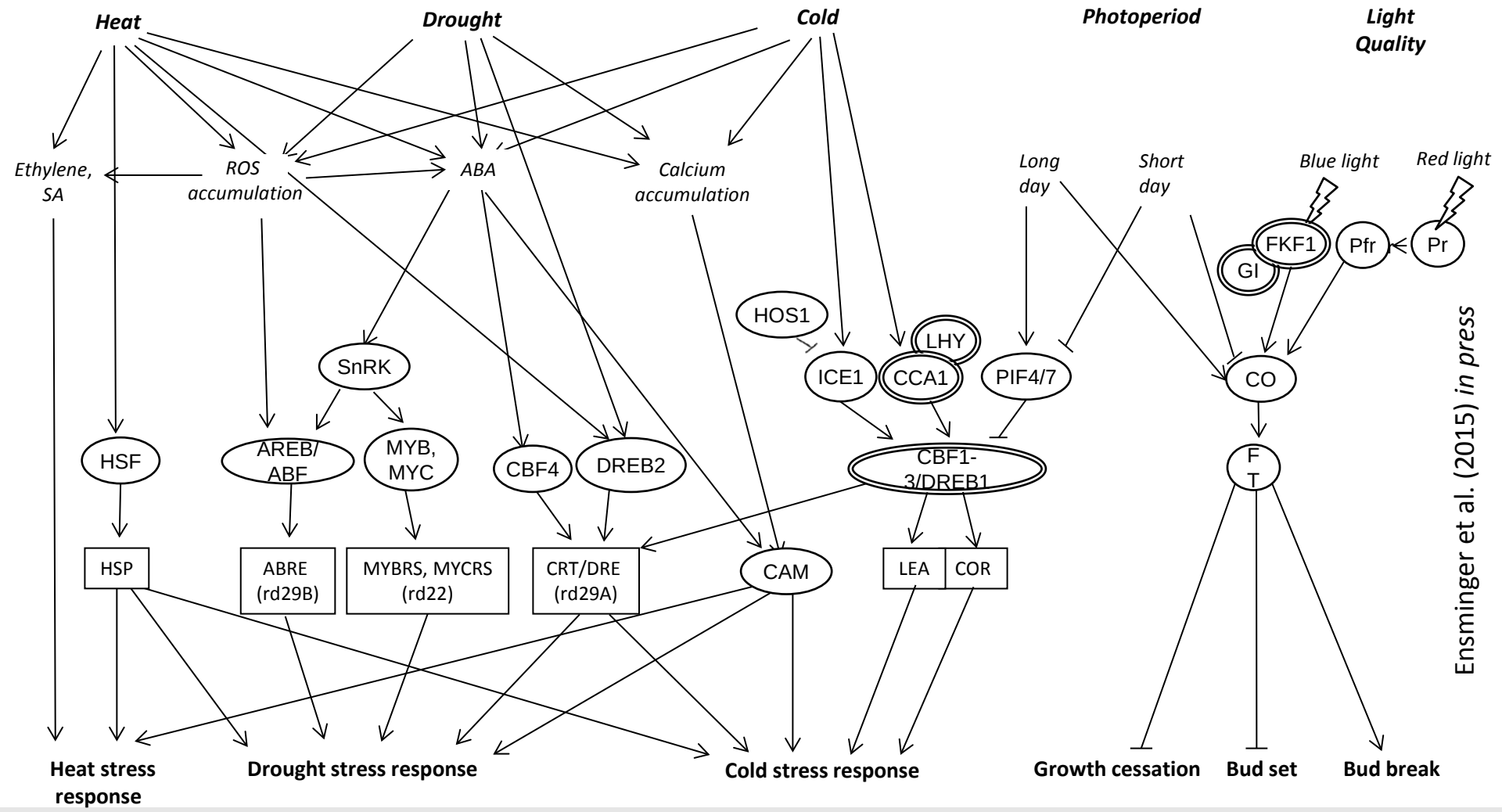


After Nicotra et al. (2010) TIPS

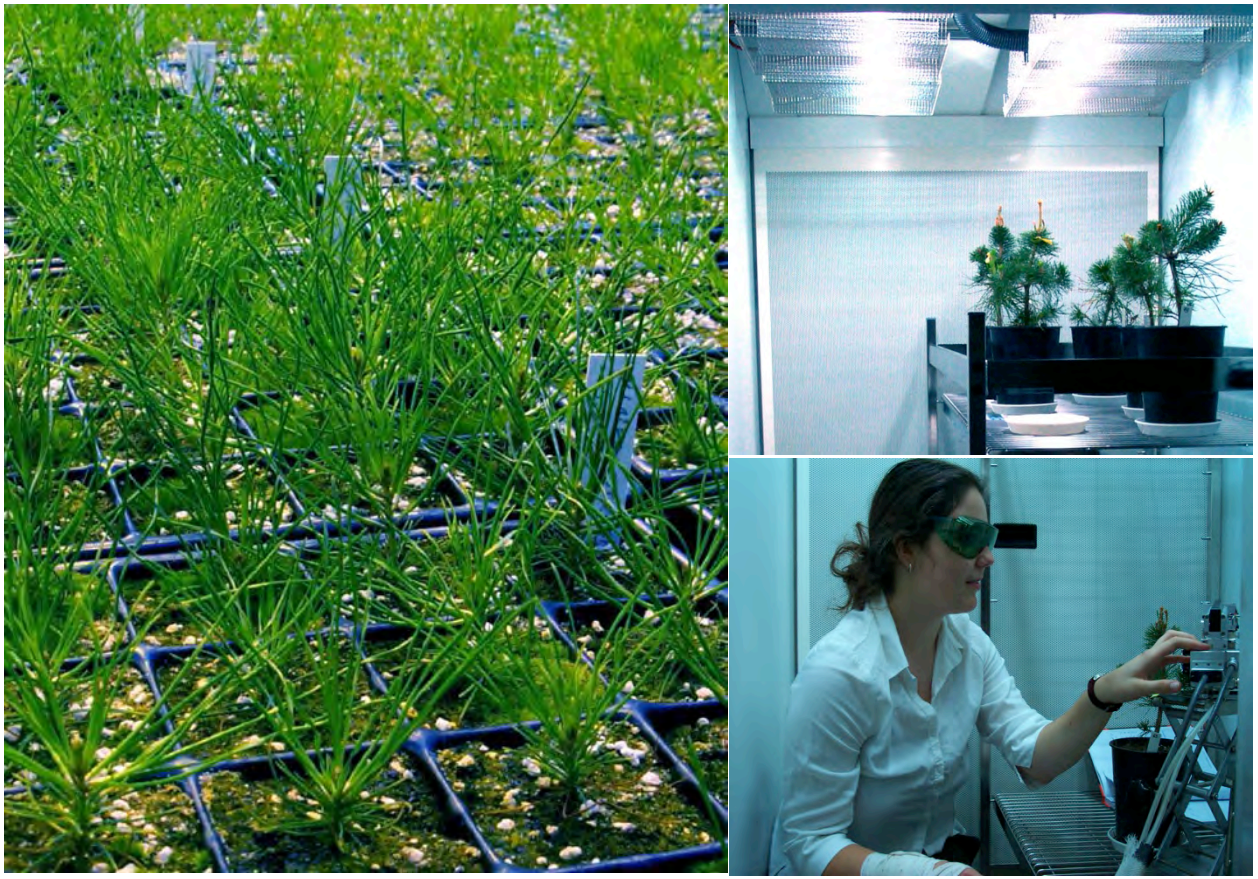
Interaction of environmental cues



Understanding plastic responses to environmental cues crucial for predicting and managing effects of climate change

Gas exchange measurements and sampling in 50 year old trees in the field

Controlled environments



Factorial experiments simulating future climate in growth chambers at UTM with white pine seedlings

Longterm monitoring sites

PRI – a leaf reflectance index for remote sensing of photosynthetic efficiency and vegetation productivity is based on xanthophyll cycle dynamics and non-photochemical quenching

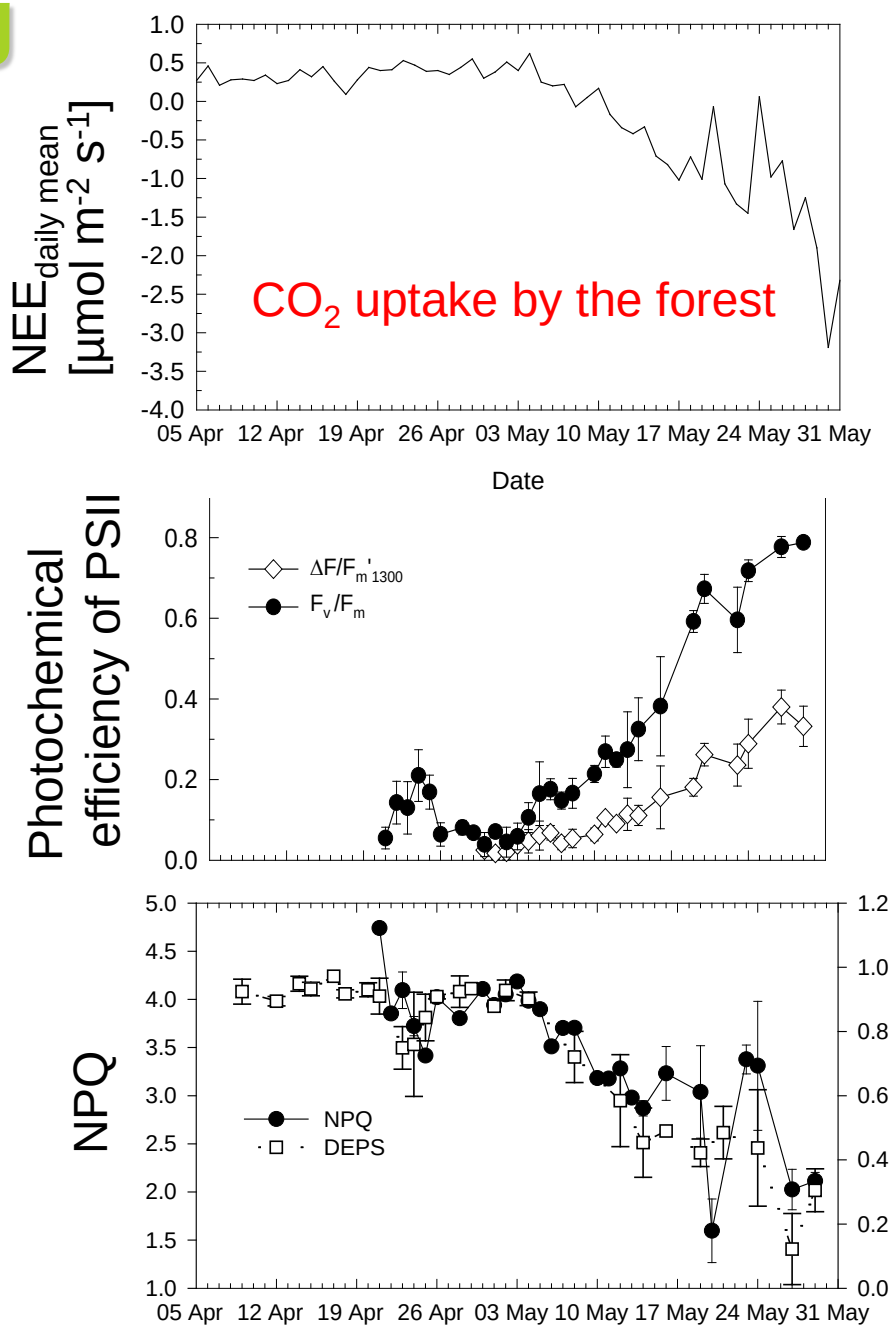
Photos: http://www.ppsystems.com/, Jon Lloyd and John Gamon

- Photochemical reflectance index (PRI)
  - Based on changes in two narrow wavebands
  - Indicates **de-epoxidation of xanthophyll cycle pigments** (531 nm) and reference band (570 nm)

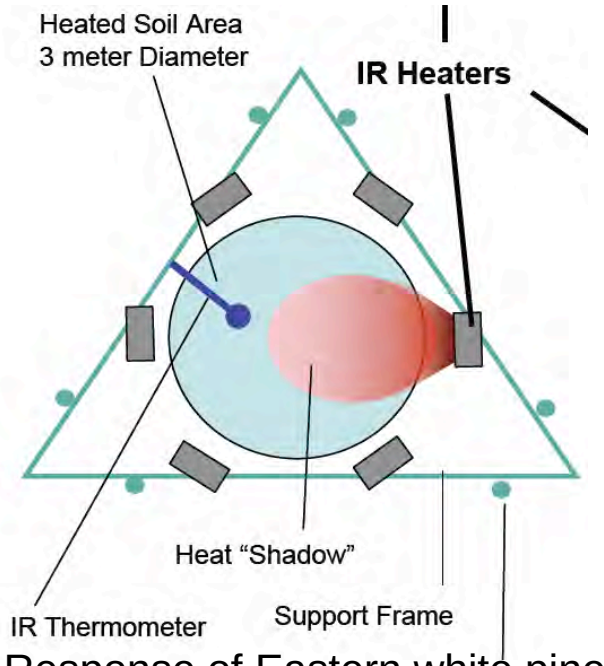
$$PRI = R_{531} – R_{570} / R_{531} + R_{570}$$

Phenology - Spring onset of photosynthesis in Scots pine

preceeded by increases in photochemical and decreases in non- photochemical processes



Response to increased air temperature

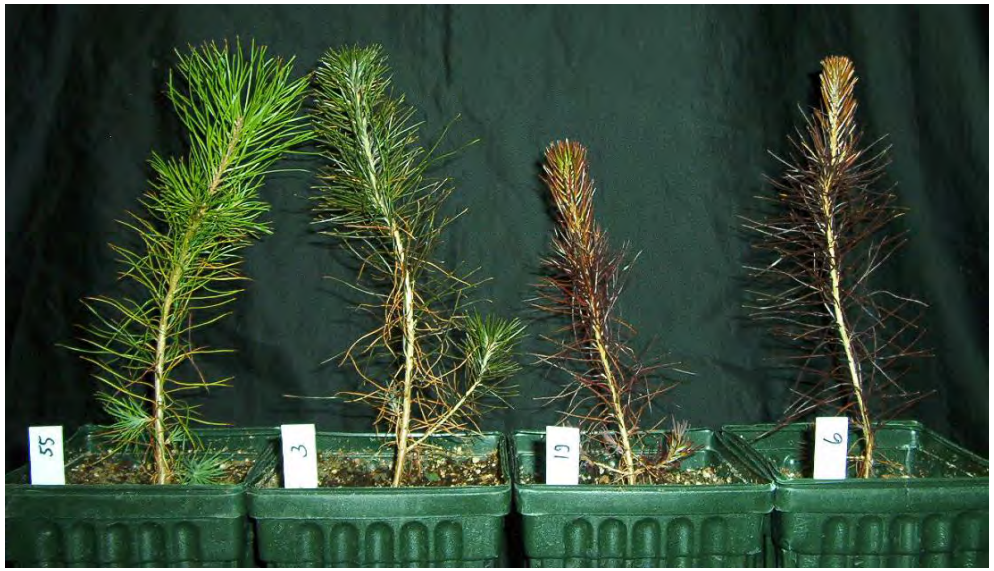


Response of Eastern white pine provenances to experimental warming, UofT's Koffler Scientific Reserve (6 heated + 6 unheated control plots)

Christine Chang

Emmanuelle Frechette

Response of gene expression to increased autumn temperature

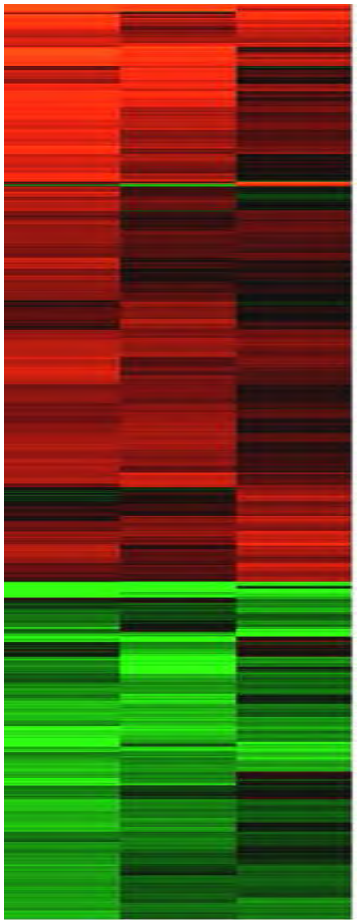


22° /18° , 22° /18° , 7° /5° , 7° /5° ,
15 h light 8 h light 15 h light 8 h light

LDHT **SDHT** **LDLT** **SDLT**

10k genes on microarray (2005)

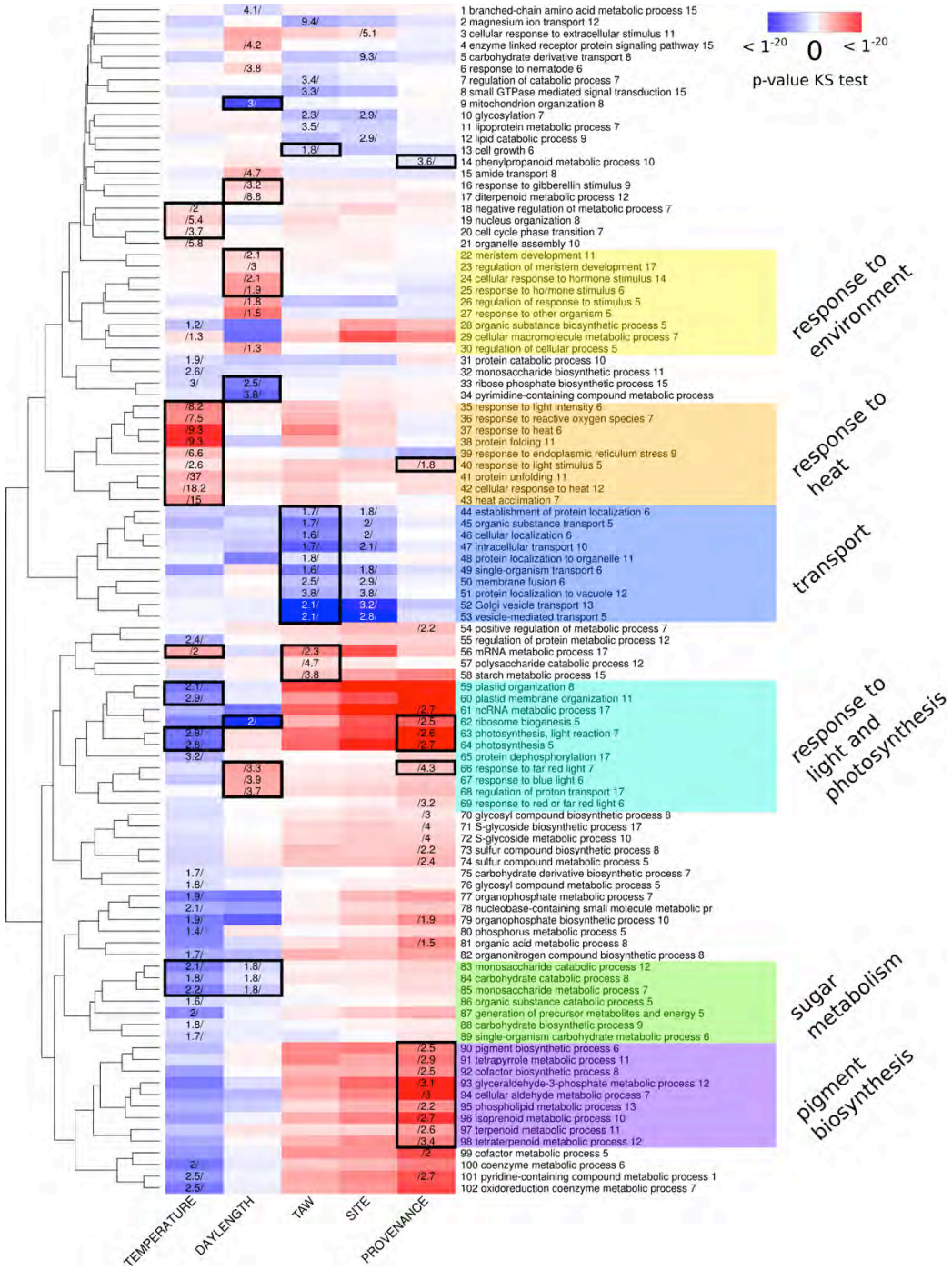
SDLT/ LDHT **LDLT/ LDHT** **SDHT/ LDHT**



989 differentially expressed genes *(p < 0.001)* in at least one contrast in response to daylength and temperature

Transcriptome responses and enrichment of GO categories revealed by Next Generation RNA Sequencing

35k genes from whole transcriptome analysis (2015)

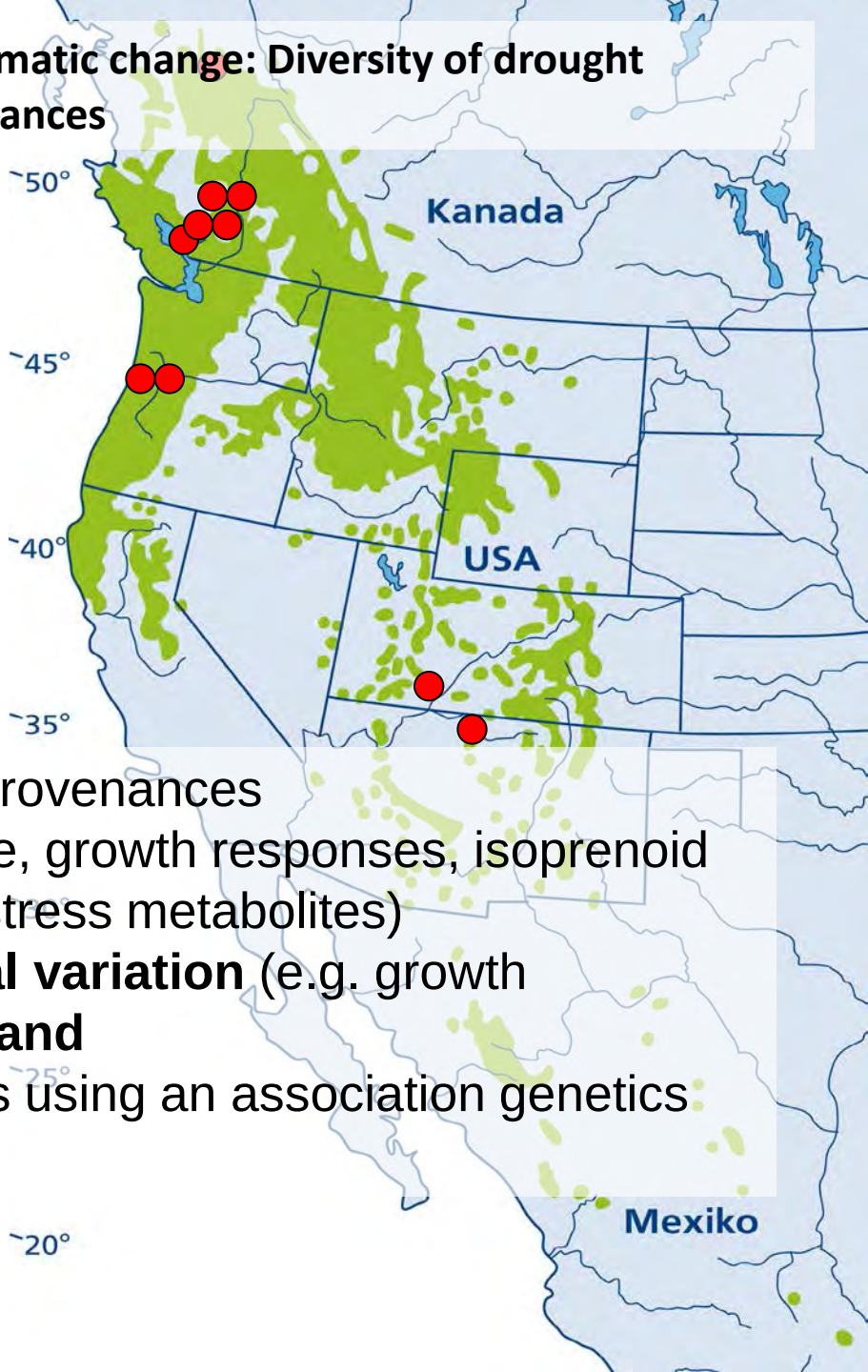


Outline

1. DougAdapt
   - *De Novo* transcriptome assembly of drought stressed Douglas-fir seedlings
   - Provenance specific physiological and transcriptome responses to a complex environment in the field
2. Eastern White Pine
   - Response to elevated temperature
   - Tissue specific *De Novo* transcriptome assembly
3. Conclusions and Perspectives

Doug AdapT

Adaptation of forest trees to climatic change: Diversity of drought responses in Douglas-fir provenances



- Identify drought responses in tree provenances (gene expression, isotopic signature, growth responses, isoprenoid metabolism, nitrogen metabolism, stress metabolites)
- **link phenotypic and physiological variation** (e.g. growth responses, isotopic discrimination) **and**
- **allelic variation** in candidate genes using an association genetics approach.

Linking phenotypic variation with allelic variation in candidate genes

Project 1

Discovery of candidate genes for drought sensitivity

Candidate genes „drought“

Project 1

Allelic variation in candidate genes

„Genotyping“

Projects 2, 3 and 4

Physiological validation of drought sensitivity on different time scales

„Phenotyping“

Project 1 with all information from Projects 2, 3, 4

Association validation of candidate genes of drought sensitivity

„Association mapping“

Project 1

Large scale field evaluation of drought sensitivity

„Screening field populations“

DougAdapt Project

Moritz Hess
Transcriptome Response to drought & elevated temperature

Laura Junker
Drought stress & photoprotective isoprenoids


Arthur Gessler & Kirstin Jansen

ZALF, Berlin (now WSL Birmensdorf, SUI)
Isotope signature


Karl Schmid & Thomas Müller, U Hohenheim
Transcriptome & SNP analysis

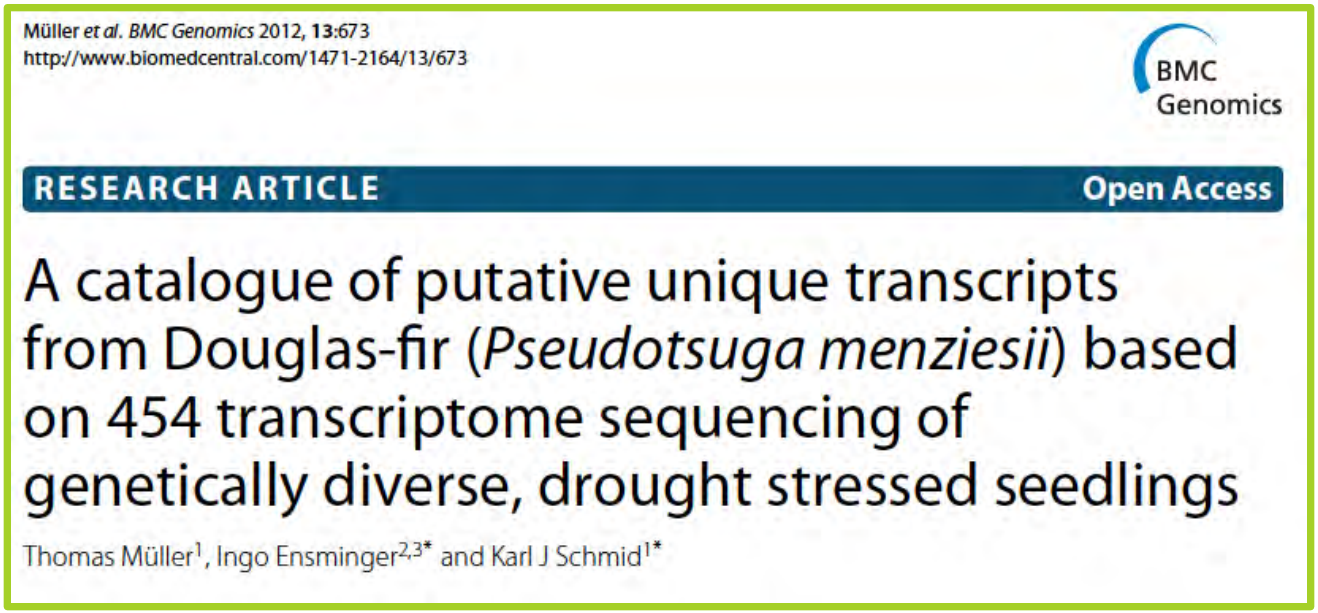

Jurgen Kreuzwieser, Anita Kleiber
U Freiburg
Volatile isoprenoids

Uli Kohnle
FVA Freiburg
Growth dynamics

Henning Wildhagen
FVA Freiburg (now U Goettingen)
Transcriptome analysis

DougAdapt

De Novo transcriptome assembly of drought stressed Douglas-fir seedlings



Müller *et al. BMC Genomics* 2012, **13**:673
http://www.biomedcentral.com/1471-2164/13/673

BMC Genomics

RESEARCH ARTICLE Open Access

A catalogue of putative unique transcripts from Douglas-fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings

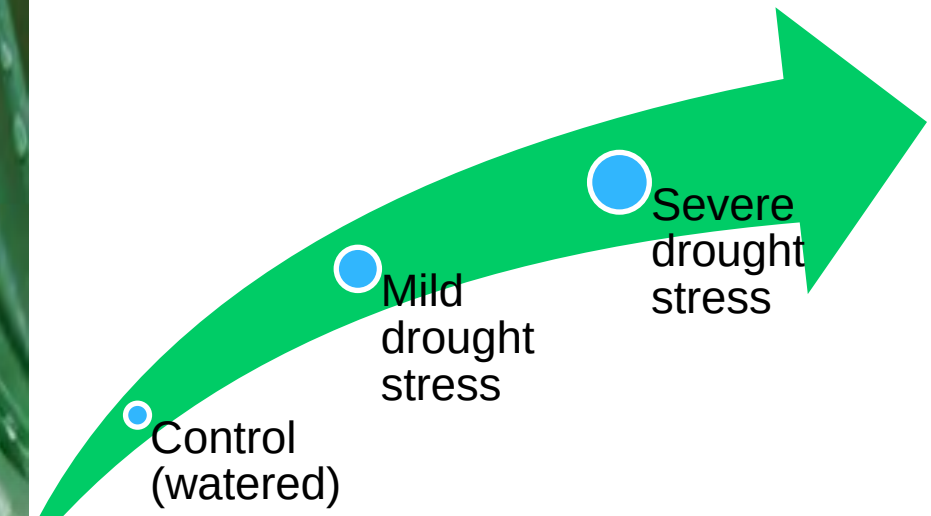
Thomas Müller[1], Ingo Ensminger[2,3*] and Karl J Schmid[1*]

Howe G, Yu J, Knaus B, Cronn R, Kolpak S, Dolan P, Lorenz W, Dean J (2013) A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation. BMC genomics 14: 137

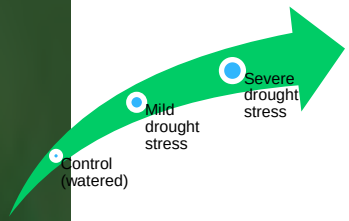
Drought stress experiments – Physiological states and genetic backgrounds used for reference library construction

Three levels of water availibility

18 provenances



De novo assembly of unigene set for Douglas-fir



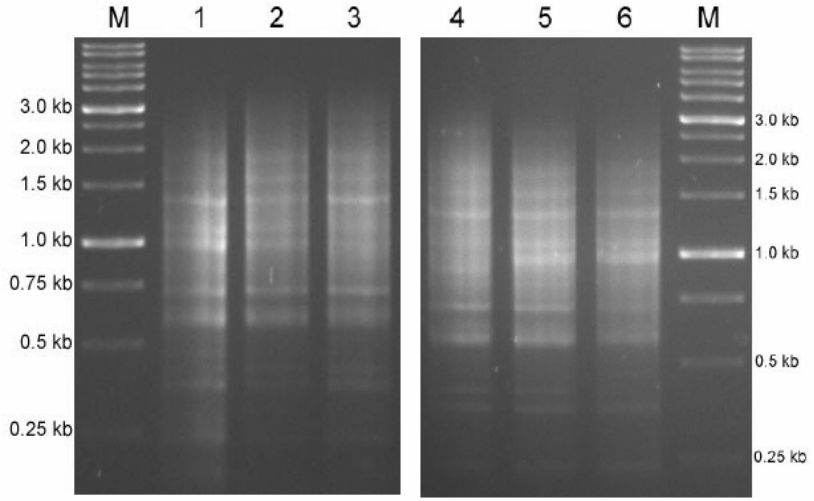
x

RNA

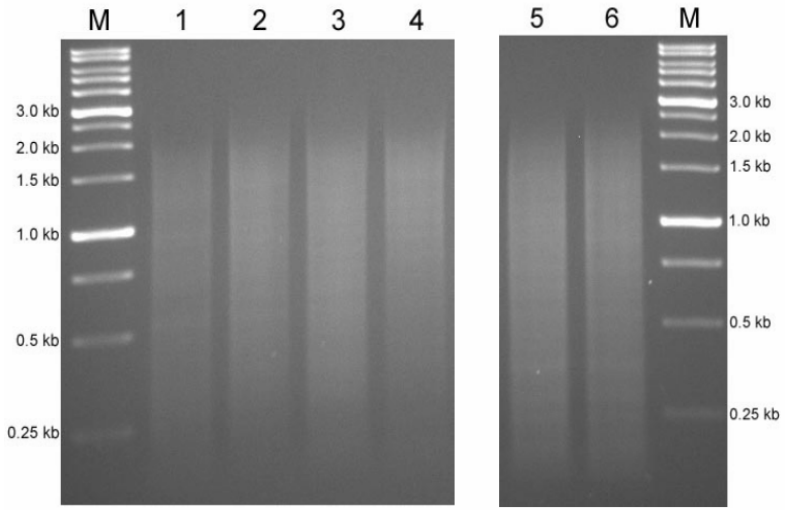
Pooling, synthesis

12 cDNA libraries

(wood, needles, coastal, interior, transitional, control, mild stress, severe stress)



amplified cDNA before normalization

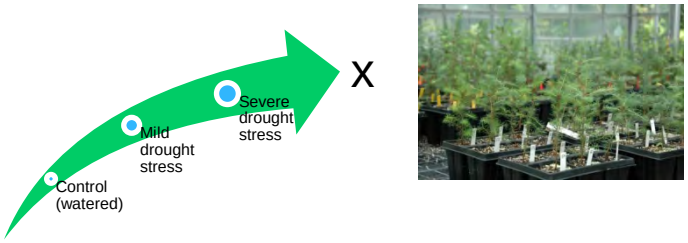


Amplified cDNA after normalization

1: int. Douglas-fir,control; 2: int. Douglas-fir, mild stress; 3: int. Douglas-fir severe, stress;

4: coast. Douglas-fir, control; 5:coast. Douglas-fir, mild stress; 6: coast. Douglas-fir, severe stress

De novo assembly of unigene set for Douglas-fir



RNA

Pooling, synthesis

cDNA libraries

454 Pyrosequencing

Sequence reads

Assembly

contigs

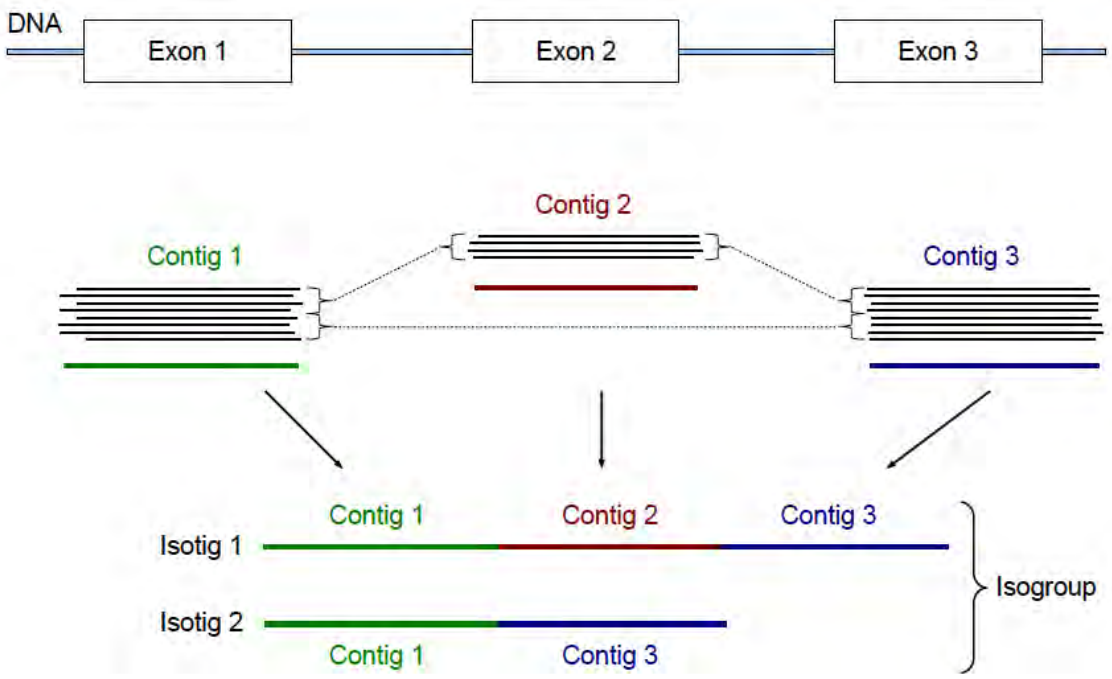
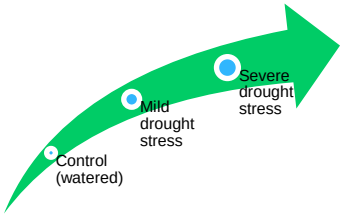


Figure 8: Schematic example of contigs, isotigs, and isogroups produced by **Newbler** (Used **Velvet** in beginning). Single reads (black lines) are assembled to contigs. The dotted lines represent subsets of reads implying connections between the contigs. The red, blue, and green line represent the consensus sequence of the contigs.

De novo assembly of unigene set for Douglas-fir



x

RNA

Pooling, synthesis

cDNA libraries

Pyrosequencing

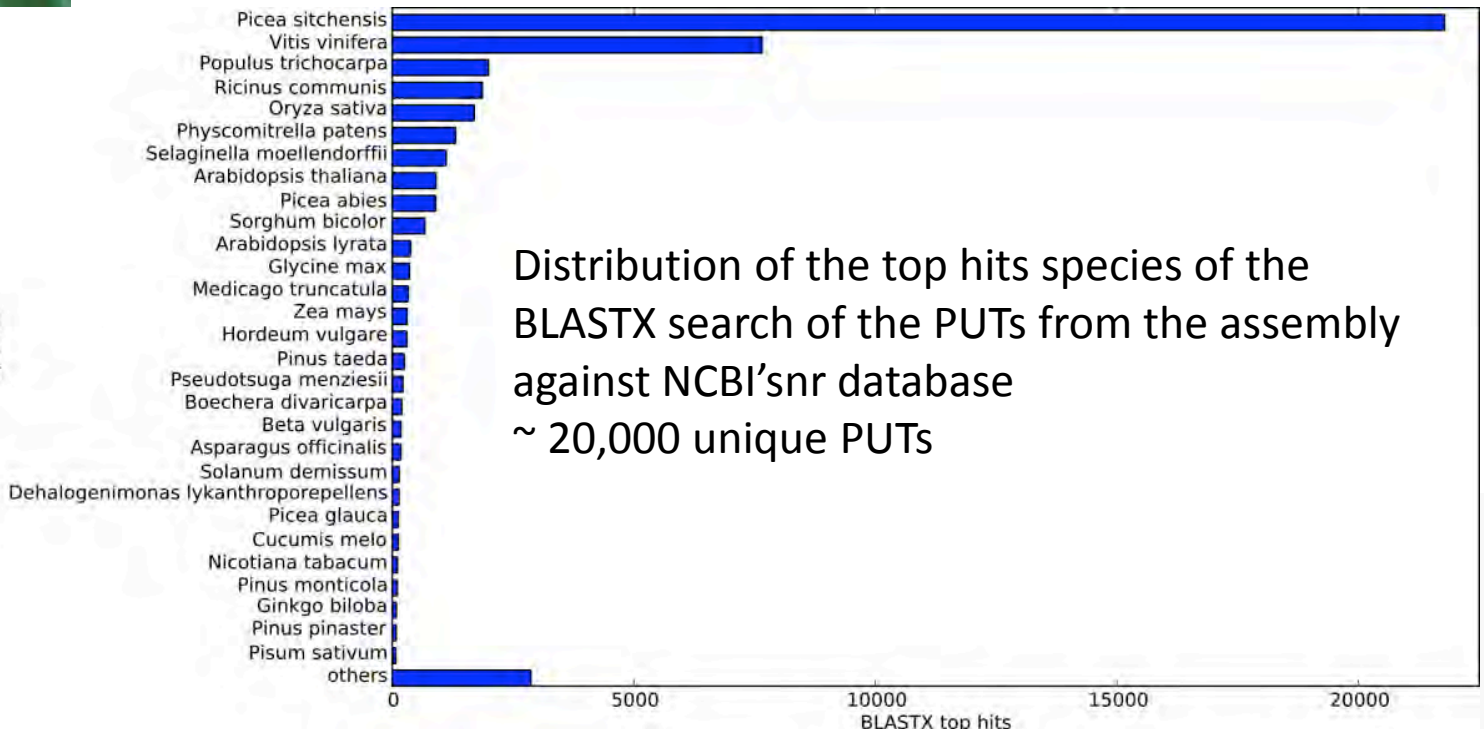
Sequence reads

Assembly

contigs

Annotation

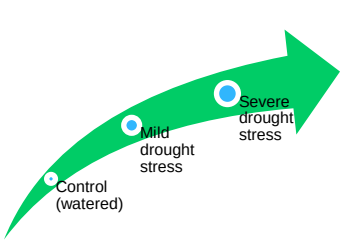
Annotated set of unigenes



Distribution of the top hits species of the BLASTX search of the PUTs from the assembly against NCBI'snr database

~ 20,000 unique PUTs

De novo assembly of unigene set for Douglas-fir



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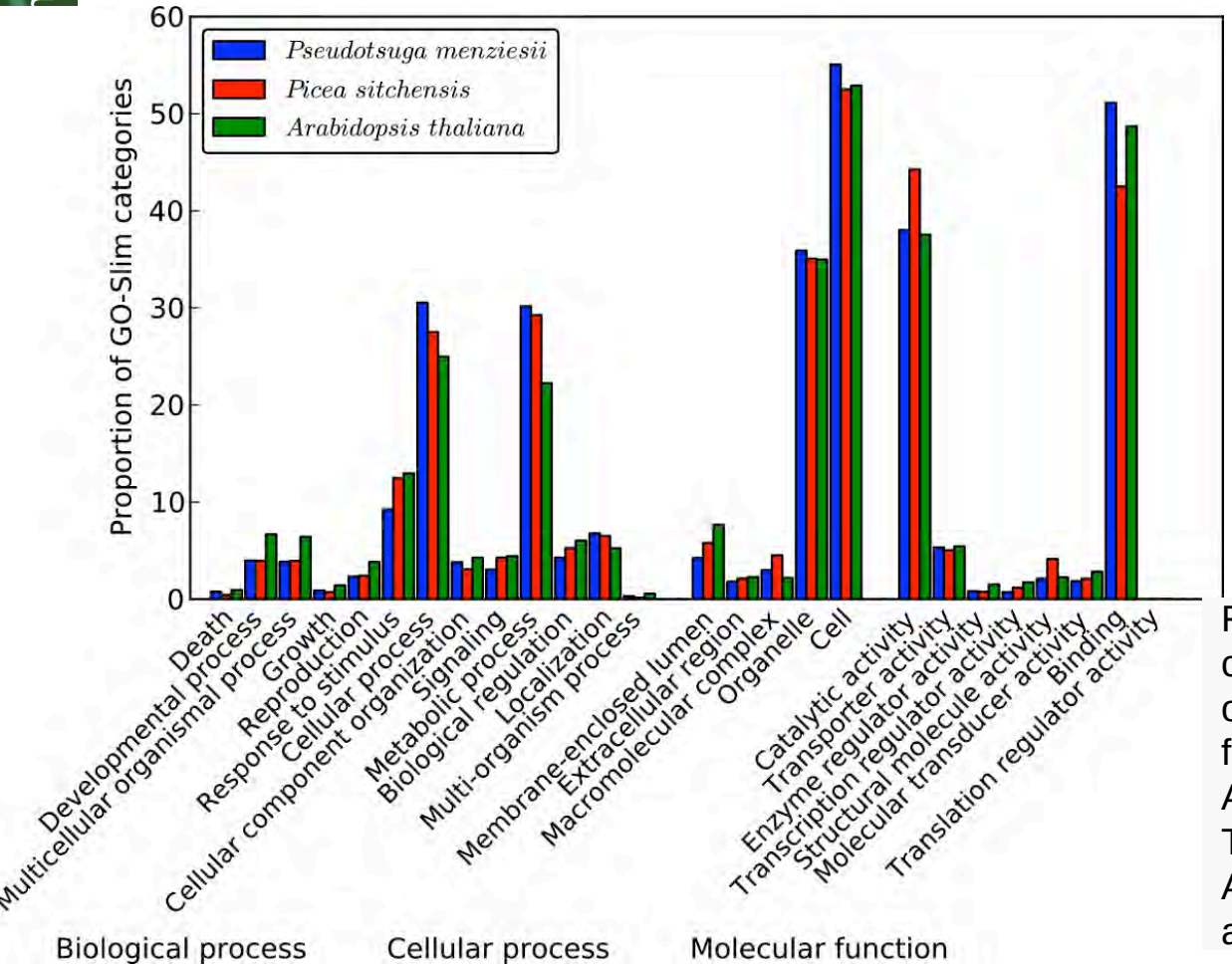
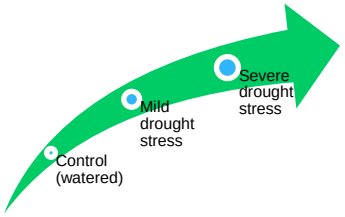


Functional annotation to **GO-Slim** categories. Comparison of the distribution of the GO-Slim categories of the Douglas-fir PUT set versus Picea sitchensis and Arabidopsis thaliana at GO level 2. Transcriptome data of P. sitchensis and A. thaliana were obtained from NCBI and TAIR databases, respectively

De novo assembly of unigene set for Douglas-fir



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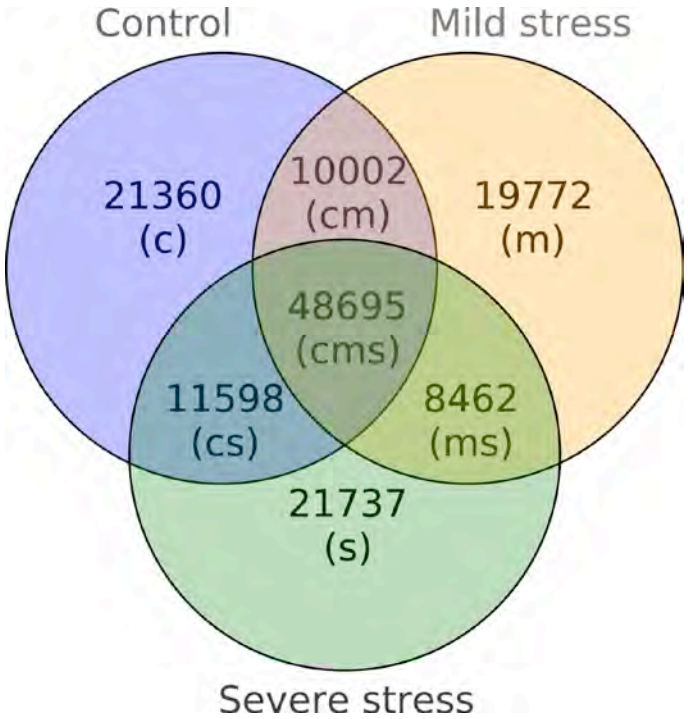
Identification of treatment specific PUTs



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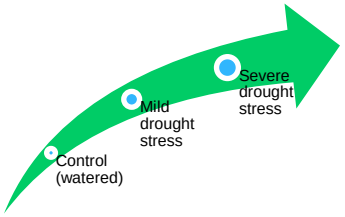

x

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Pooling, synthesis

cDNA libraries

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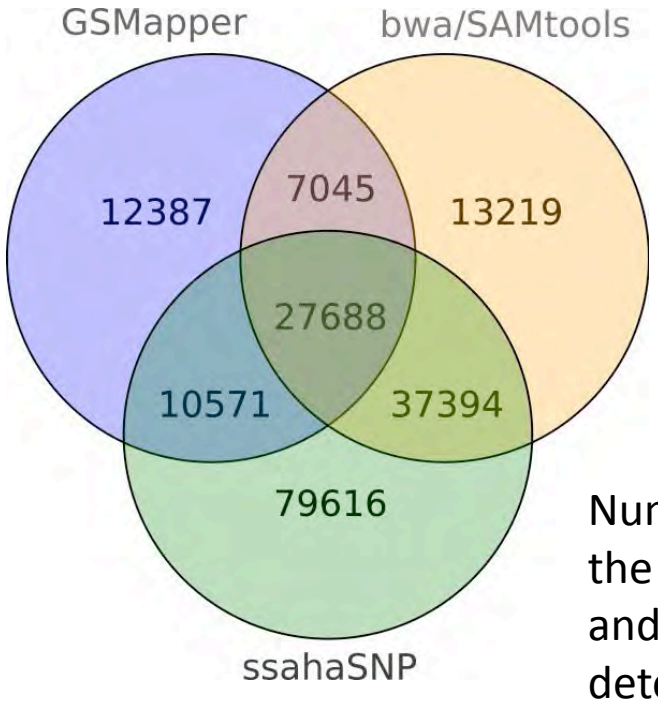
SNP identification



Number of SNPs. Number of SNPs identified by the SNP detection tools GSMapper, ssahaSNP, and bwa/SAMtools. 27,688 SNPs were detected by all three tools and are considered to be the most reliable SNPs

Case study De Novo transcriptome assembly

SNP chip development for Coastal Douglas-fir

Keith JS Jayawickrama[a], Glenn Howe[a], Stephanie Guida[b] and Callum J. Bell[b]
[a] Oregon State University, Corvallis, OR, [b] National Center for Genome Resources, Santa Fe, NM

Introduction

We are using a breeding approach called genomic selection to improve Douglas-fir growth and bioenergy traits. Genomic selection, or whole-genome marker-assisted selection (Meuwissen et al. 2001), could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. Genomic selection is a type of marker-assisted selection that uses dense marker coverage to track alleles for most or all quantitative trait loci (QTL) in the genome (Meuwissen et al. 2001). If very large numbers of markers are used, most or all QTL will be in linkage disequilibrium with at least one marker, particularly in small populations. Genomic selection involves two steps (Hayes and Goddard 2010). First, a genomic prediction model is developed using phenotypes and marker genotypes measured on a test or 'training' population. Second, superior individuals are selected from a related breeding population based on marker genotypes alone. We will use **single nucleotide polymorphic** (SNP) genetic markers to pursue genomic selection in Douglas-fir. These markers will be assayed in breeding populations using an Illumina Infinium genotyping array.

Goals

(1) Build a next-generation chip for coastal Douglas-fir with ≥ 15,000 SNP markers

(2) Identify SNP genetic markers in Douglas-fir associated with useful variations in biofuel production potential. These would be traits (height, diameter) controlling the volume of wood produced and wood chemistry traits (total carbohydrates, lignin extractives, recalcitrance to pretreatment); and

(3) Select for increased biofuel production in woody residuals of trees developed for use as saw logs using a combination of phenotypic and SNP genetic marker data.

Genetic Markers

Howe et al. (2013) identified **278,979** potential SNPs in Douglas-fir, and based on a SNP validation experiment, estimated they had as many as ~69,000 SNPs that could be genotyped at ~20,000 gene loci using an Infinium II array. Because Douglas-fir may contain 30,000 to 40,000 genes, we decided to augment our SNP database with other Douglas-fir SNPs identified by Müller et al. (2012). They identified 170,859 putative unique transcripts (PUTs) and 187,653 SNPs, 27,688 of which were identified by all three SNP detection methods they used.

We used SNPs identified by Muller et al. (2012) to augment our existing SNP resource (Howe et al. 2013). The objective of this work was to increase the number of genes that could be assayed, thereby increasing genome coverage for genomic selection. The Douglas-fir transcriptome (454 sequence data) and SNPs identified by Muller et al. (2012) were downloaded from http://www.treeversity.org. These data contain ~170,000 putative transcripts and ~188,000 SNPs.

SNP bioinformatic analyses

Objective – increase the number of genes that can be assayed, thereby increasing genome coverage for genomic selection

Downloaded transcriptome from Muller et al (2012)

↓

Conducted a BLAST analysis against Howe et al. (2013) dataset and identified 92K new transcripts found @ BLAST e-value = 10^{-10}

↓

Analyzed 42K new SNPs using the Illumina Assay Design Tool

↓

22K SNPs with successful design scores were added to our SNP resource, increasing the total to 301,379 SNPs which will form the foundation for constructing a SNP genotyping array

↓

Ranked all SNPs for inclusion on the genotyping array (final umber of SNPs genotyped depends on array costs)

Tree Breeding Plan

We are using simulation studies to optimize genomic selection given the available resources. These simulations will be used to answer the following questions: What is the best sampling strategy within each breeding program? What are the expected results for various numbers of SNP markers and genotyped trees? How might genomic selection be combined with early phenotypic selection? Should NWTIC breeding programs be altered to increase the performance of genomic selection? The resulting answers will form the basis of our tree breeding plan.

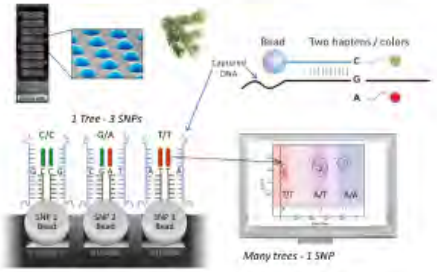
NARA Year 3-5 plans: Genotyping has already been committed to for the following populations (1) Families used in the phenomics study (75 families), (2) families from the populations assayed in year 1-2 (90), and (3) families for which biomass recalcitrance is to be studied in year 3 (50) making a total of 215 families. We will use the phenotypic data (growth rate, drought hardiness, wood chemistry) to select appropriate trees for genotyping.

Genomic selection simulations. Our simulation population was designed to mimic a typical NWTIC first-generation progeny test, plus their advanced generation progeny. We started with 300 parent trees plus their open-pollinated progeny (100 progeny per family), and then built multiple generations with 30 forward selections and 30 full-sib families each which can be repeated indefinitely. The next step was to develop the software needed to simulated realistic breeding populations, including phenotypes and SNP genotypes. We first evaluated a program called QMSIM (Sargolzaei and Schenkel 2009), but after recognizing several limitations for application in our situation, we modified an existing program instead.

This in-house program (Tree Genome Simulator) can simulate the types of mixed-matings that occur in natural populations of Douglas-fir. We added a module to the program that simulates a recurrent double-pair mating design, and modified its functions and the output to allow for the types of genomic selection analyses described below. We are now evaluating the output to confirm that it produces simulated data that accurately mimics data expected from Douglas-fir, such as expected patterns of linkage disequilibrium (LD).

Infinium genotyping array

(Individual figures are from Illumina publications)



Building the chip and subsequent genotyping

Preliminary information from Illumina suggests that we should be able genotype ~15,000 SNPs for about $100 per tree. This estimate is for projects of about 2,000 trees or less. If we genotype more trees the per-sample cost would be less. Therefore, we have contacted other Douglas-fir breeders and researchers to see if they are interested in collaborating in a genotyping consortium. Illumina's Consortia Management Service helps bring different research groups and breeders together to achieve economies of scale. Ultimately, this may allow us to genotype more trees with more SNPs than we would be able to achieve alone. Once we know the final number of SNPs and trees we will genotype, we will negotiate with Illumina on the final price for the genotyping. We intend to begin genotyping by March 1 2014 and complete genotyping by the end of NARA Year 3.

References

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NARA
Northwest Advanced Renewables Alliance

NARA is led by Washington State University and supported by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture.

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   - Provenance specific physiological and transcriptome responses to a complex environment in the field
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   - Response to elevated temperature
   - Tissue specific *De Novo* transcriptome assembly
3. Conclusions and Perspectives

Two contrasting field sites in Southern Germany

Physiology: 4 provenances
sampling in May and Juli 2010 & 2011

Transcriptome: 2 Provenances
sampling in May, June, July, September 2010

50 year old trees in international Douglas-fir provenance trial

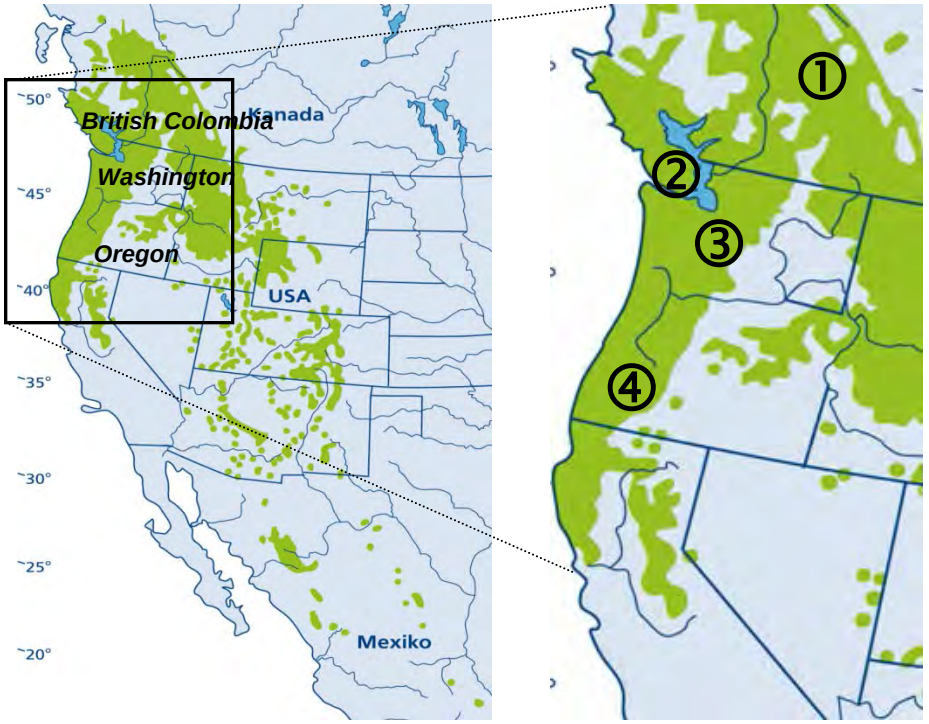
From Muller et al. (2014)

	Dry	Humid
	Wiesloch	**Schluchsee**
Elevation a.s.l.	105 m	1050 m
Precipation (annual sum)	660 mm	1345 mm
Mean annual temperature	9.9° C	6.1° C

Origin of provenances

Physiology: 4 provenances
sampling in May and Juli 2010 & 2011

Transcriptome: 2 Provenances (1+2)
sampling in May, June, July, September

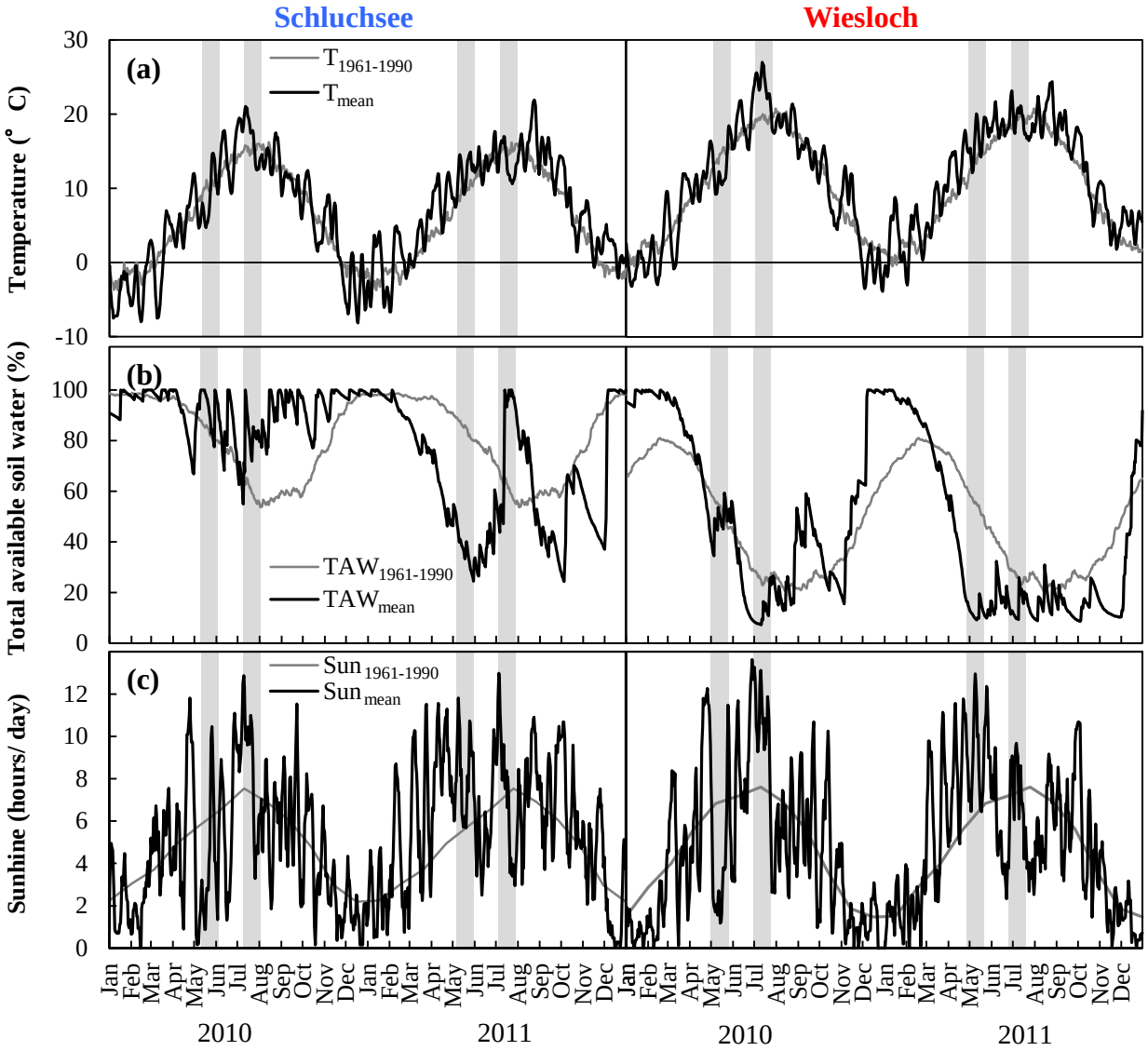


(Map: Aas 2008, modified)

	Physio-geographical area	Elevation a.s.l.	Mean annual precipitation sum	Mean annual temp.
Salmon Arm ①	Southern interior landside	580 m	500 mm	7,8 °C
Cameron Lake ②	Vancouver Island (East coast)	210 m	1475 mm	10,0 °C
Conrad Creek ③	North Cascades	280 m	2300 mm	9,5 °C
Santiam River ④	West Cascades	800 m	1780 mm	9,5 °C

Gas exchange measurements and tissue sampling in the canopy of 50 year old trees of a common garden experiment

Temperature, Soil Water Availability and Sunshine Duration at the two field sites



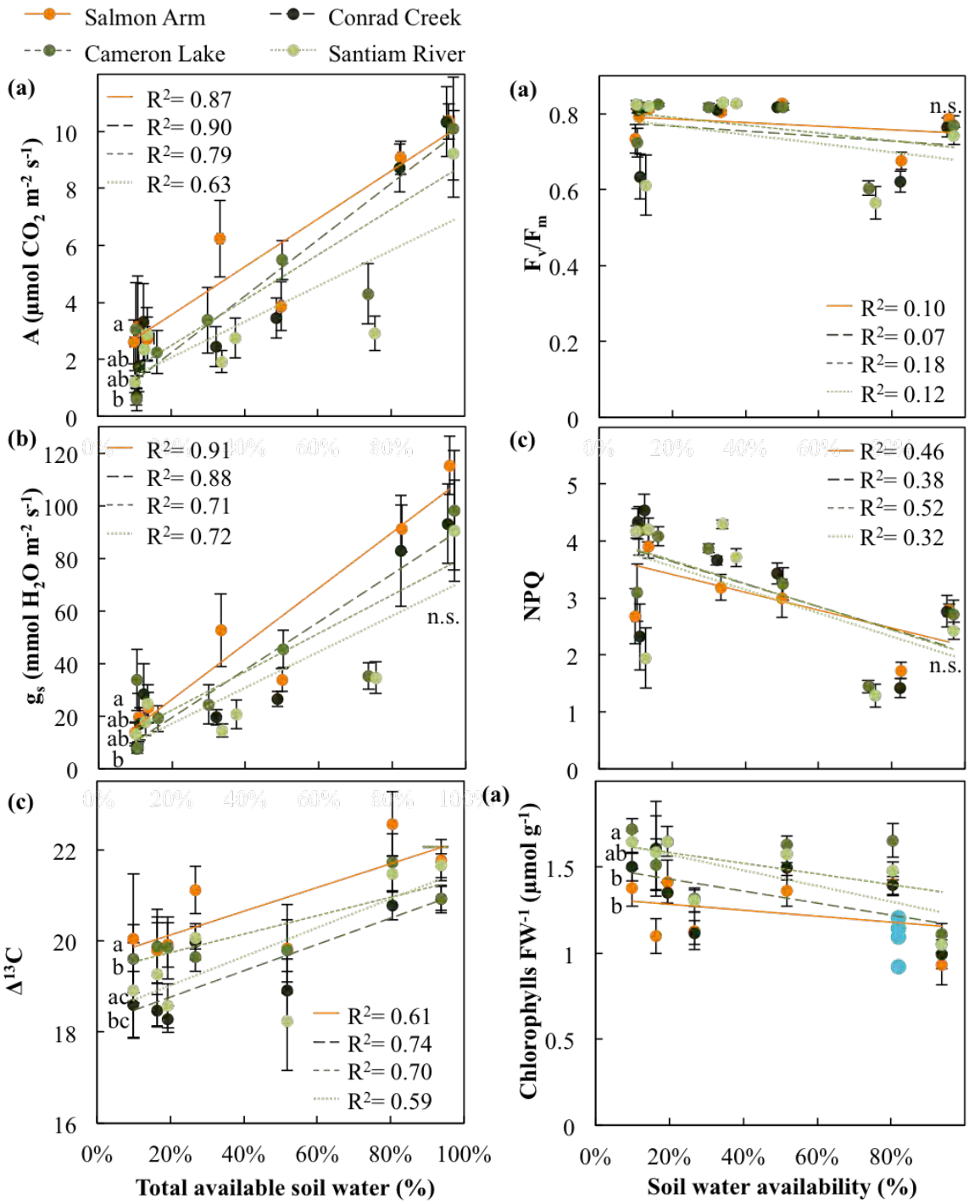
Effect of provenance, site and climatic parameters on physiology

	ANOVA			Spearman-Correlation		
	Provenance	Site	Prov:Site	TAW	Temperature	Sun
R	0.54	0.09	0.19	0.05	-0.10	-0.14
A	**0.02**	**0.00**	**0.04**	**0.67**	**-0.42**	**-0.55**
g_s	**0.04**	**0.00**	**0.00**	**0.68**	**-0.37**	**-0.54**
IWUE	0.07	0.49	0.07	0.08	-0.17	**-0.15**
$\Delta^{13}C$	**0.00**	**0.00**	**0.01**	**0.56**	**-0.33**	**-0.24**
F_v/F_m	0.13	**0.00**	0.63	**-0.26**	**-0.17**	-0.03
Φ_{PSII}	0.15	0.69	**0.02**	0.01	**-0.25**	**-0.23**
NPQ	0.73	**0.00**	0.96	**-0.56**	0.13	**0.29**
Chl a+b	**0.00**	**0.00**	0.40	**-0.35**	0.08	-0.12
Carotenoid	**0.00**	**0.00**	0.10	**0.35**	-0.20	-0.15
VAZ	**0.00**	0.24	0.93	-0.10	**0.29**	**0.29**
DEPS	0.19	0.27	0.32	**-0.09**	**0.36**	**0.58**
VOC store	**0.00**	**0.04**	**0.00**	0.06	-0.07	-0.02
VOC emitt	0.31	0.07	0.27	0.00	-0.06	**0.17**

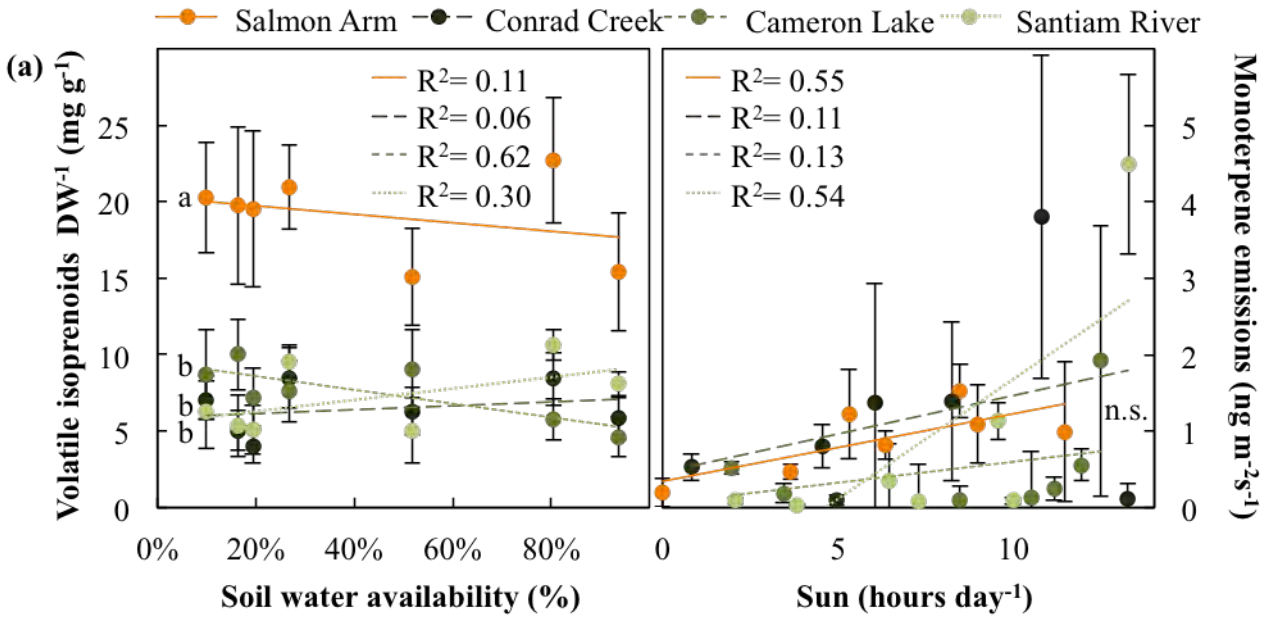
Provenance effects assimilation, isotopic discrimination, pigments, stored monoterpene pools

No provenance effect on fluorescence and VOC emissions

Provenance effects on assimilation, stomatal conductance, water use efficiency and chlorophyll content of 50 year old Douglas-fir



Provenance effects on assimilation, stomatal conductance, water use efficiency and chlorophyll content of 50 year old Douglas-fir



Outline

1. DougAdapt
   - *De Novo* transcriptome assembly of drought stressed Douglas-fir seedlings
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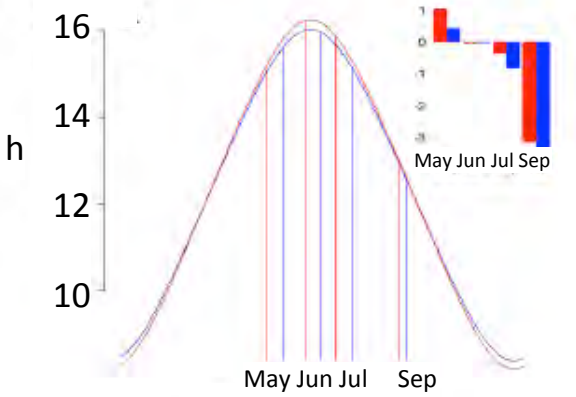
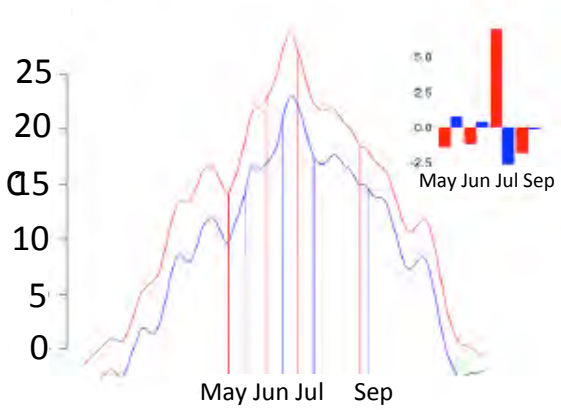
Gene expression (2 provenances, 2 field sites, 4 sampling points) in a complex environment with variation in environmental parameters

Salmon Arms (interior)
Cameron Lake (coastal)

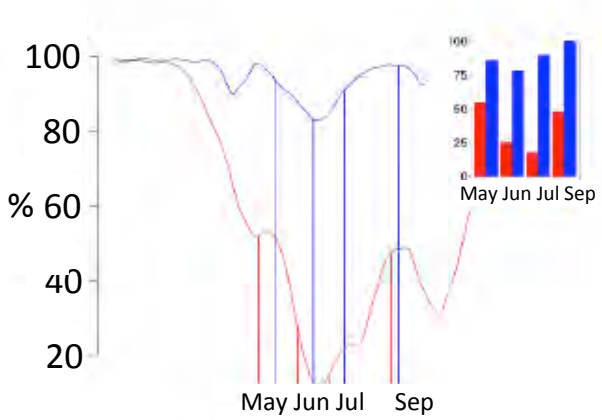
Field site Schluchsee (humid)
Field site Wiesloch (dry)



Daylength

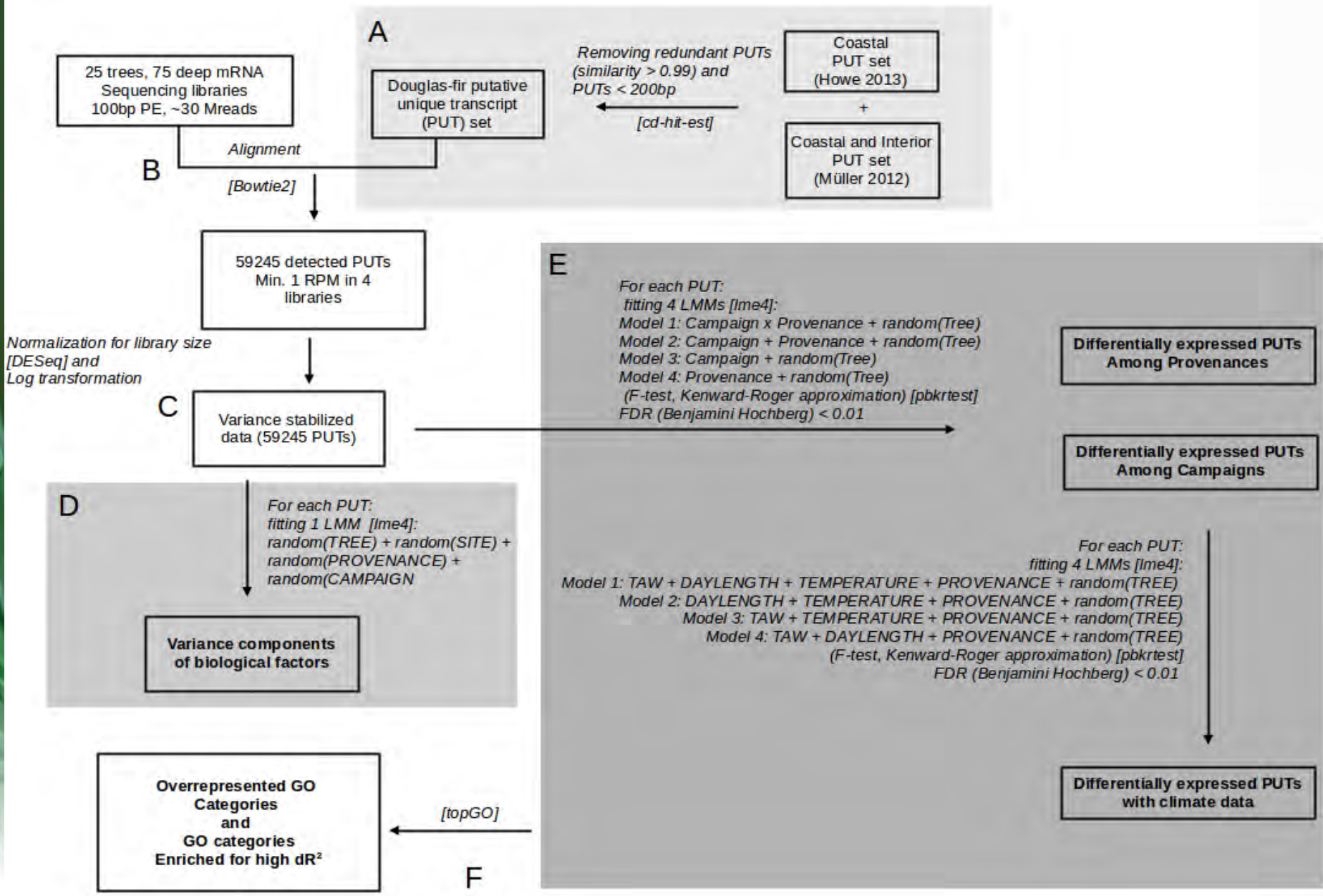


Temperature



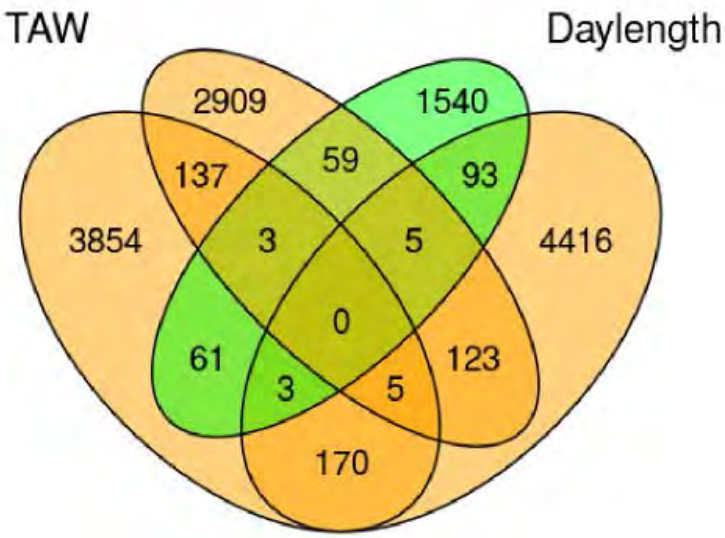
Water availability

Pipeline Transcriptome Analysis



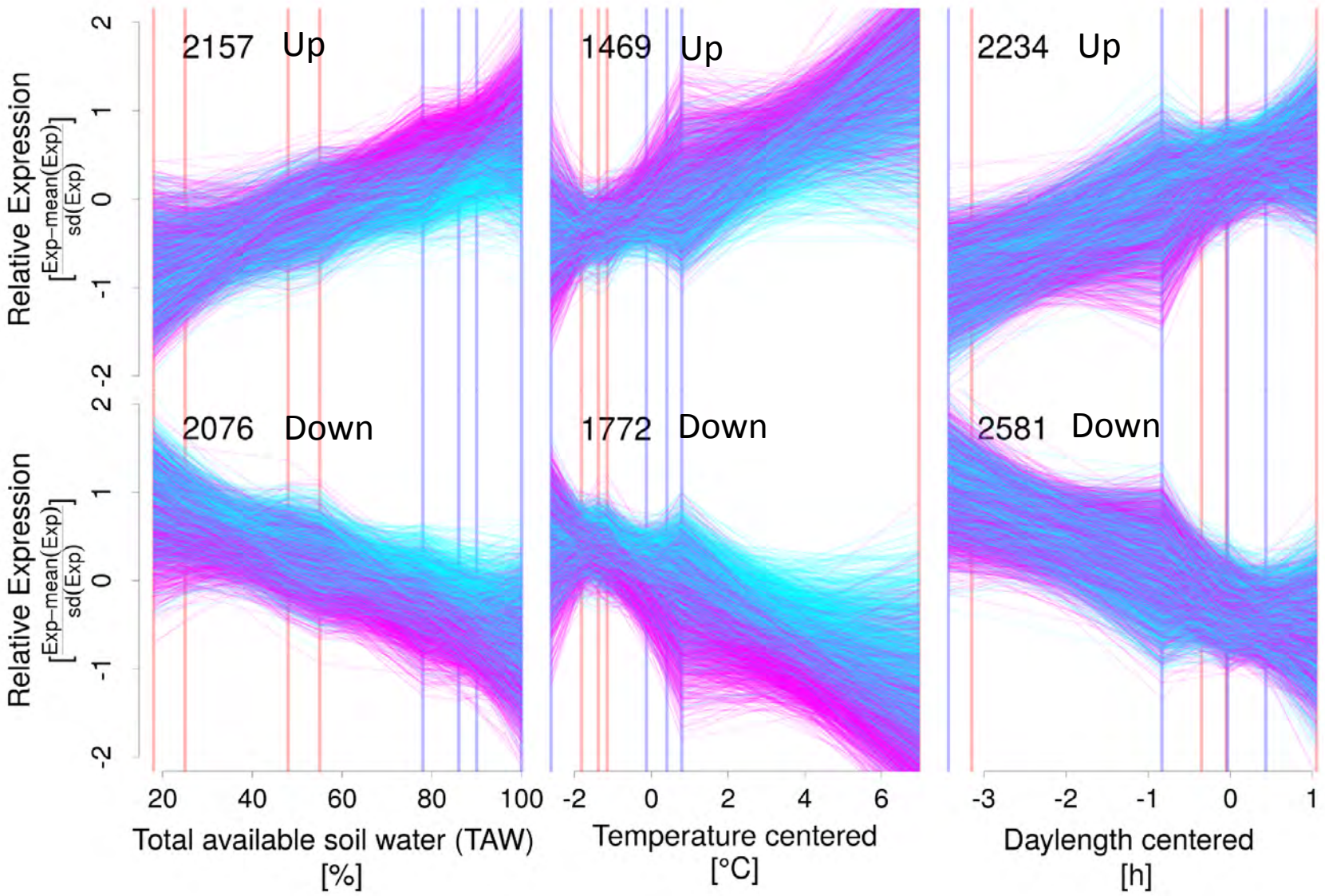
Differentially expressed genes (F-Test p<0.01)

Temperature Provenance



Use of environmental parameters (as proxies for campaign) and provenance as predictors for expression of PUTs

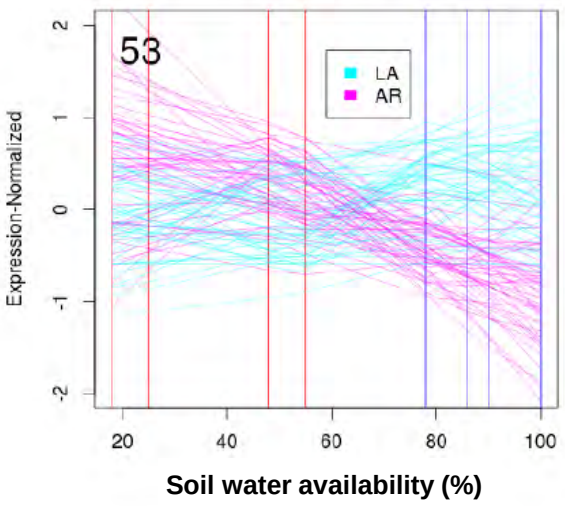
Effect of E on PUT normalized expression on Salmon Arm (Interior) or Cameron Lake (Coastal)



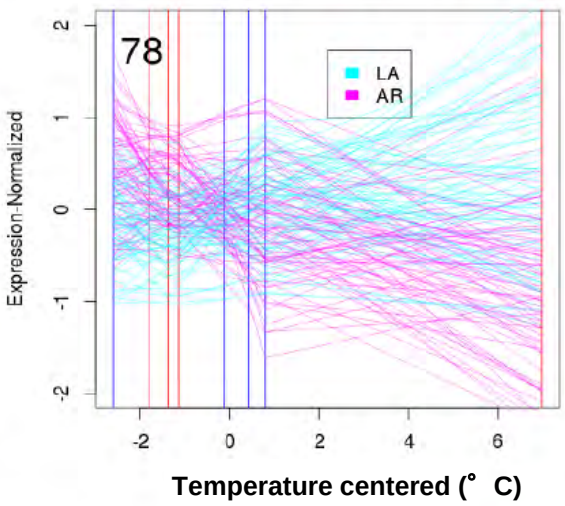
Hess et al. (in preparation)

Effect of G x E interaction on normalized PUT expression

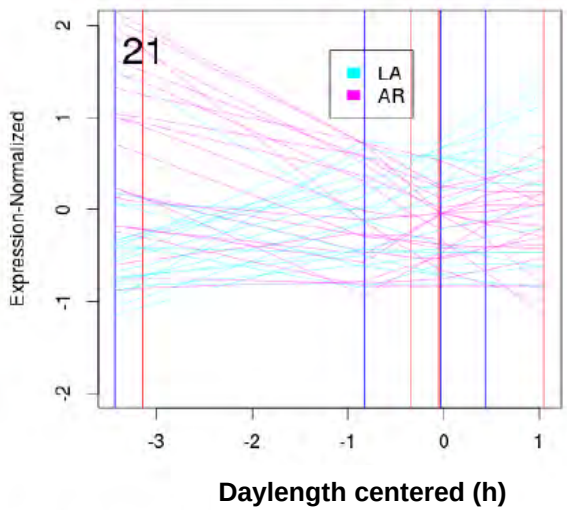
SWA x Provenance up dAIC < -10, dR2 > 0.1



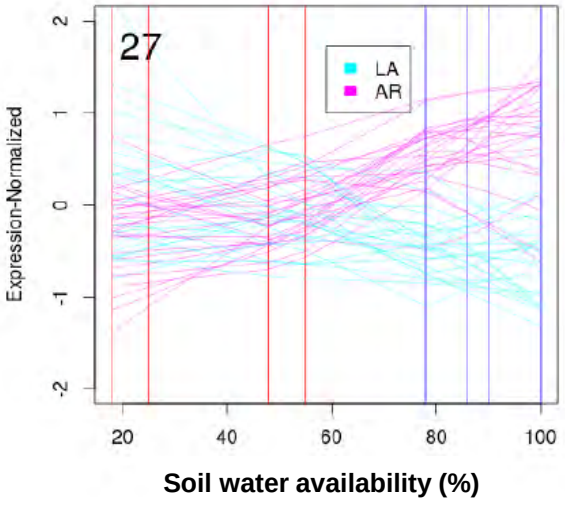
Temp. x Proven. up dAIC < -10, dR2 > 0.1



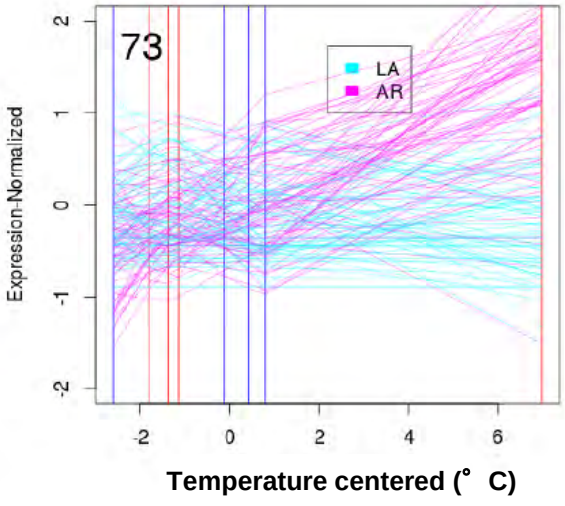
Day. x Proven. up dAIC < -10, dR2 > 0.1



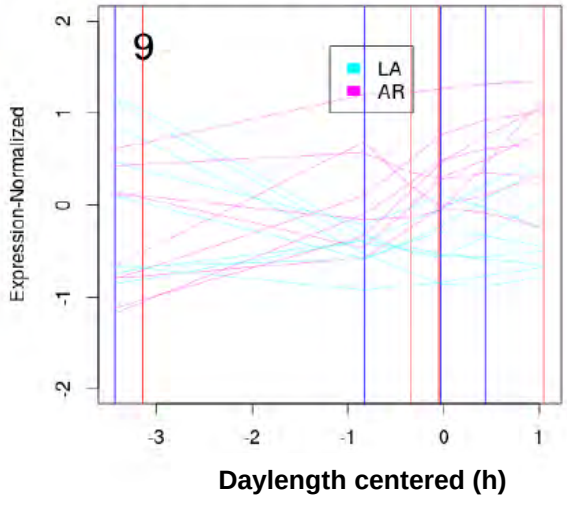
SWA x Proven. down dAIC < -10, dR2 > 0.1



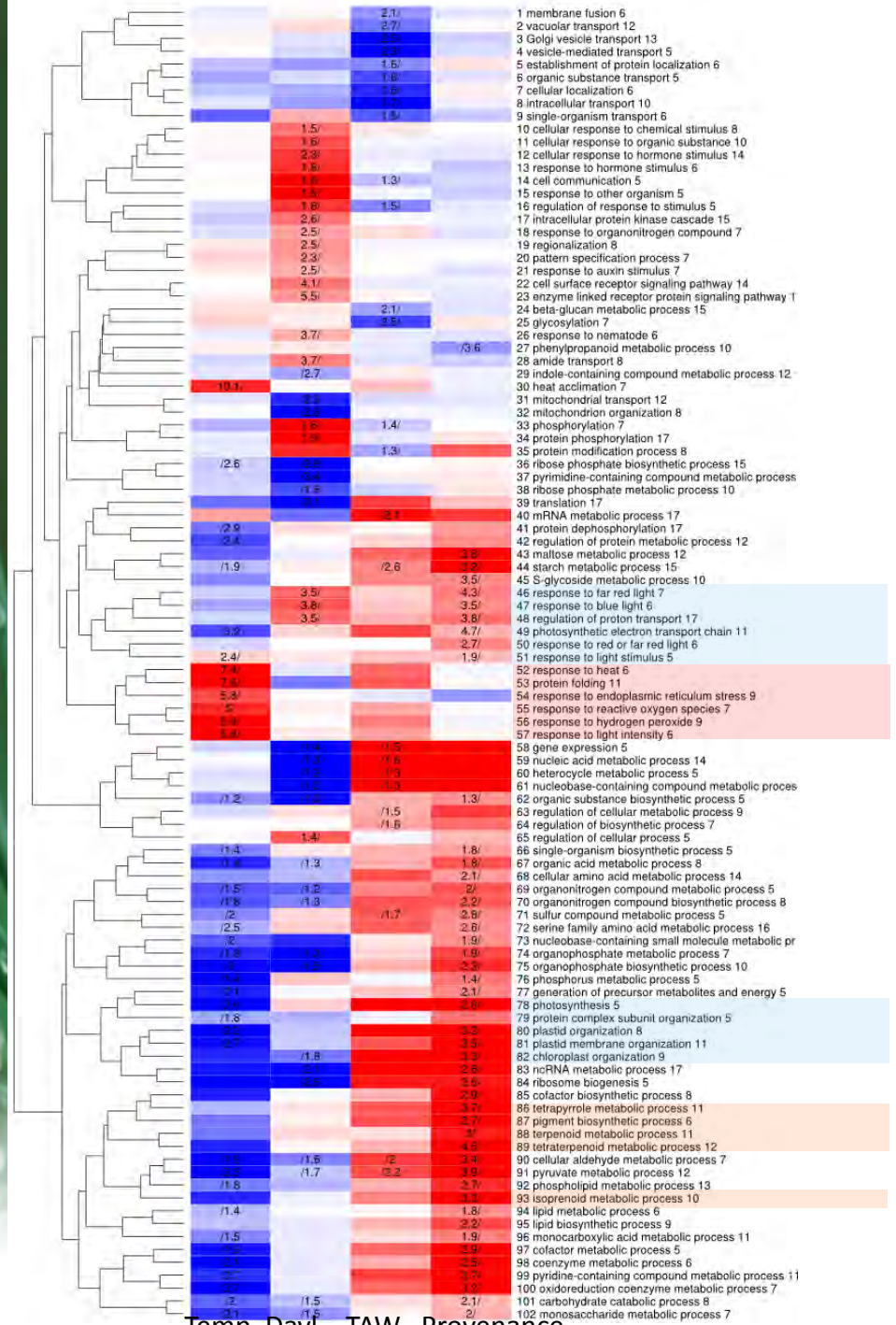
Temp. x Proven. down dAIC < -10, dR2 > 0.1



Day. x Proven. down dAIC < -10, dR2 > 0.1



GO Enrichment (E or G effects)



Response to light and photosynthesis

Response to heat

Response to light and photosynthesis

Pigment biosynthesis and isoprenoids

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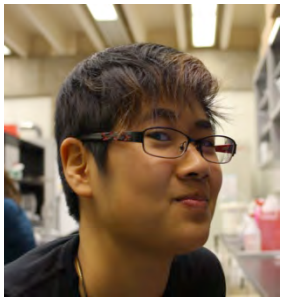
White pine project – Phenology and response to elevated temperature

Leaf spectral reflectance and carbon cycling

Regulation of Photosynthesis & carbon metabolism

Emmanuel Frechette - *Xanthophyll cycle dynamics, leaf spectral reflectance & PRI*

Chris Wong – *Leaf spectral reflectance, carbon cycling (and remote sensing)*



Christine Chang - *Elevated temperature, autumn cold acclimation & senescence*

Claire Depardieu-Rasheed - *Transcriptomics and bioinformatics*

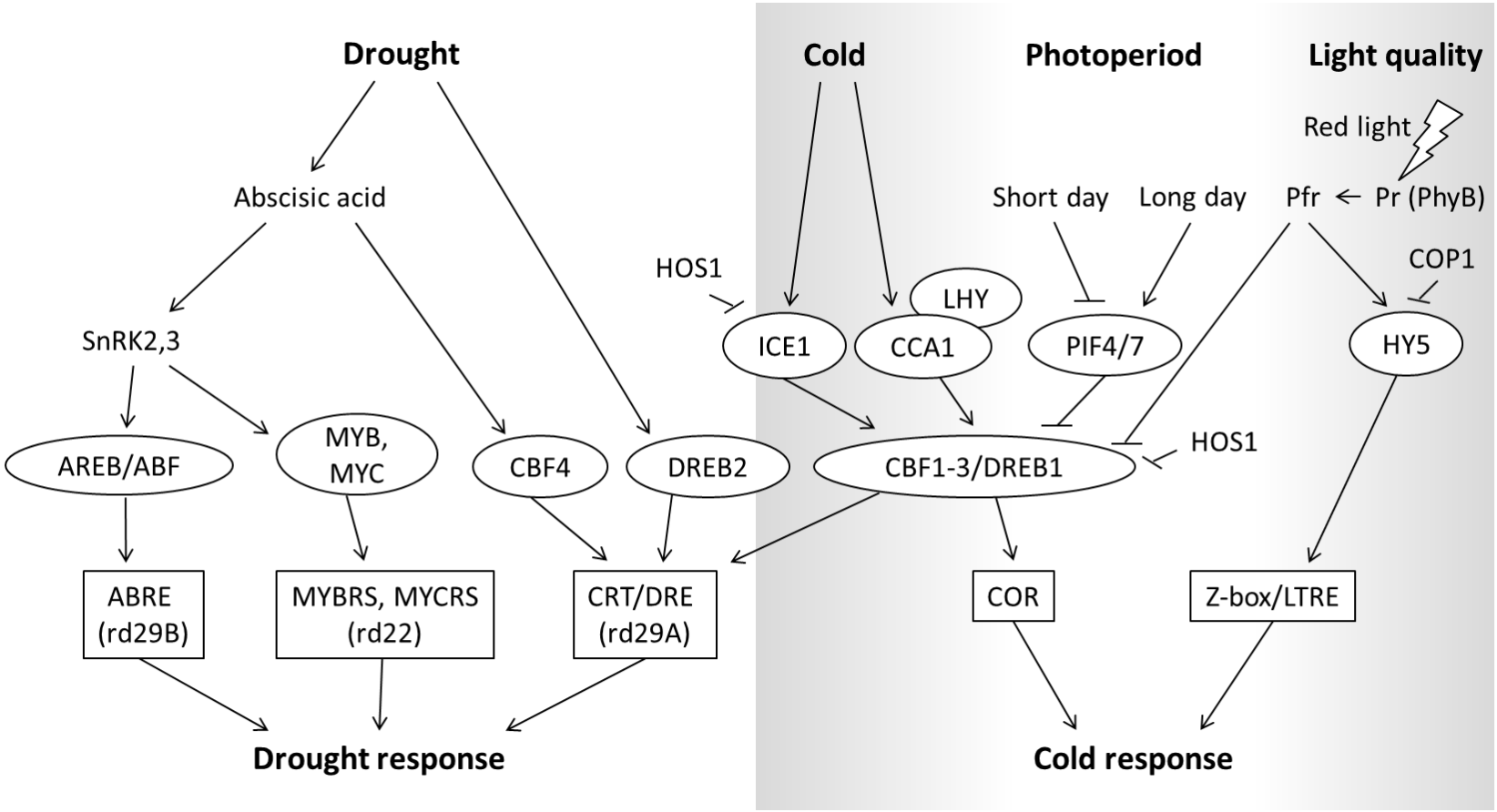
Altaf Arain

John Gamon

Shawn Mansfield

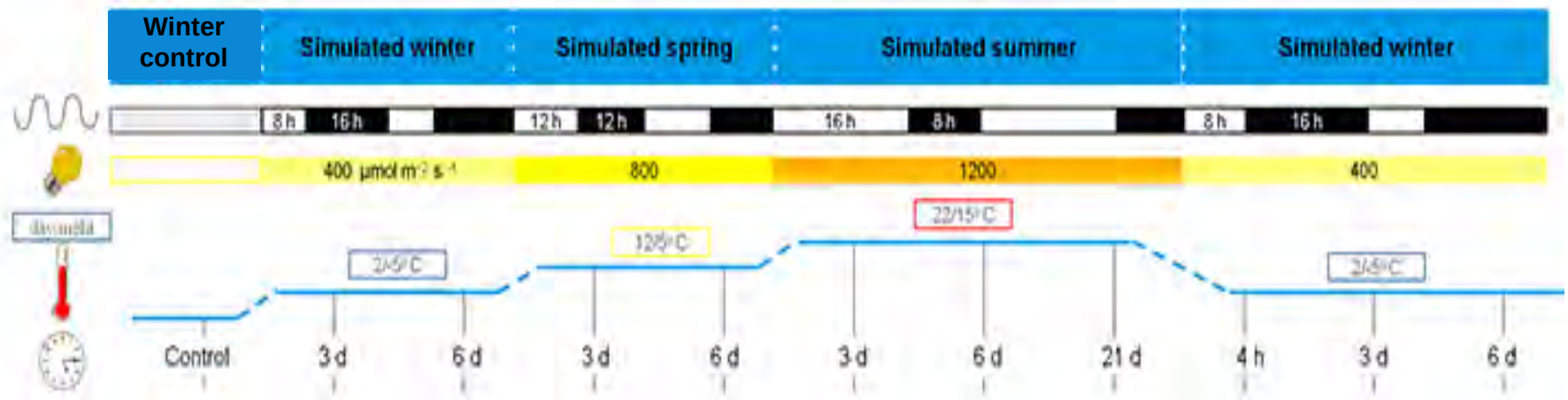
Faride Unda

Establish a catalog of Eastern White Pine unigenes and identify genes involved in response to drought, cold and photoperiod



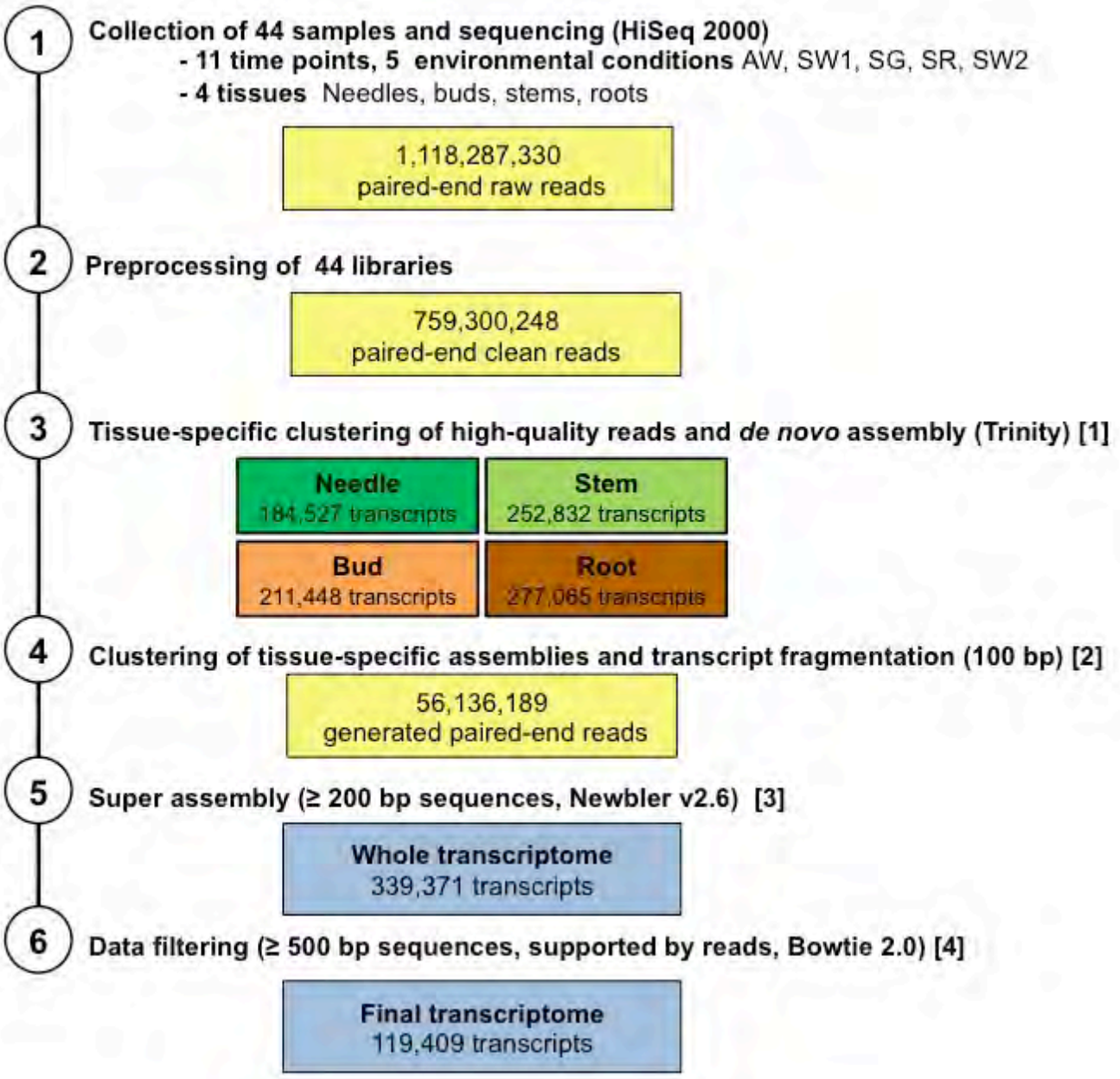
Chang et al. (in preparation)

Experimental design



Workflow for the generation of the EWP transcriptome

Rasheed-Depardieu et al. (in preparation)



CW: Winter control
SW1: Simulated winter
SG: Simulated spring
SR: Simulated summer
SW2: Simulated winter

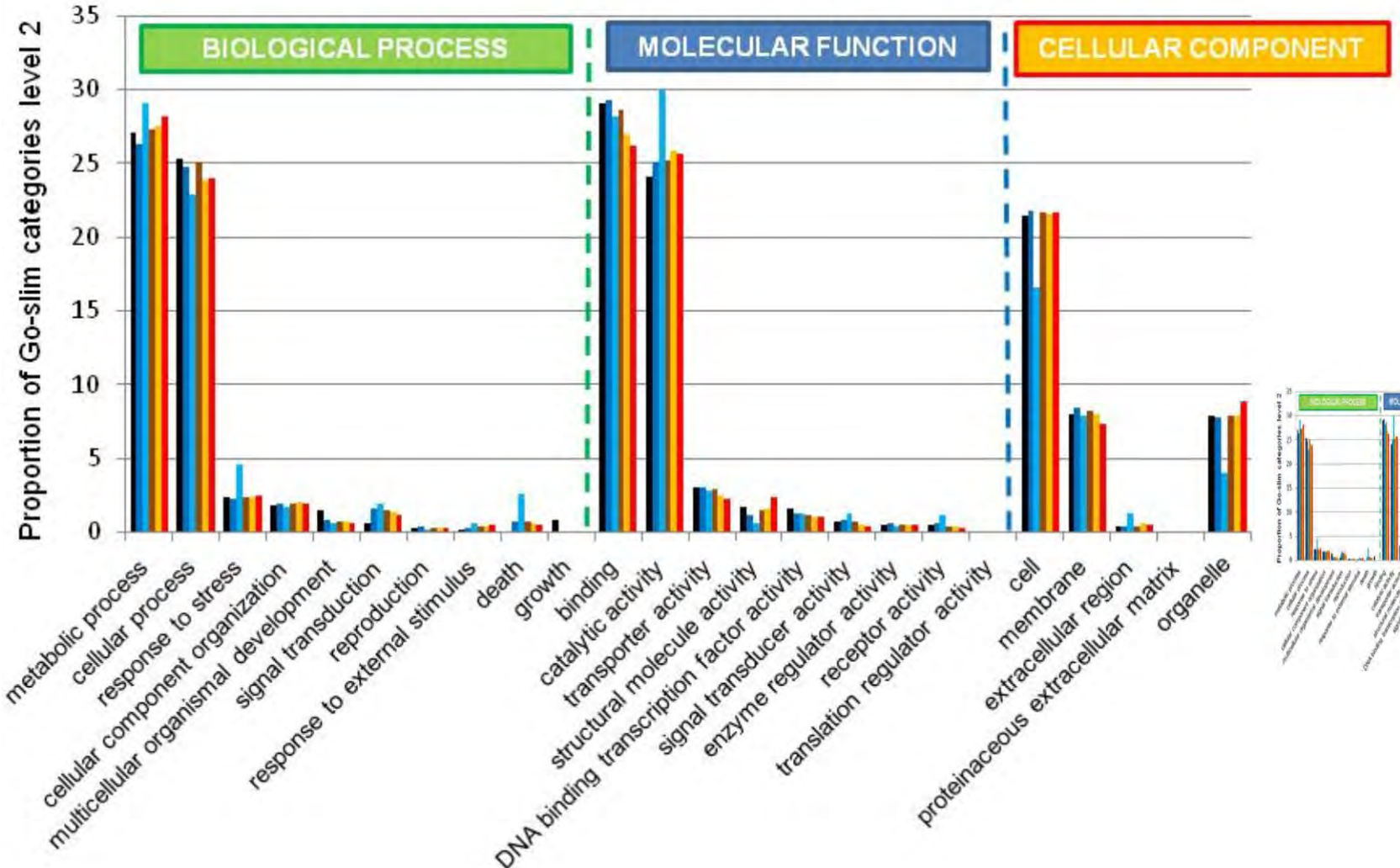
SEQUENTIAL ASSEMBLY & ANNOTATION

- Tissue specific *de novo* assembly of 759,136,189 clean reads produced 925,872 transcripts
- Fragmentation of tissue specific transcripts produced 56,136,189 paired-end reads (100 bp)
- Super assembly using 100 bp fragments produced 339,371 transcripts (unfiltered). Filtering removed more than 200,000 transcripts
- Upon filtering, 119,409 high quality transcripts were obtained through sequential *de novo* assembly
- For 76,685 (64.2%) of the high quality transcripts a significant hit was detected in the *Pinus taeda* database

	Tissue-specific assemblies (Trinity)					Super assembly (Newbler)	
	Needle	Bud	Stem	Roots	Total	Before filtering	After filtering (Bowtie)
Average contig length (bp)	758	757	713	701	733	1068	1900
Genes	142,171	161,627	198,032	218,762	720,592	229,487	41,358
Transcripts	184,527	211,448	252,832	277,065	925,872	339,371	119,409
Minimum sequence length (bp)	201	201	201	201	201	200	500
Maximum sequence length (bp)	17,674	18,236	17,908	17,924	17,935	18,265	18,265
Contig N50 (bp)	1384	1338	1268	1207	1299	1174	2466
Contig N90 (bp)	283	284	270	272	277	334	949
Total length of all contigs in assembly (bp)	140,022,573	160,079,216	180,262,841	194,368,369	674,732,999	305,661,821	226,890,561
Mean GC (%)	38.8	38.9	39.2	39.8	39.2	40.32	39.47
Average bitscore[a]	302.8	297.9	275.4	270.6	286.7	315.6	436.5
Transcripts with significant hits (%)[b]	70,852 (38.4)	78,263 (37.0)	88,812 (35.1)	94,328 (34.0)	83,063 (36.1)	129,924 (38.3)	76,685 (64.2)

GO TERM DISTRIBUTION

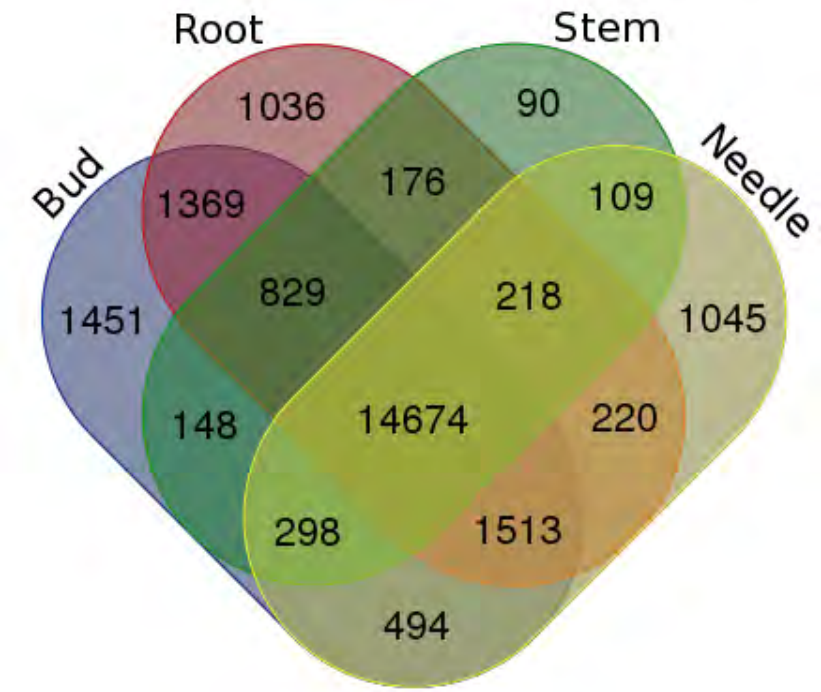
- GO terms were assigned to 45,627 transcripts
- GO term distribution shows great similarity with other conifer species, indicating that the majority of transcriptome has been tagged reliably



TISSUE & PHASE SPECIFIC RESPONSES

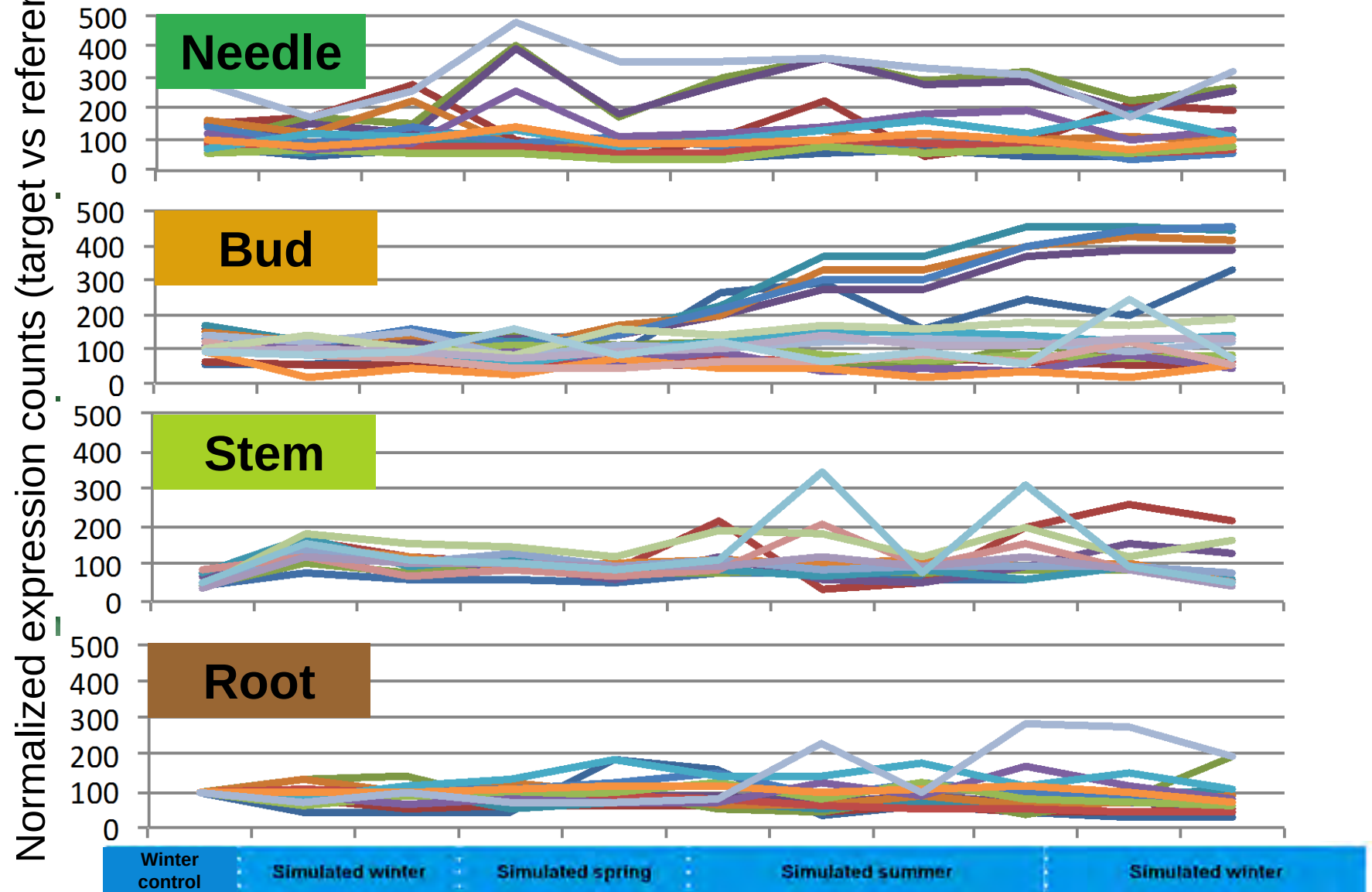
14,674 unigenes present in all tissues

A large number of unigenes detected only in one tissue during all phases



TISSUE & PHASE SPECIFIC RESPONSES

- Expression of unigenes dependent on seasonal phase
- Members of the MYB transcription factor family reveal differences between tissues, but also vary during simulated seasonal phases



Conclusions

Douglas Fir

- Assessed provenance specific responses of physiology and gene expression in complex field environment
- Observed changes in physiology linked to TAW; signature of provenance detected in isotopic signature, pigments, VOC pools, but not in VOC emissions
- Mixed linear models for each PUT allowed identification of DE and assessing effects of G, E on gene expression
- Provenance specific differential expression of many GO categories consistent with physiological data
- Association study is underway (Karl Schmid, U Hohenheim, Germay)

Conclusions

White pine

- Used a sequential assembly approach to generated a draft *Pinus strobus* transcriptome
- Unigenes represent tissue and seasonal phase specific transcriptome responses
- Majority of transcripts have best hits in *Pinus taeda* database
- Comparison of GO term distribution between *Pinus strobus* unigenes and other conifers indicates reliable functional annotation
- The draft assembly and annotation of *Pinus strobus* transcriptome is a resource for further gene expression analysis, and for understanding cold-acclimation, de-acclimation and the development of frost tolerance

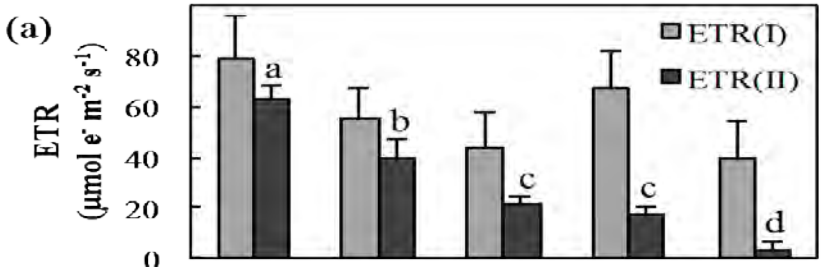
Perspectives

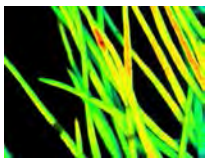
Phenotyping bottleneck (seedlings, trees)

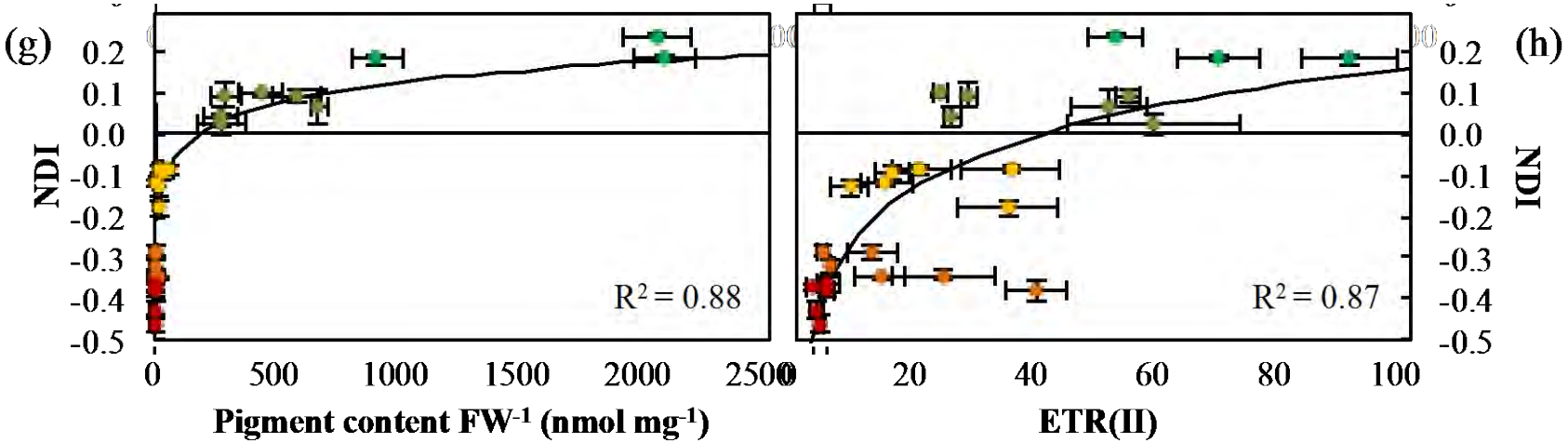
- Use of vegetation indices to assess phenology of individual plants
- Use of vegetation indices to assess drought stress
- Use of imaging PAM fluorescence to assess freezing damage

Perspectives

Phenotyping bottleneck







Junker et al. (submitted)

Perspectives

Characterization of gene function by comparison

- Characterization of gene families and transcription factors
- Co-expression analysis and identification of tree specific genes
- Gene browser