Functional Genomics of *Populus* Growth & Development

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Outline

- Environmental regulation of growth, dormancy, flowering
  - Regulatory genes and differentiating functions of paralogs
  - Multi-tissue time series transcriptomics to gene regulatory networks

- Wood-associated protein-protein and protein-DNA interactions
  - Have we identified new regulators of wood formation?

Phenology is closely matched to local climates

- Common garden studies
  - Latitudinal clines
  - Genetic differentiation between populations
- Ecotypes –controlled environment studies
  - Different critical daylengths for bud set & dormancy induction
  - Phytochrome: night breaks of white or red light disrupt SD response

Native range of *P. trichocarpa*

- **Dormancy**: a meristem is insensitive to growth promoting conditions until it is released from dormancy by an environmental cue
- **Depth of Dormancy**: refers to the quantitative nature of this phase
- “Classic terms”
  - Ecodormancy: meristem is quiescent, but will rapidly resume growth if the limiting environmental factor is altered
  - Endodormancy

- **Reproductive phenology**: Seasonal timing of the floral transition, anthesis and fruiting
  - In temperate zones, flowering is indirect

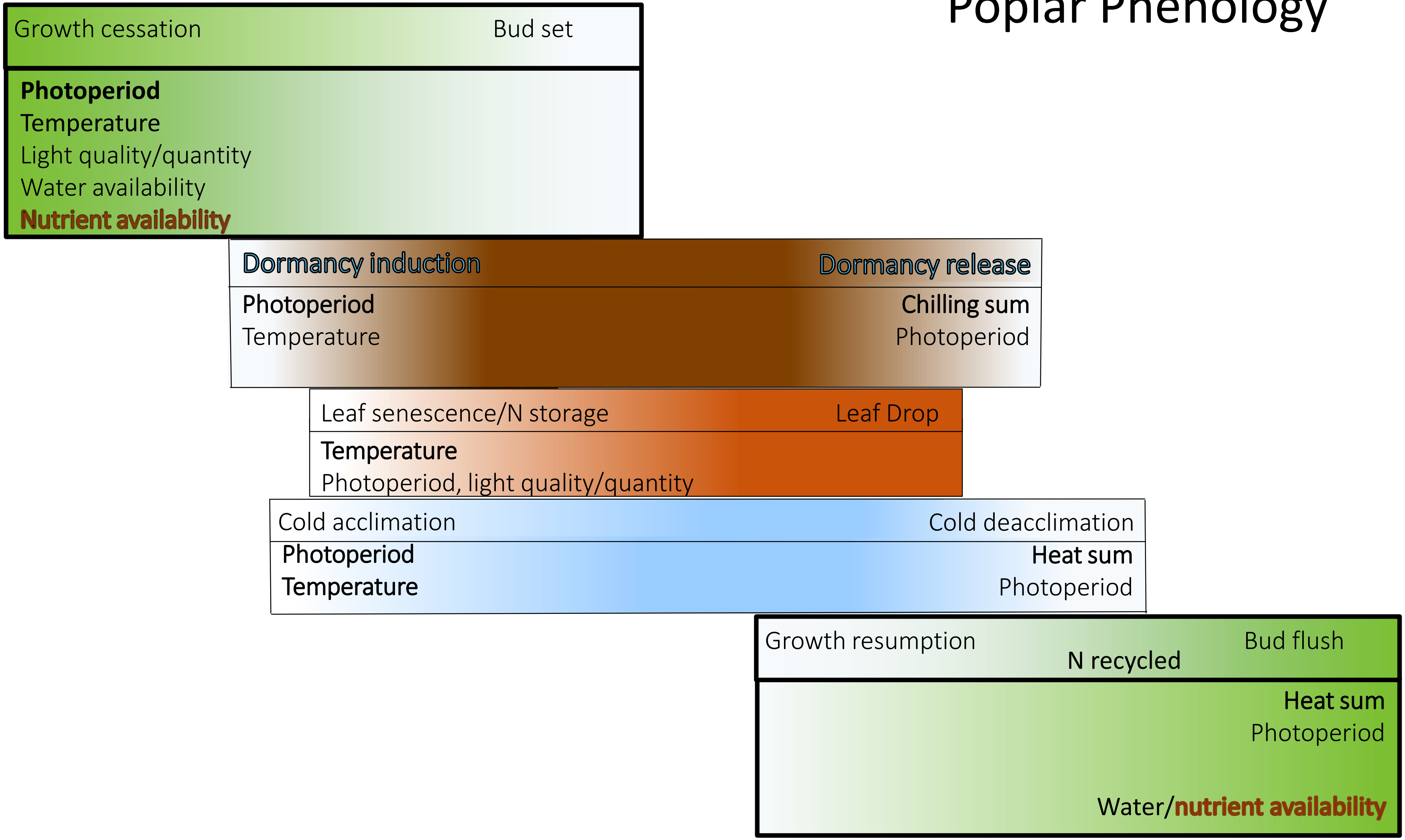
The environmental signals regulating vegetative and flowering phenology in temperate zones are generally the same

Environmental Factor	Reliability in temperate zones
Photoperiod	High
Prolonged chilling period	High
Ambient temperature	Moderate
Light intensity	Moderate
Water availability	Moderate
Nutrient availability	Low
Light quality	Low

Adapted from Bernier and Perilleux (2005) A physiological overview of the genetics of flowering time control. Plant Biotechnol J 3 (1): 3-16

Less predictable factors modulate the effects of the primary signals and sometimes can substitute for the primary signal

Poplar Phenology



Growth cessation
Bud set

Photoperiod
Temperature
Light quality/quantity
Water availability
Nutrient availability

Dormancy induction
Dormancy release

Photoperiod
Temperature

Chilling sum
Photoperiod

Leaf senescence/N storage
Leaf Drop

Temperature
Photoperiod, light quality/quantity

Cold acclimation
Cold deacclimation

Photoperiod
Temperature

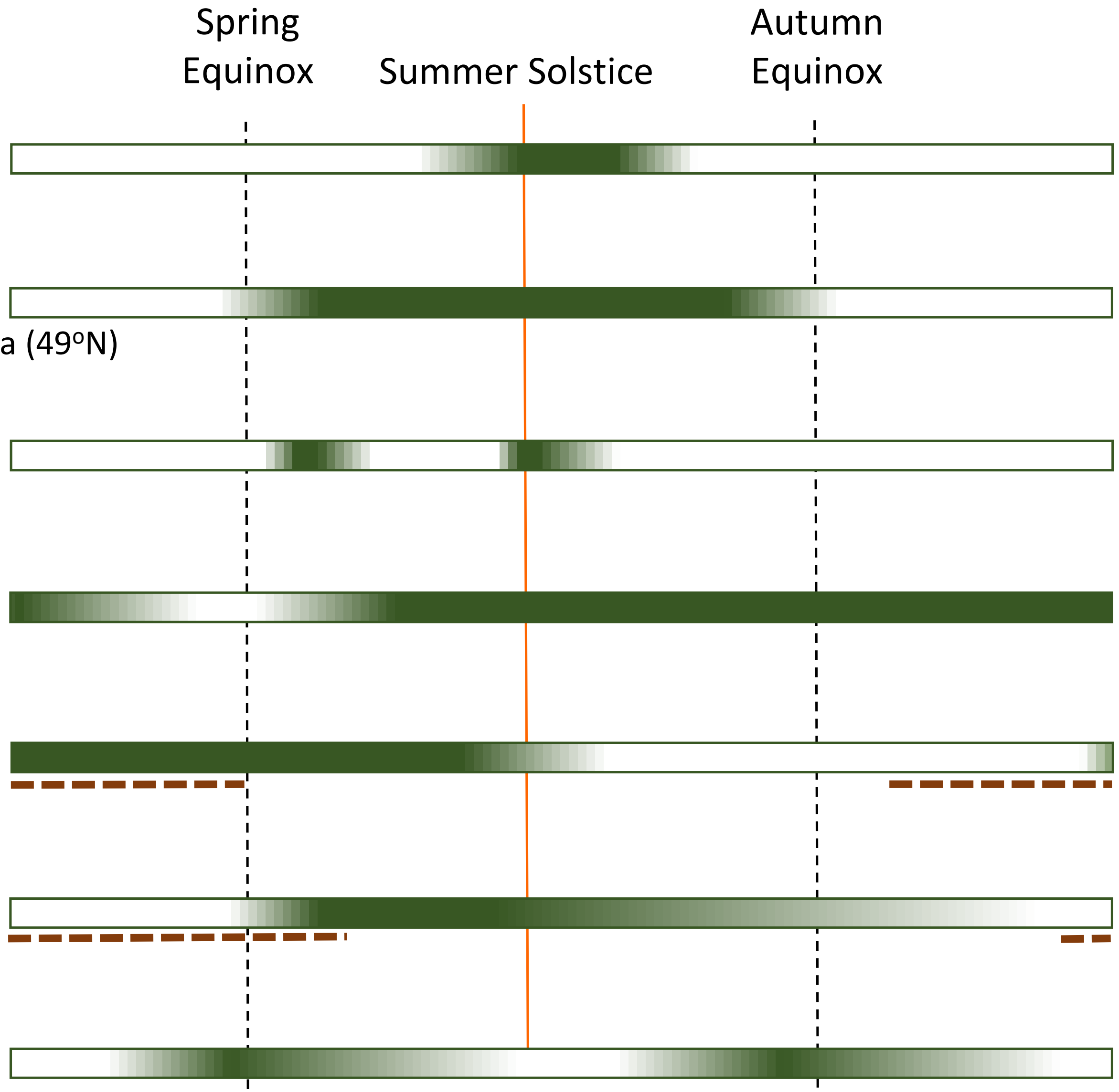
Heat sum
Photoperiod

Growth resumption
N recycled
Bud flush

Heat sum
Photoperiod

Water/**nutrient availability**

Tree annual growth patterns



Free growing, *Populus balsamifera*
Boreal: Alaska (65°N)

Free growing, *Populus trichocarpa*
Maritime temperate: British Columbia (49°N)

Rhythmic growth, *Quercus robur*
Warm temperate: Serbia (45°N)

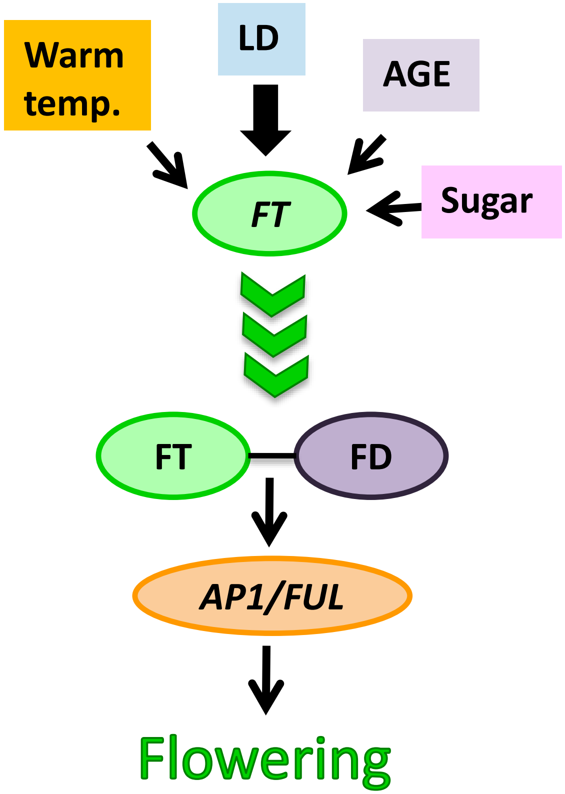
Brevideciduous, *Dillenia indica*
Sub-tropical highland: India (25°N)

Evergreen, *Eucalyptus miniata*
Tropical wet and dry: Australia (13°S)

Deciduous, *Pulmeria rubra*
Tropical wet & dry: Costa Rica (10°N)

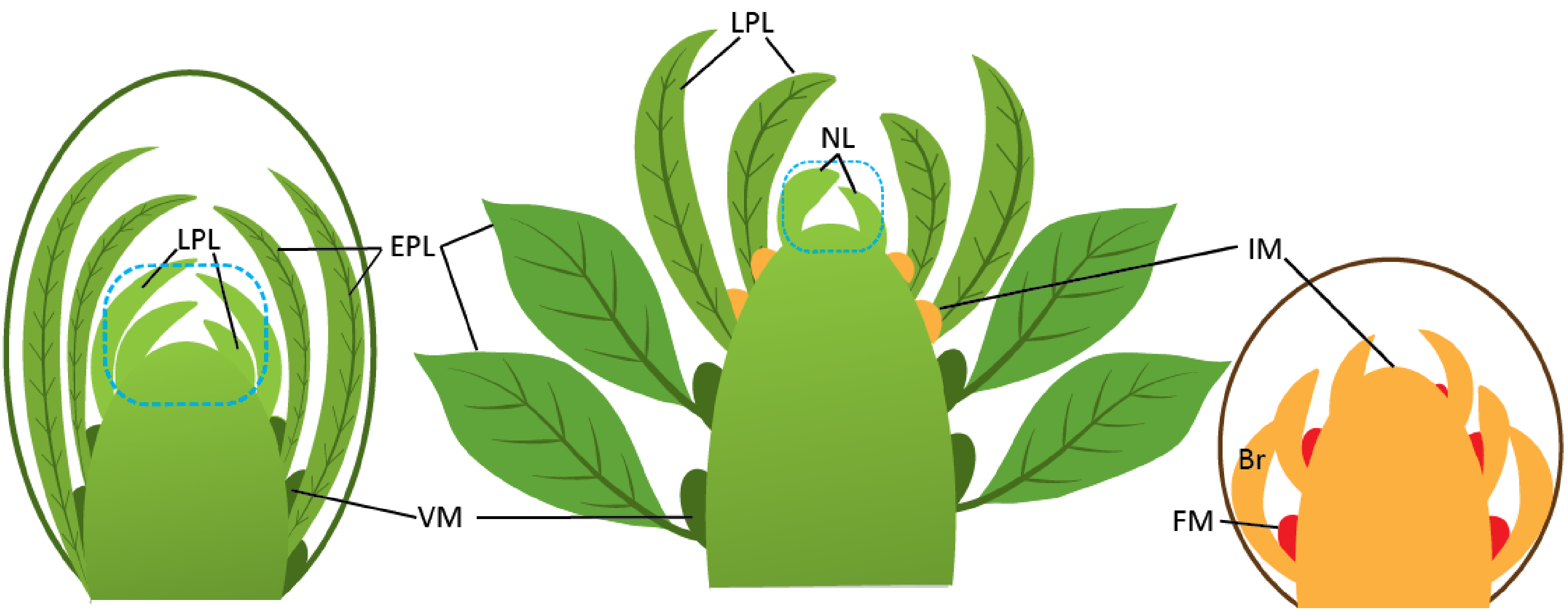
Deciduous, *Parkia javanica*
Tropical rain forest: Singapore (1°N)

Adapted from Brunner, Varkonyi-Gasic and Jones 2017
https://link.springer.com/chapter/10.1007/7397_2016_30



FT is an integrator of environmental and internal signals regulating flowering time

Transition to flowering in *Populus*



Dormancy release

Bud flush

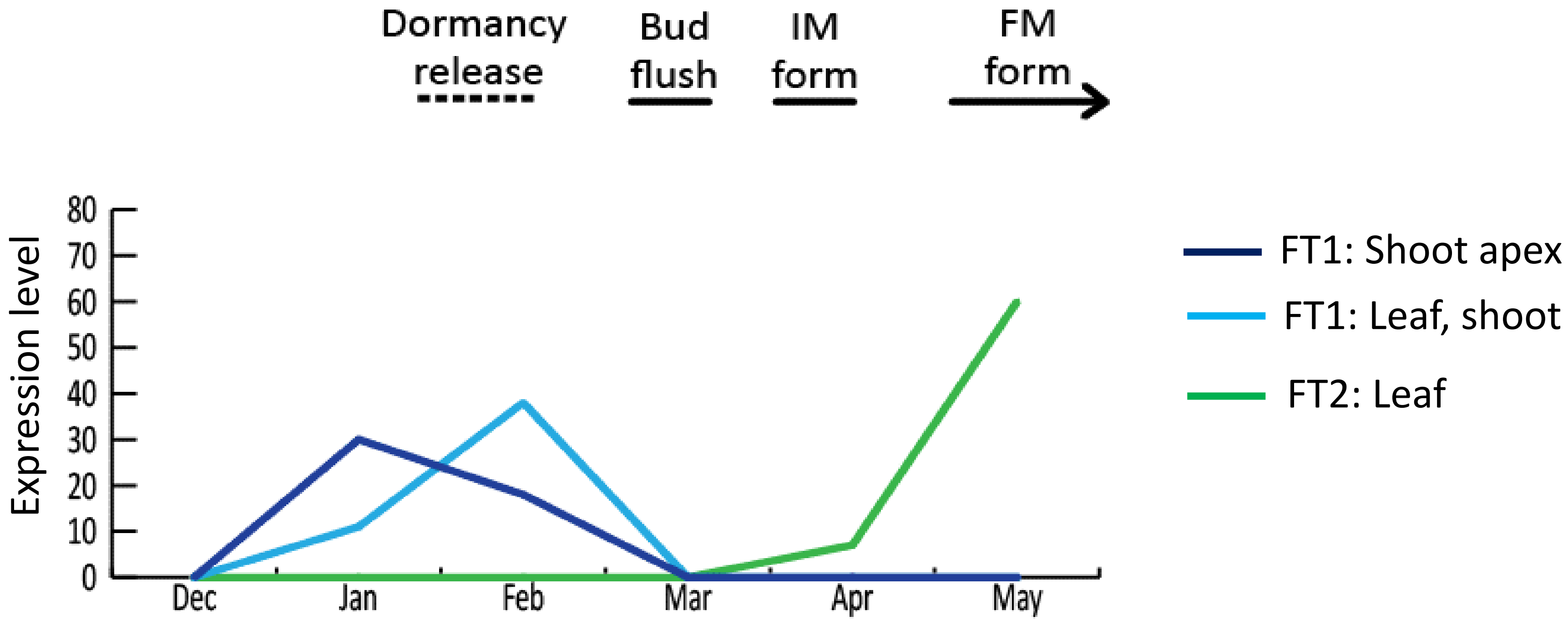
Inflorescence meristems form

Floral meristems form

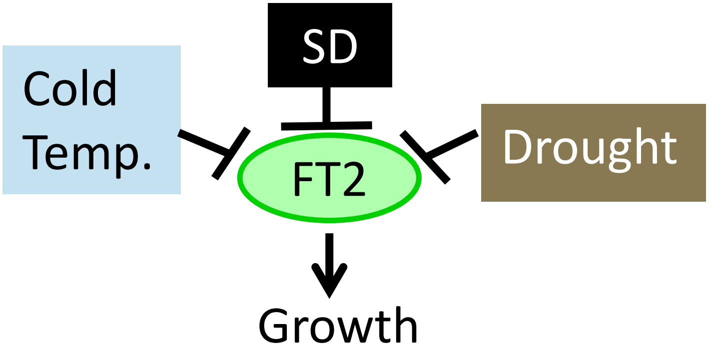
Meristems commit to flowering during a limited seasonal time

FT1/FT2 seasonal expression in mature *P. deltoides*

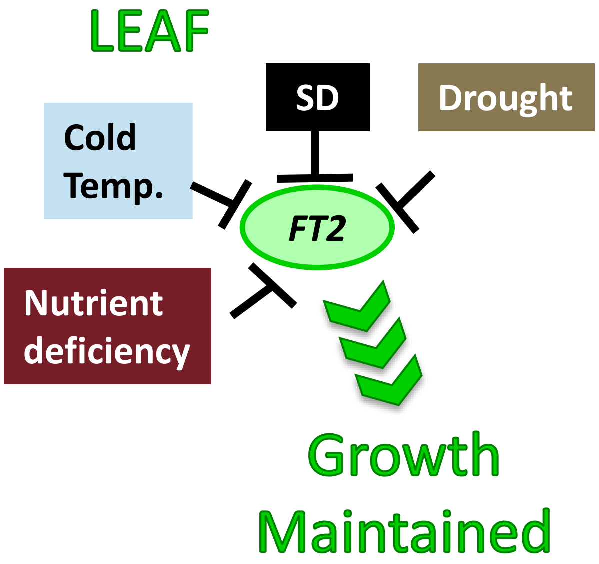
Hsu et al. 2011 PNAS 108: 10756-61



FT1 → Flowering



Do FT1 and FT2 have distinct roles in vegetative phenology?



LEAF

SD

Drought

Cold Temp.

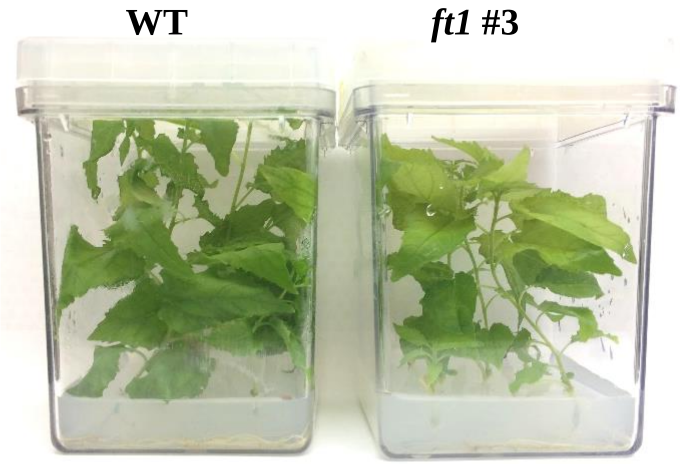
FT2

Nutrient deficiency

Growth Maintained

CRISPR/Cas9-induced mutations in both *FT2 and FT1*: reduced shoot elongation and terminal bud set in tissue culture under 16 hr daylengths

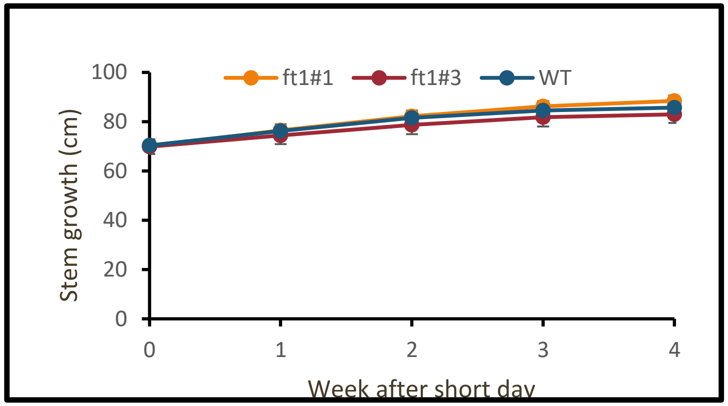
FT1-specific mutants appear WT



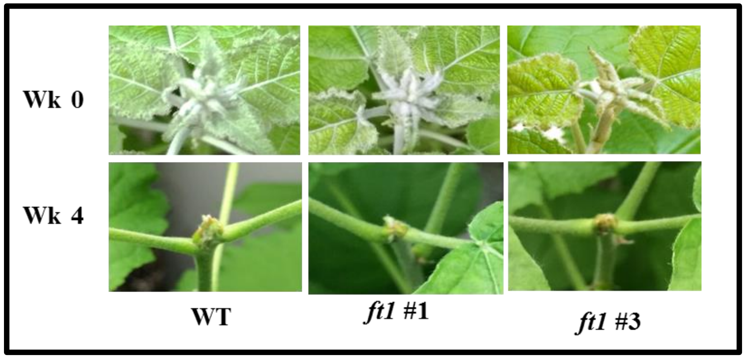
ft1 mutants have WT-like phenotype in both long and short daylengths

Growth under long days

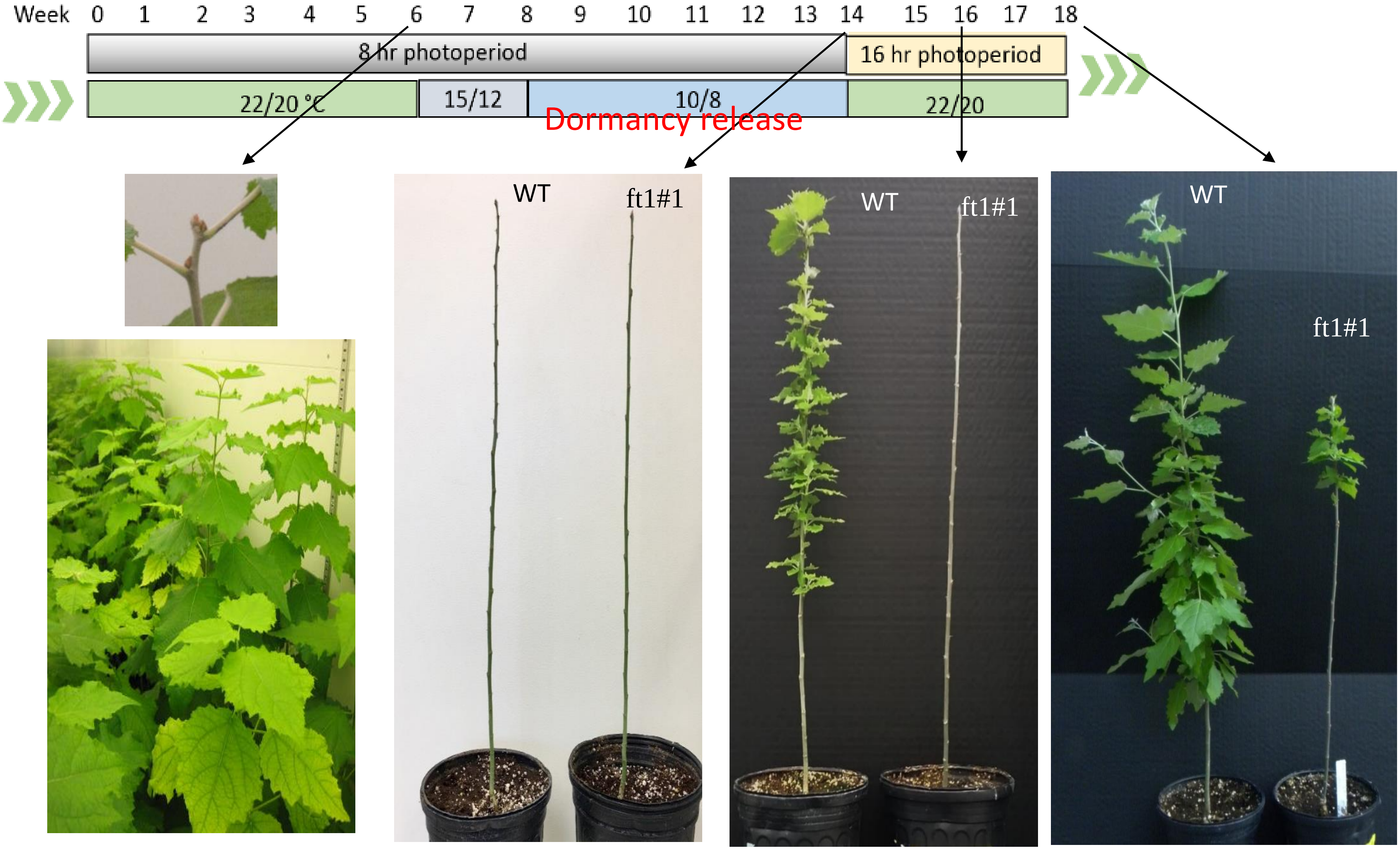
Growth cessation under short days



Bud set



Dormancy release is delayed in ft1 mutants



Week 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

8 hr photoperiod

16 hr photoperiod

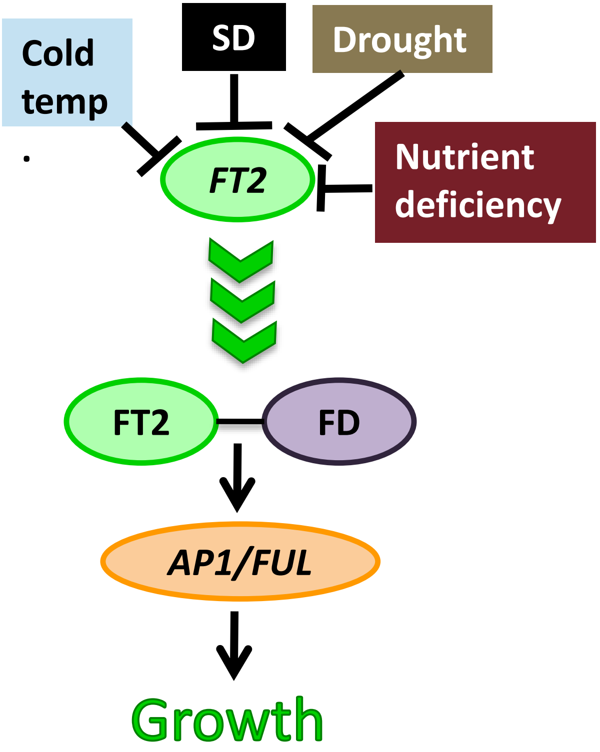
22/20 °C

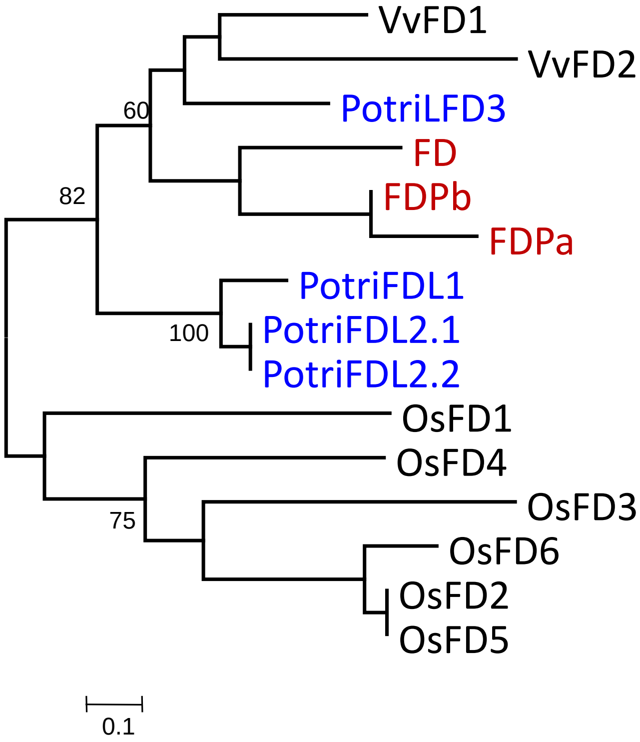
15/12

10/8

22/20

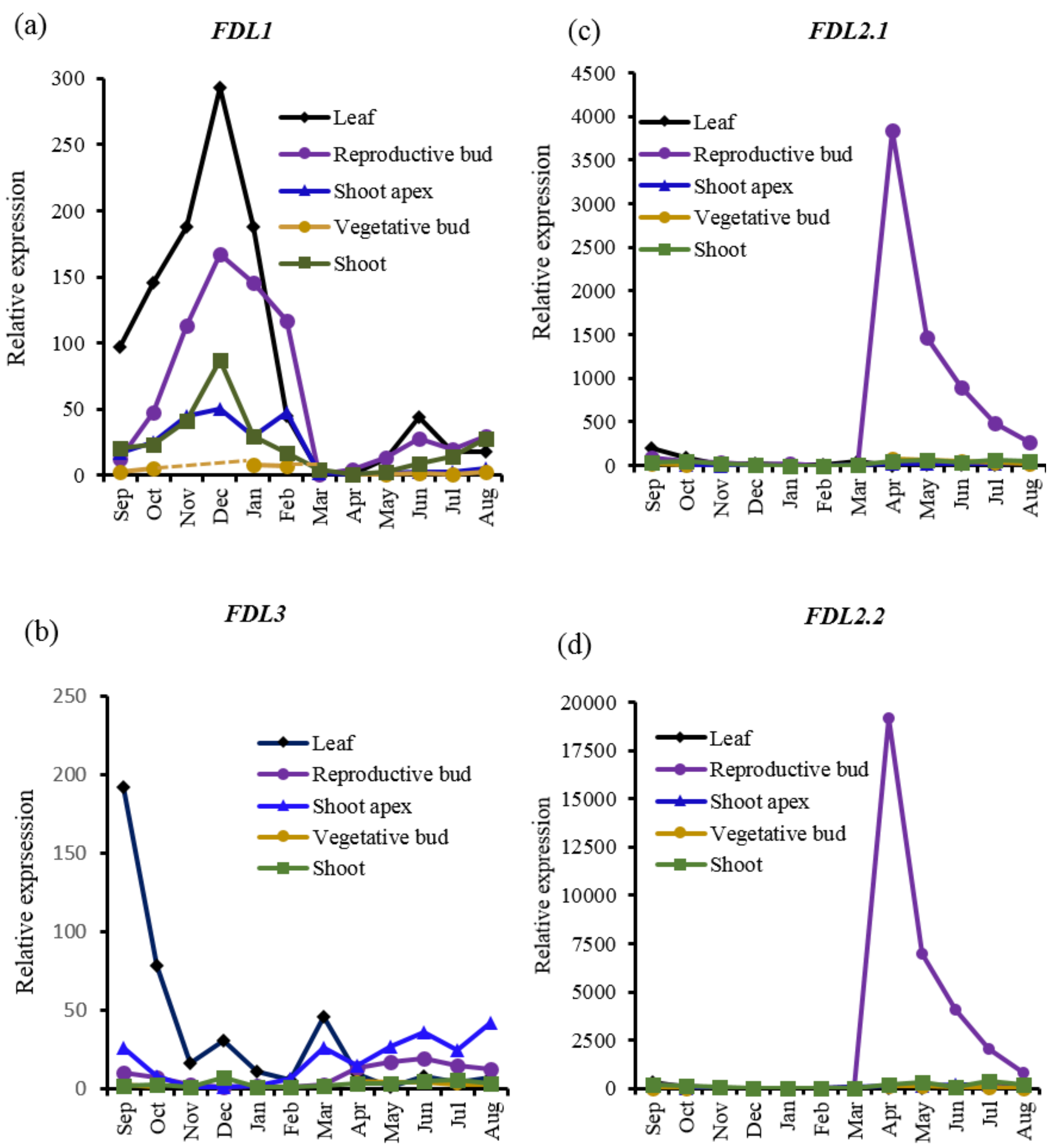
Dormancy release





FDL2.2: Parmentier-line and Coleman 2016

FDL1 and FDL2.1 :Tylewicz et al. 2015



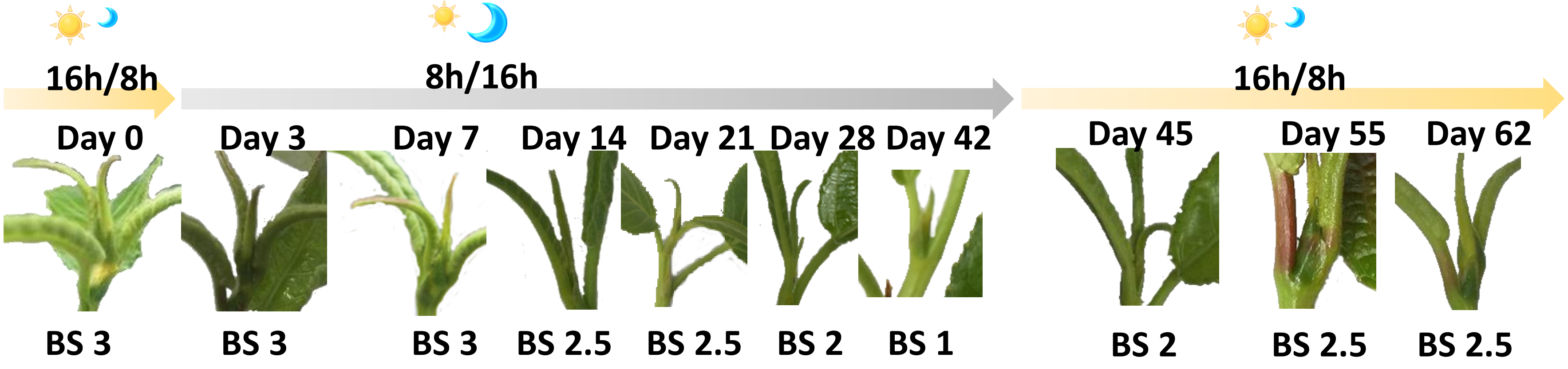
- All *Populus FDLs* delay SD-induced bud set
- Only *FDL2.2* induced flowering under LDs
- *FDL2* and *FDL3* affect shoot development under long days
- Diverged in regulation and proteins have partial functional equivalency

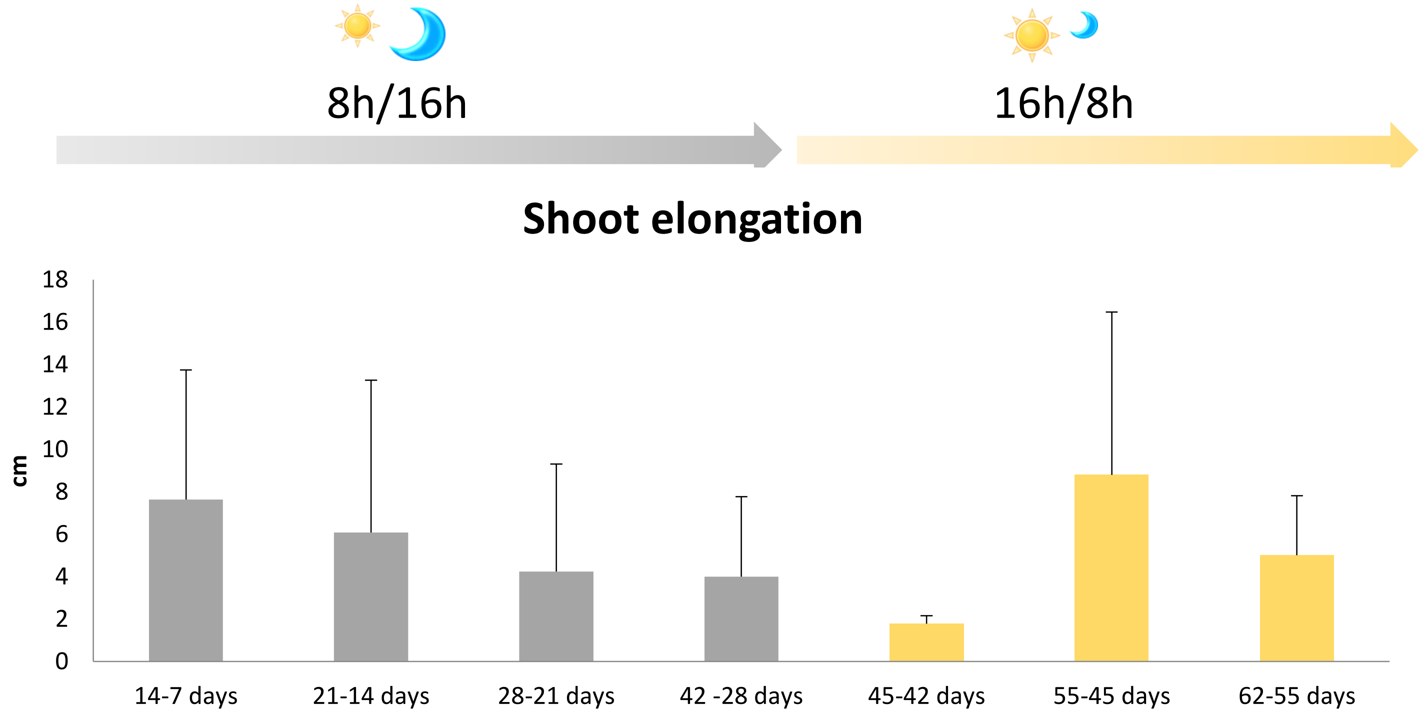
Multi-tissue time series transcriptomes

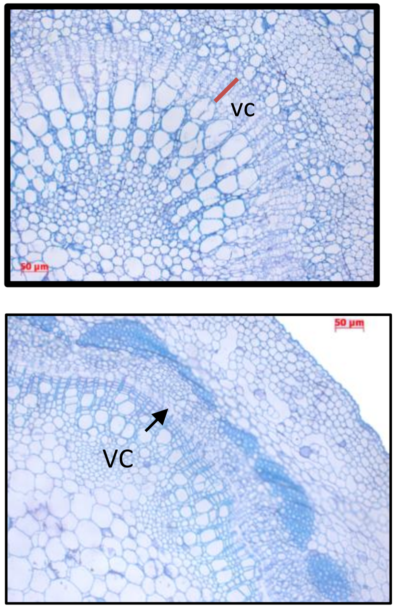
- *540 P. trichocarpa Nisqually1*
- Daylength
  - LD➔SD➔LD
- Nutrient
  - HN➔LN➔HN
  - Tissues/organs:
    - Shoot apex
    - Leaf
    - Root
    - Cambial zone (daylength only)

Goals/Questions

- GRNs for organismal-level processes and phenotypes relatable to field conditions/natural populations
- Among different organs/tissues and environmental treatments:
  - To what extent are there common modules, transcriptional regulators (context dependent), paralogous modules/regulators etc.?
- Regulatory network context of adaptive variation


16h/8h
8h/16h
16h/8h
Day 0
Day 3
Day 7
Day 14
Day 21
Day 28
Day 42
Day 45
Day 55
Day 62
BS 3
BS 3
BS 3
BS 2.5
BS 2.5
BS 2
BS 1
BS 2
BS 2.5
BS 2.5

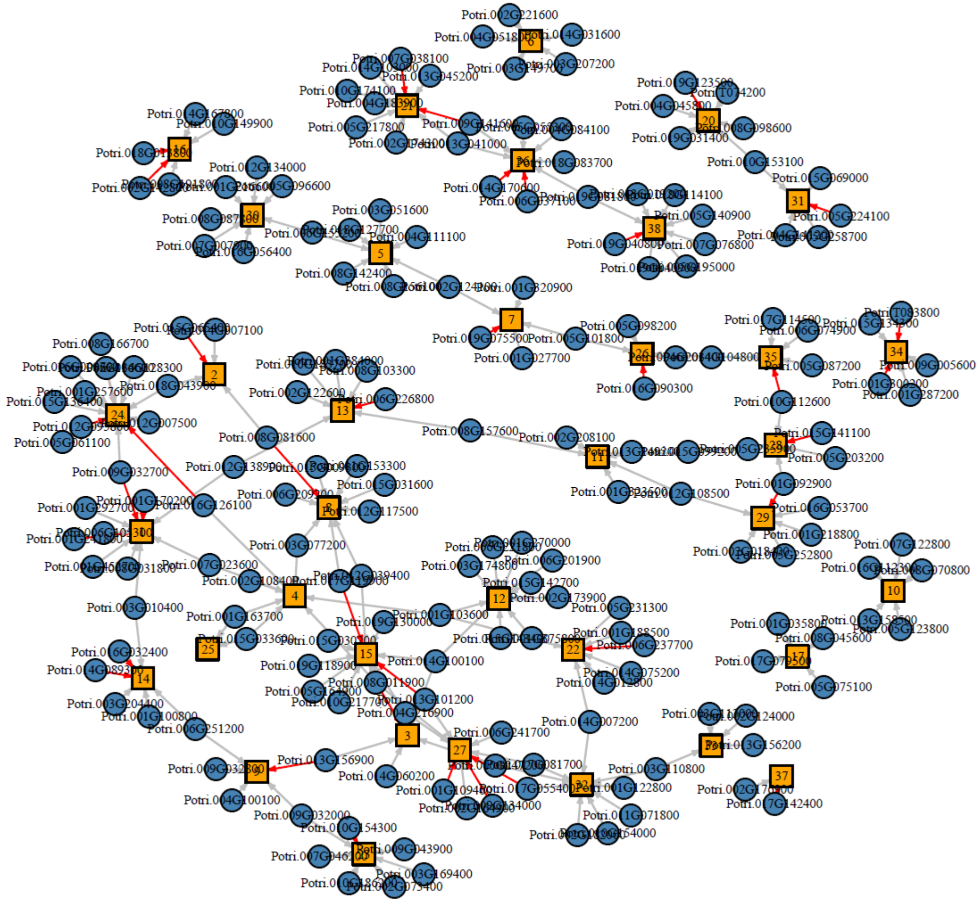

8h/16h
16h/8h
Shoot elongation
cm
0
2
4
6
8
10
12
14
16
18
14-7 days
21-14 days
28-21 days
42 -28 days
45-42 days
55-45 days
62-55 days


VC
50 µm
VC
50 µm

Gene Regulatory Network (GRN) Prediction: Ensemble method

- Uses 5 different algorithms: ARACNE, Random Forest, Least angle regression, Partial correlation, Context likelihood relatedness (Redekar, N. et al. 2017)

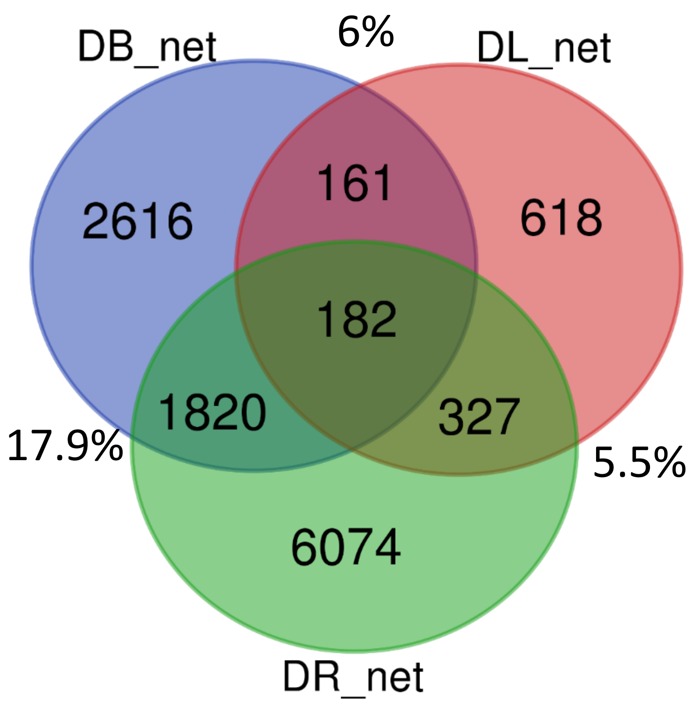
Transcriptional regulators predicted by at least 4 of the 5 algorithms



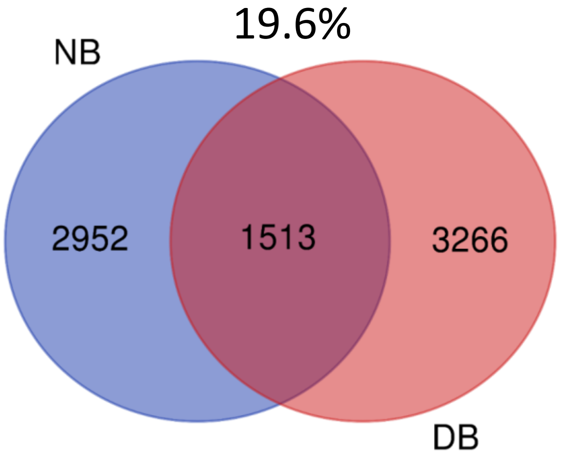
More unique genes per network, but also common genes between:

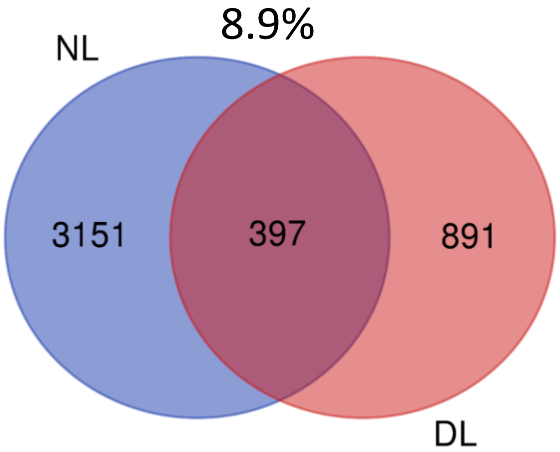
- Different organs in same treatment
- Same organ in different treatments

3 Daylength organs

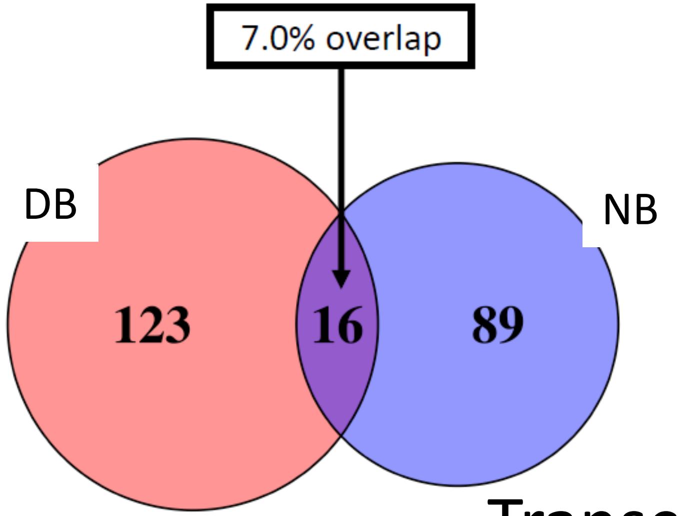


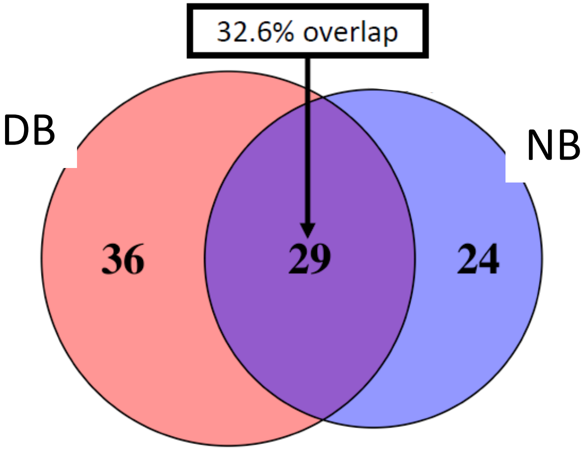
Same organ, nutrient & daylength

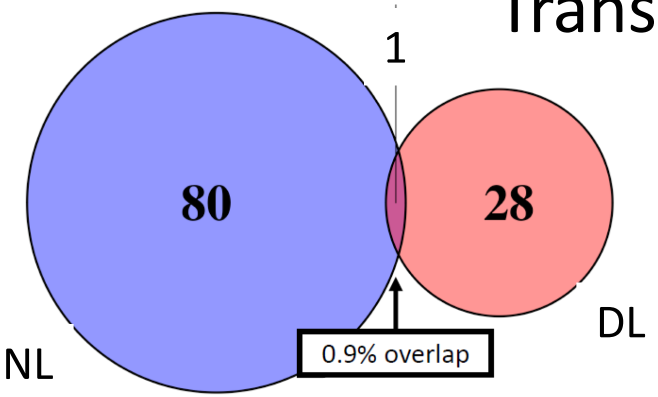


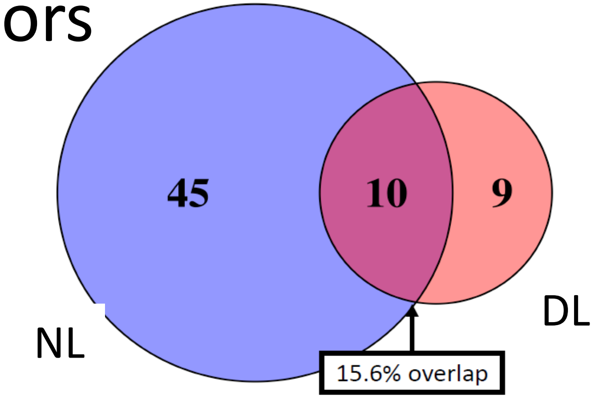


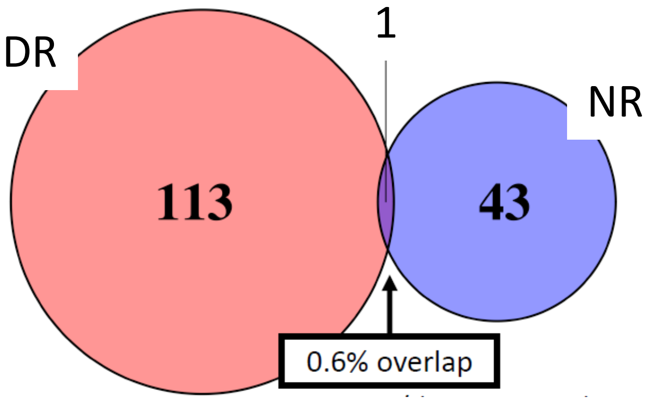
Transcriptional regulators

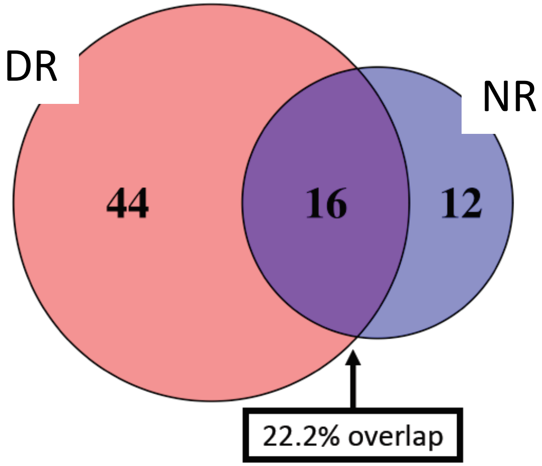




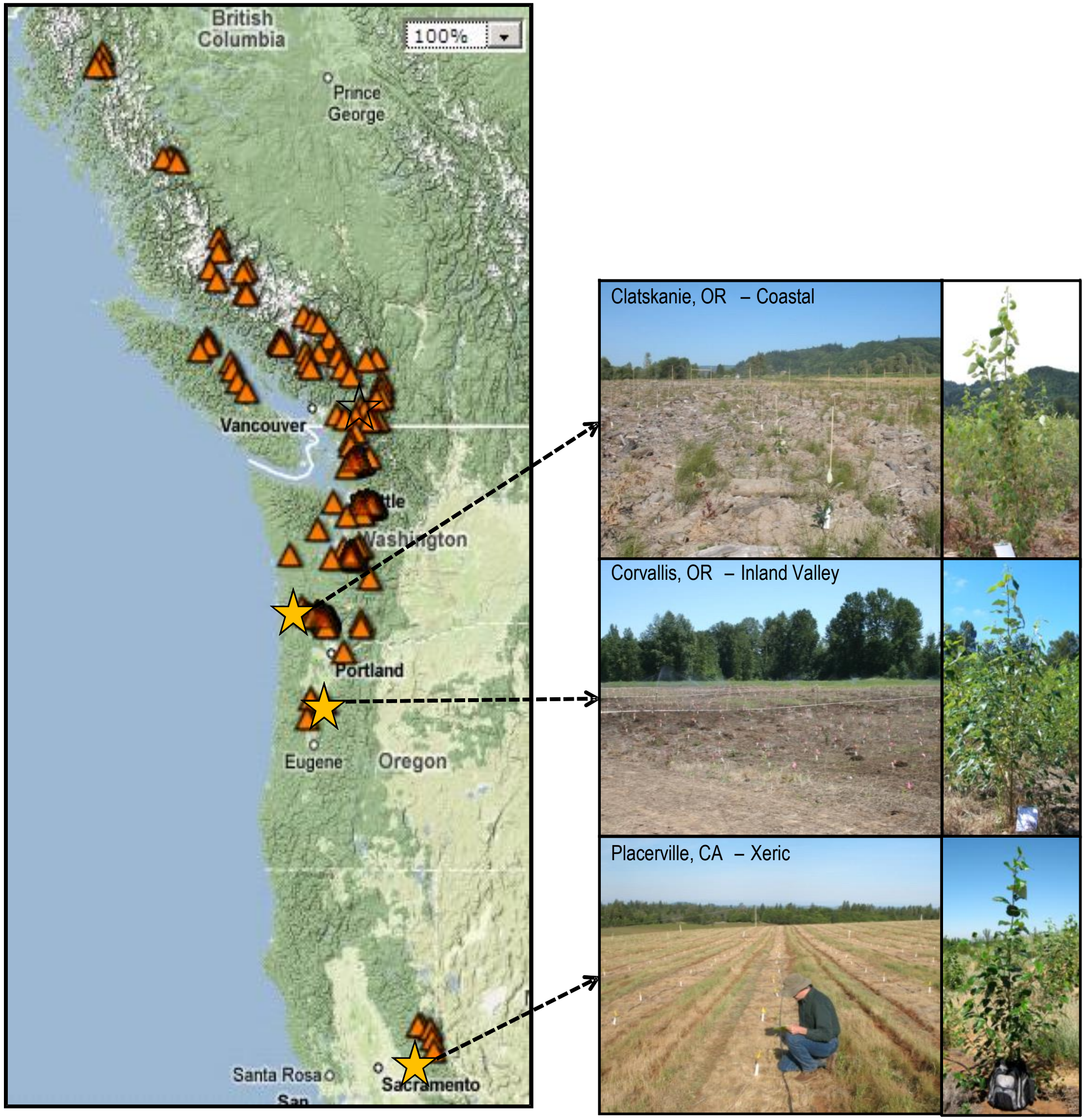








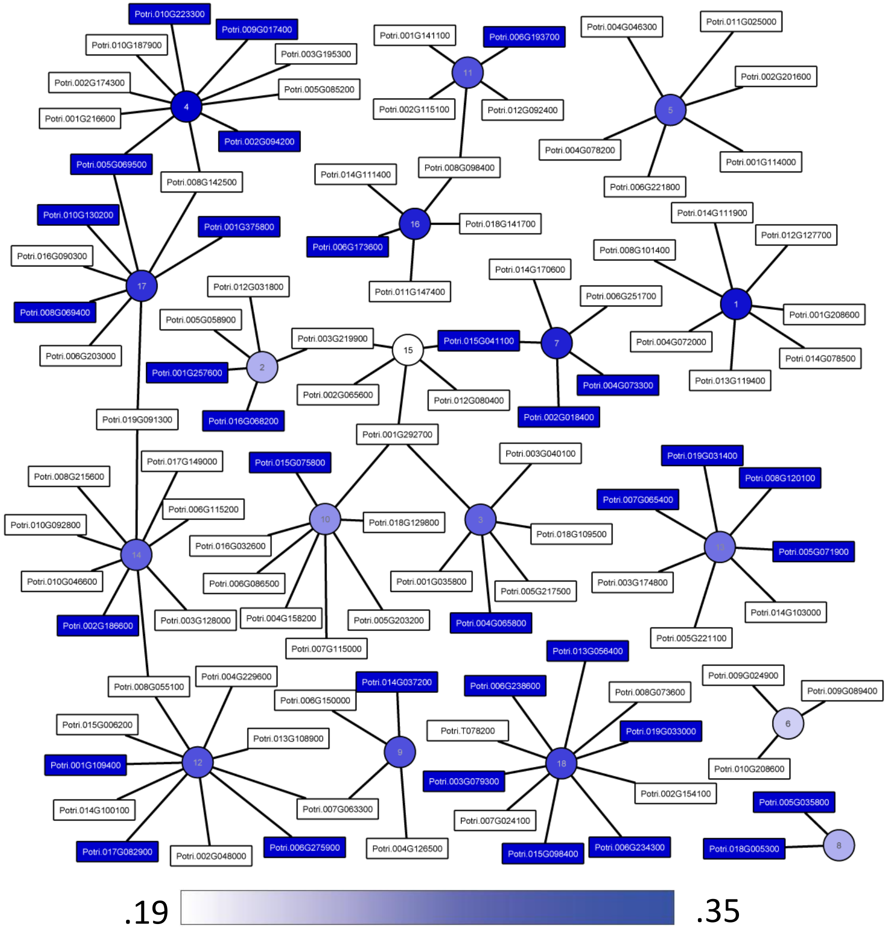
Regulatory context of adaptive variation



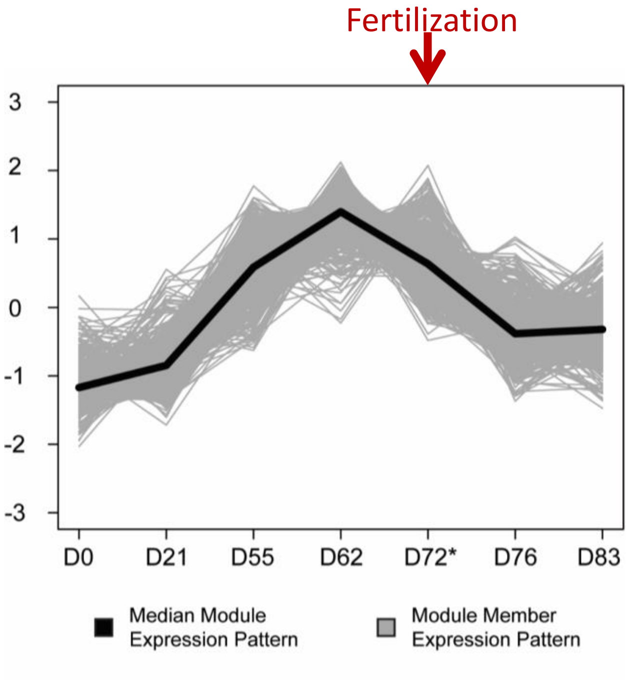
(Evans et al. 2014)

- GWAS traits
  - Bud set
  - Bud flush
  - Height
- Selection scans

Nutrient Bud Network - GWAS

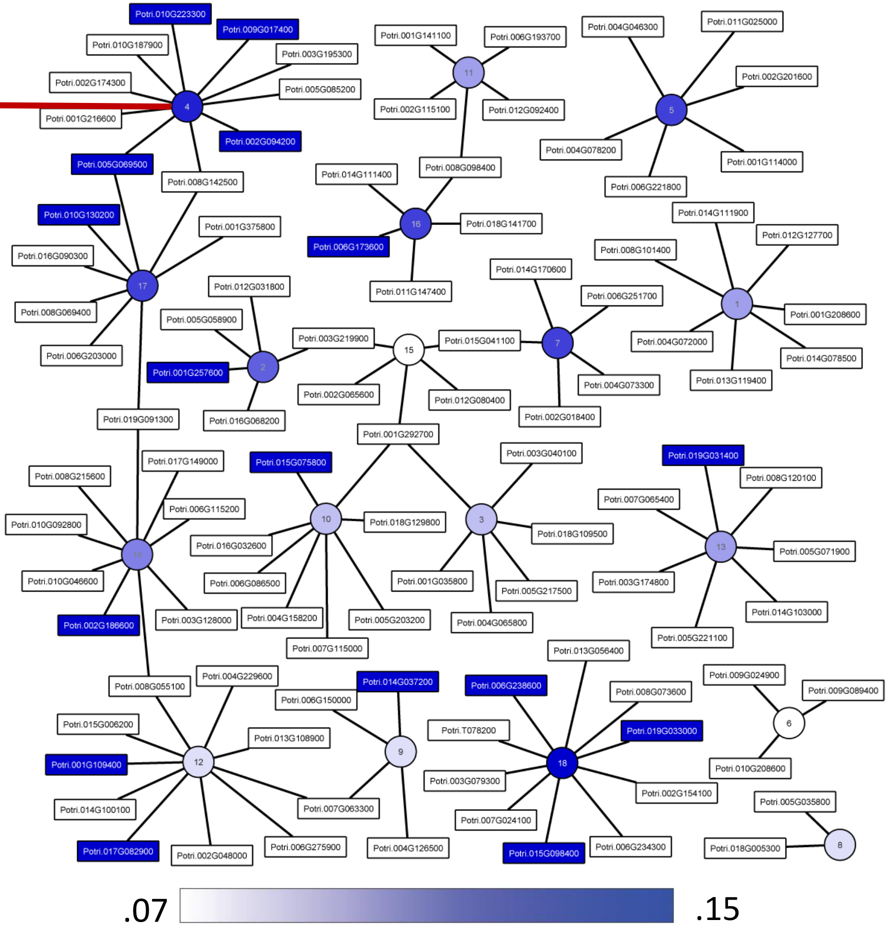


- 115 Transcriptional regulators
  - 33 GWAS
- 18 modules (4465 genes)
  - 96-404 genes/module
  - 1318 GWAS



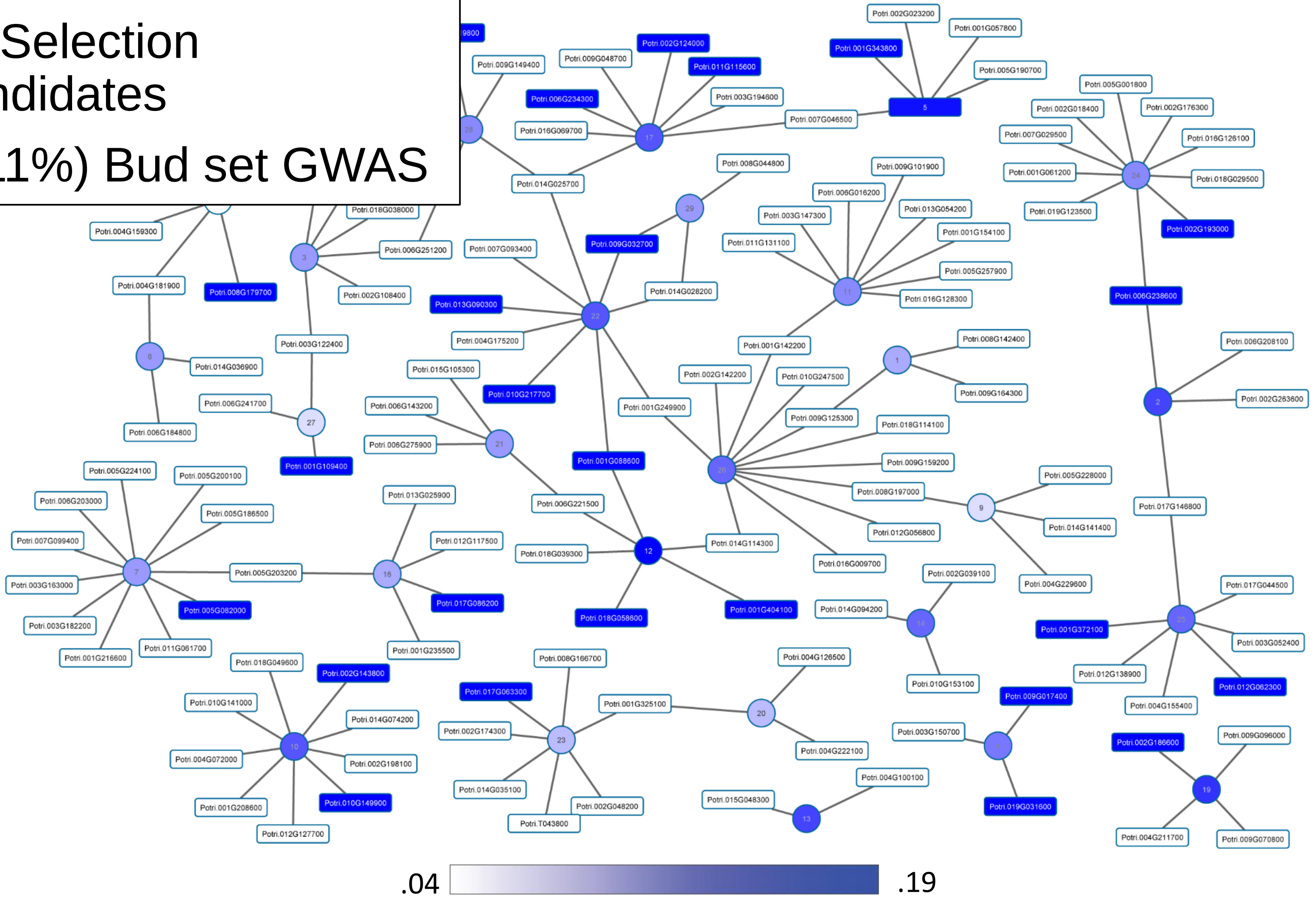
- Tale transcriptional regulator
  - Bud set associated gene
  - Selection candidate
  - Also regulator in the daylength bud network

Nutrient Bud Network - BS-GWAS

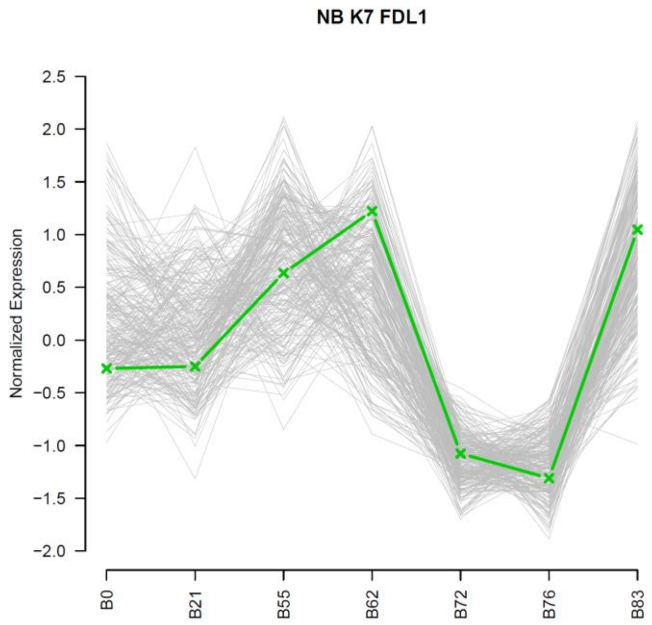


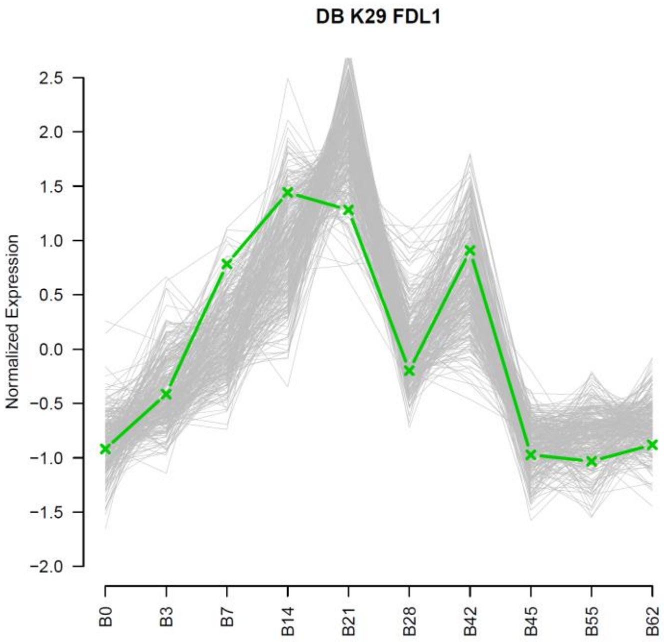
Day Length Bud – BS-GWAS

- 163 transcriptional regulators
  - 27 Bud set GWAS
  - 24 Selection candidates
- 529 (11%) Bud set GWAS



FDL1 is a predicted regulator in nutrient and daylength bud networks

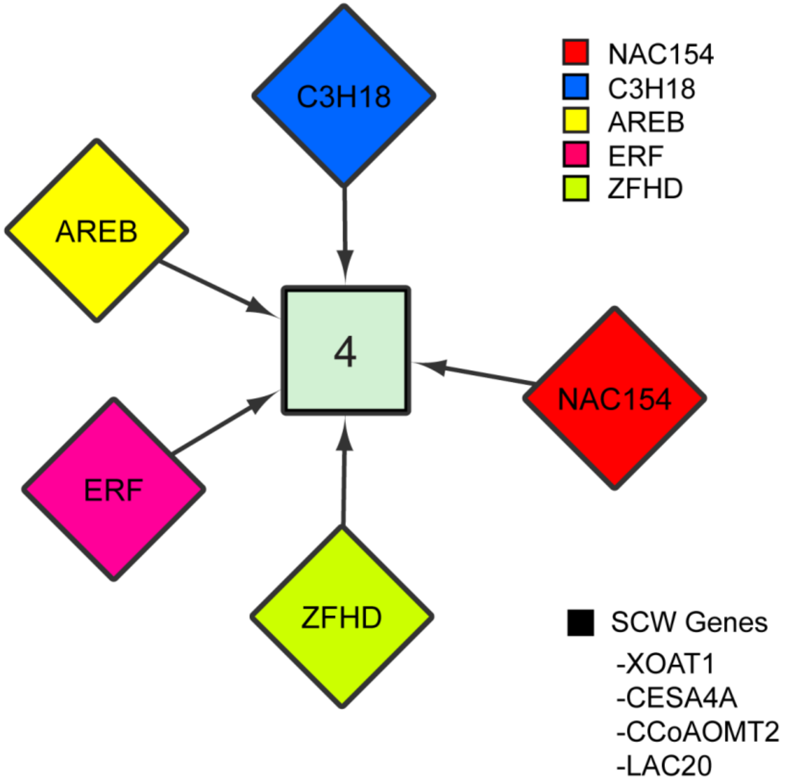

NB K7 FDL1

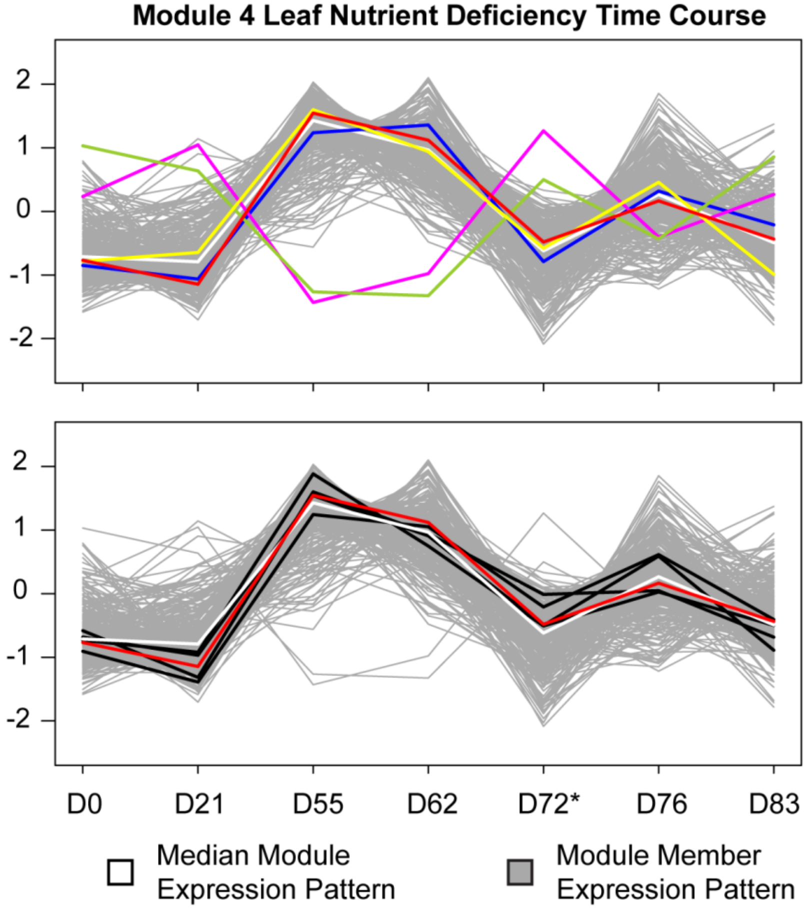

DB K29 FDL1

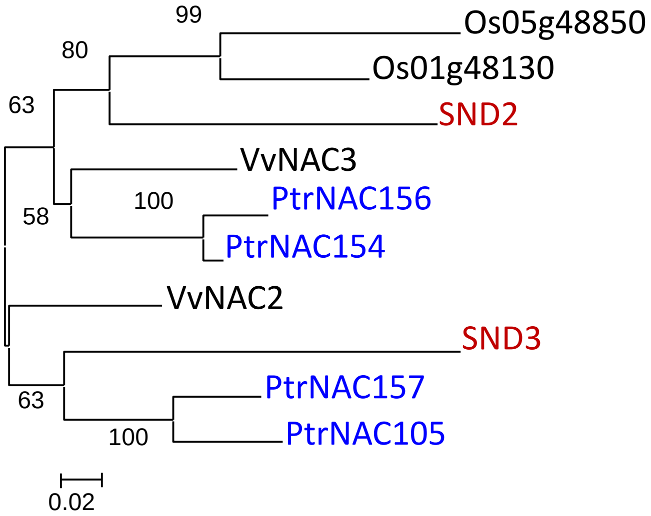
- Response to Chitin
- Regulation of JA signaling

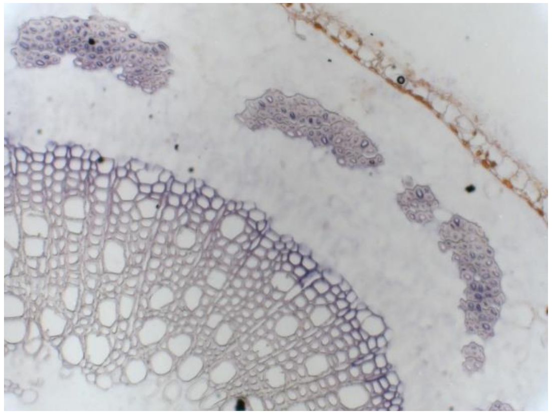
- Response to water stress
- Response to ABA

Module from leaf nutrient response GRN







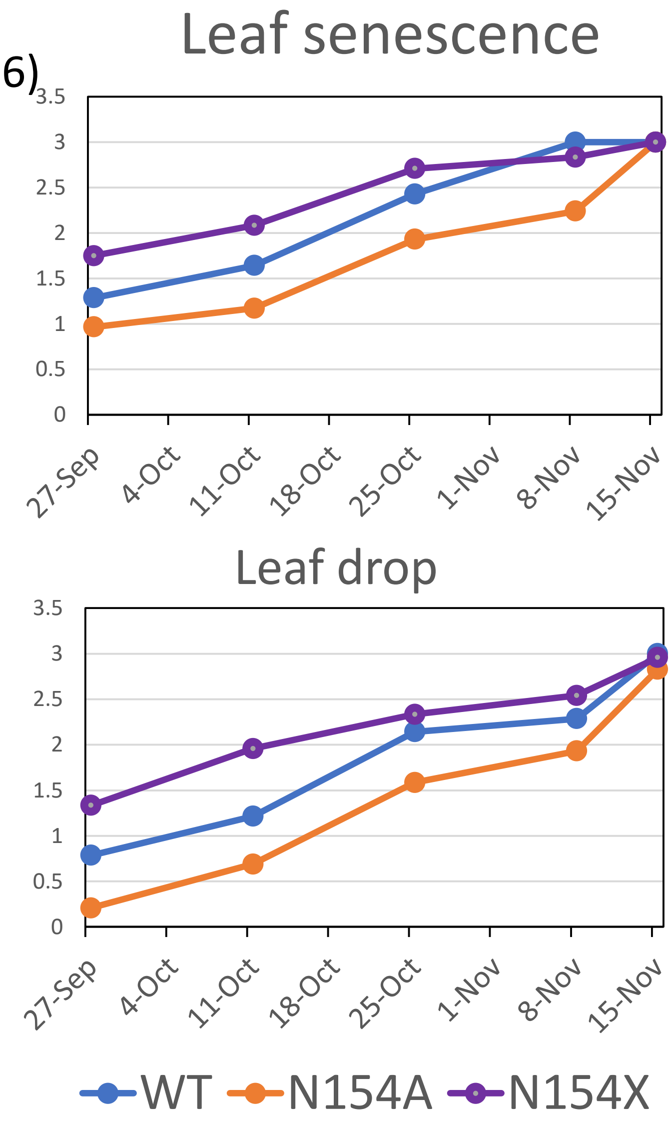


- *35S:NAC154* poplar (Grant et al. 2010; Jervis et al. 2015)
  - Reduced size
  - Elevated levels of arginine in stems

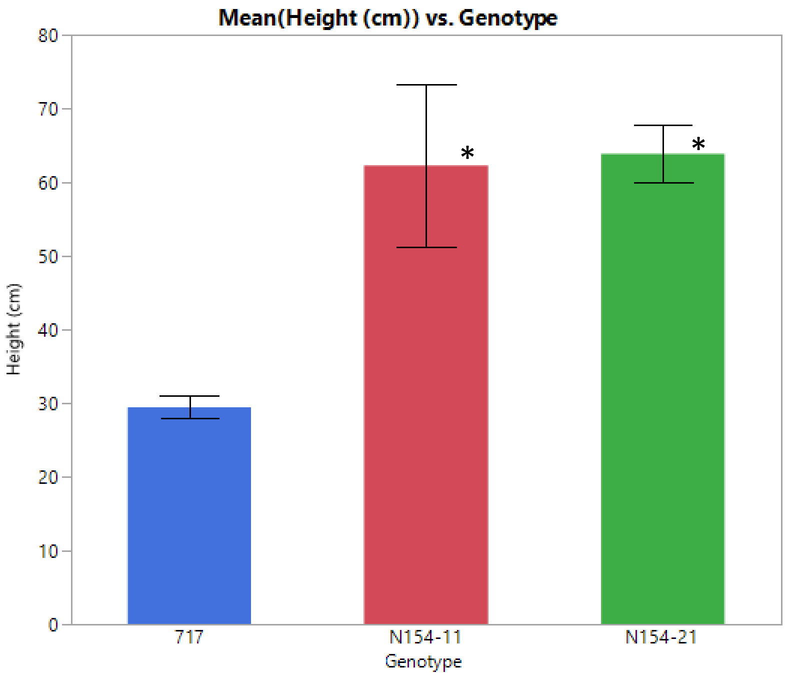
- *35S:PtrSND2-SRDX* poplar (Wang et al. 2013)
  - Reduced growth
  - Reduced secondary cell wall thickening

amiRNA-SND2 (targets paralogs NAC154 and NAC 156)

Field trial established Nov 2013, photo taken Dec 4, 2015

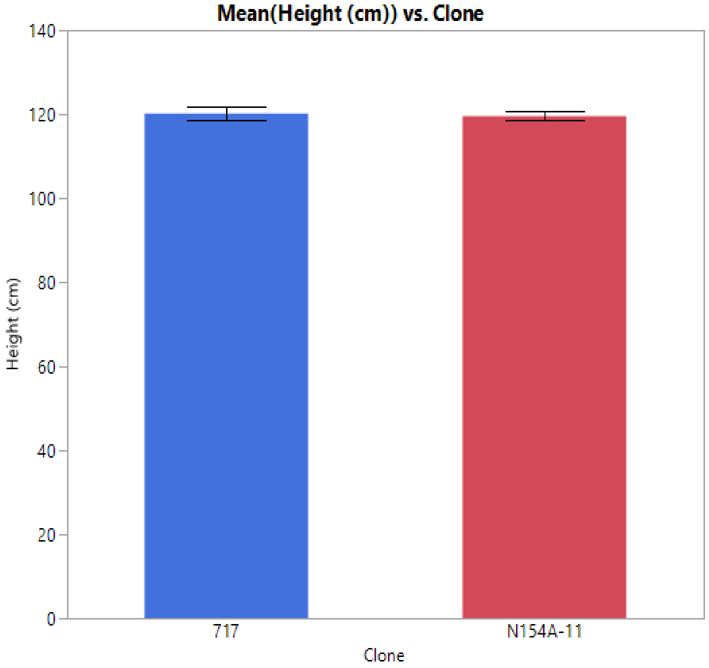


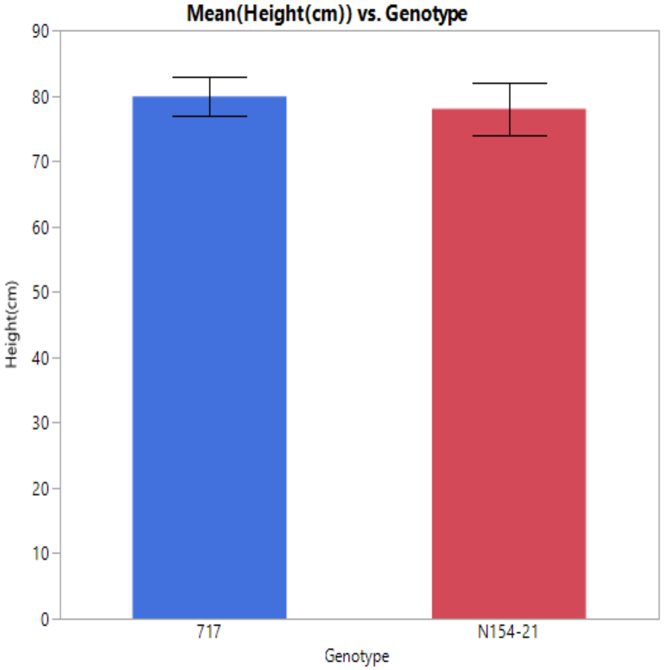
N154-LN study



P-value < 0.001

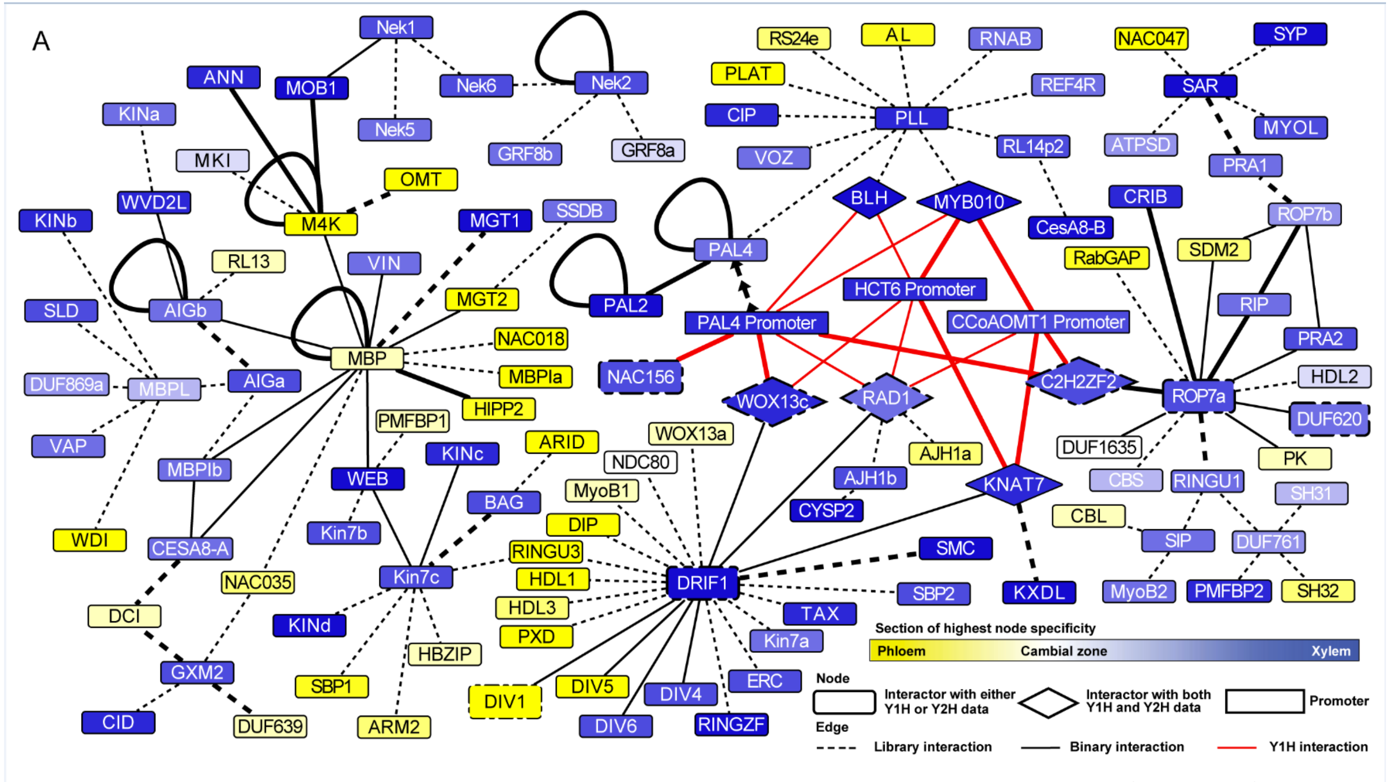
- In short days, amiRNA-SND2 transgenics cease growth and set bud the same time as WT
- After temperature lowered, leaf senescence and drop is slower in transgenics





Results consistent with presence of NAC154 in nutrient network, but not the daylength network

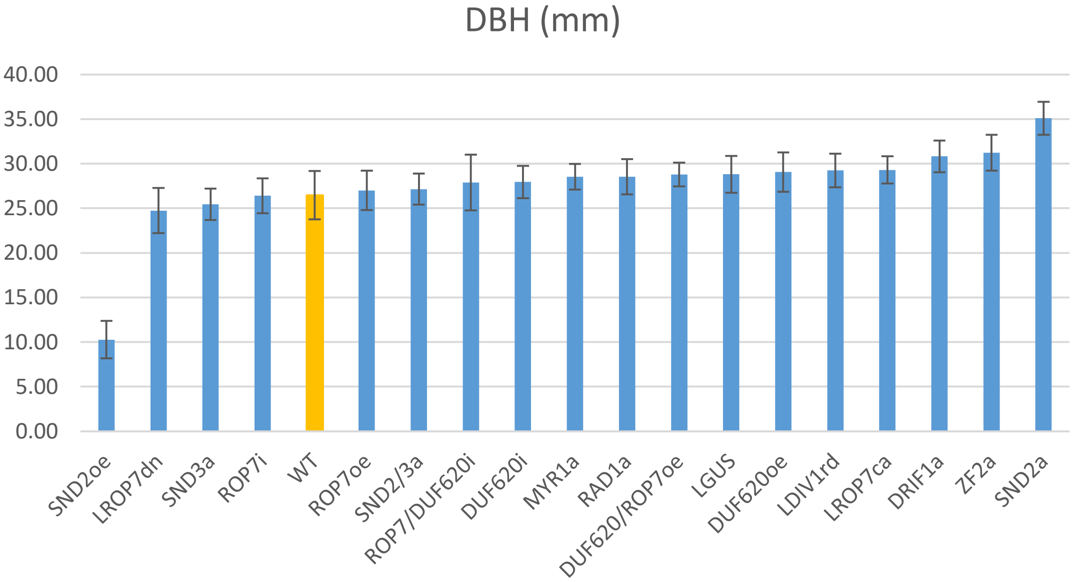
Protein-Protein and Protein-DNA interactions linked to wood formation



Petzold et al. 2018

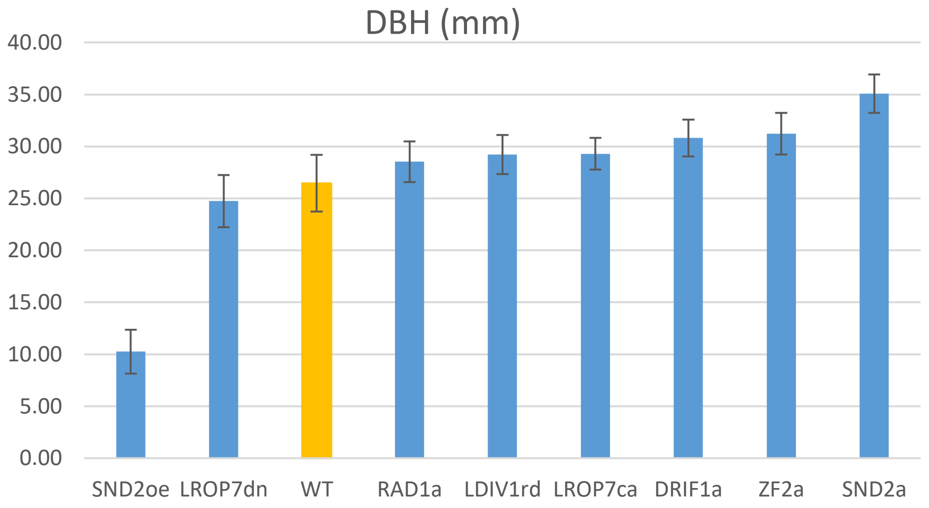
Did we identify new regulators of wood formation?

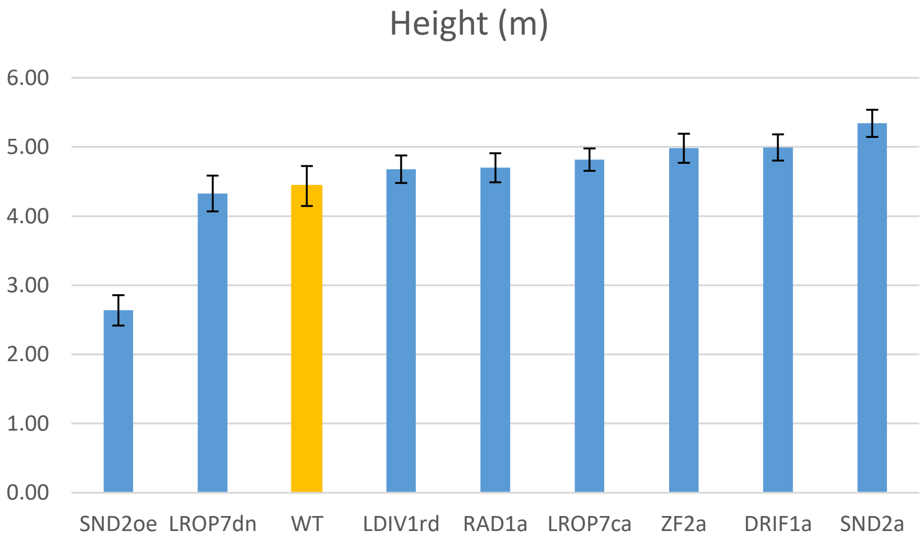
Transgenic field trial (Nov 2013-July 2017) included trees with 18 different constructs

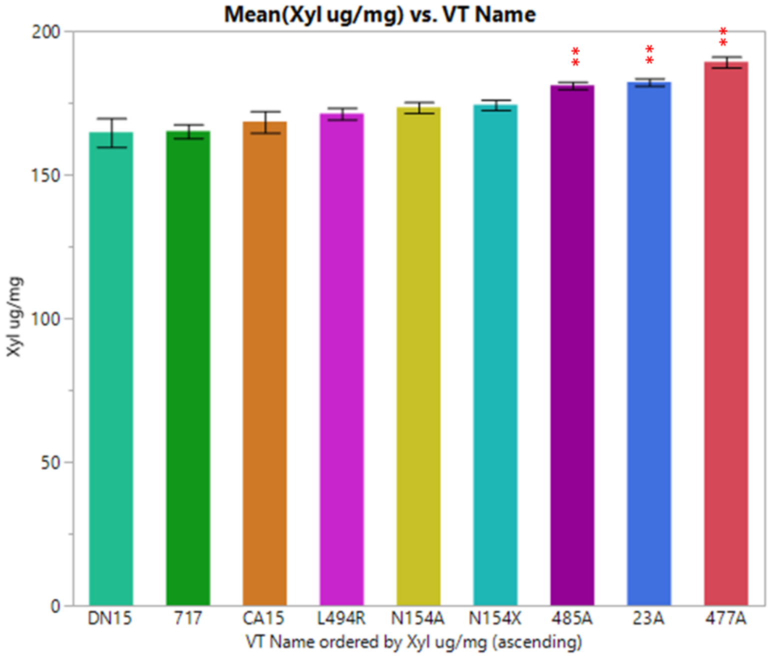


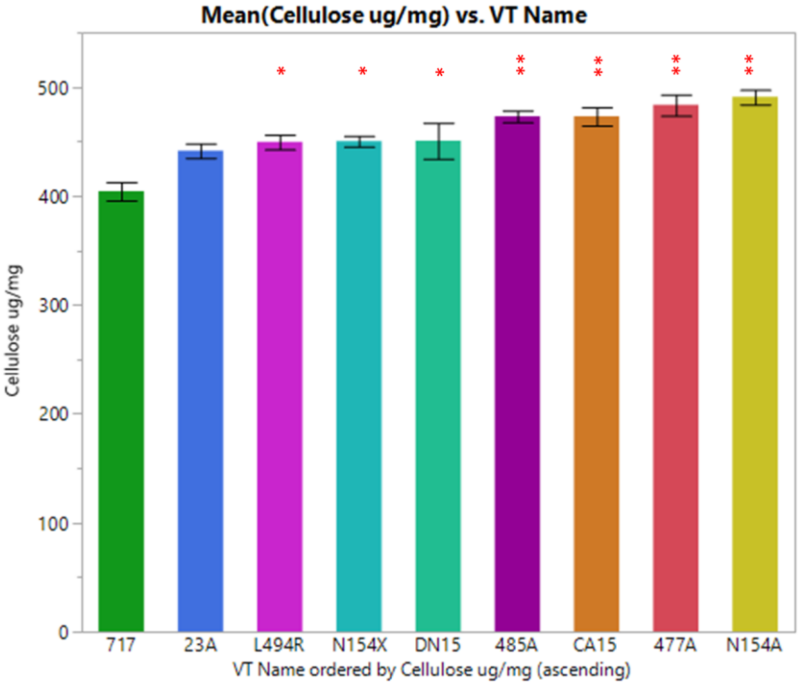
8 transgenic and WT were selected for wood chemistry analysis

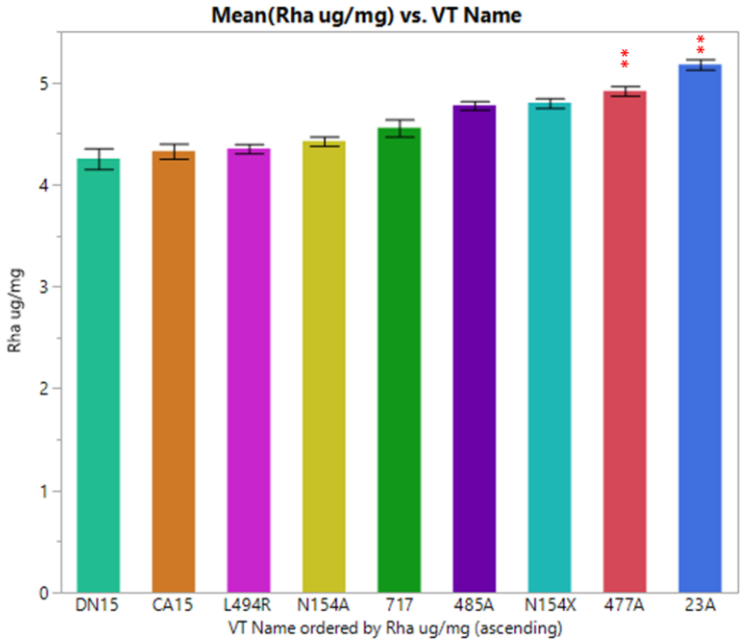
- Performed by the GLBRC

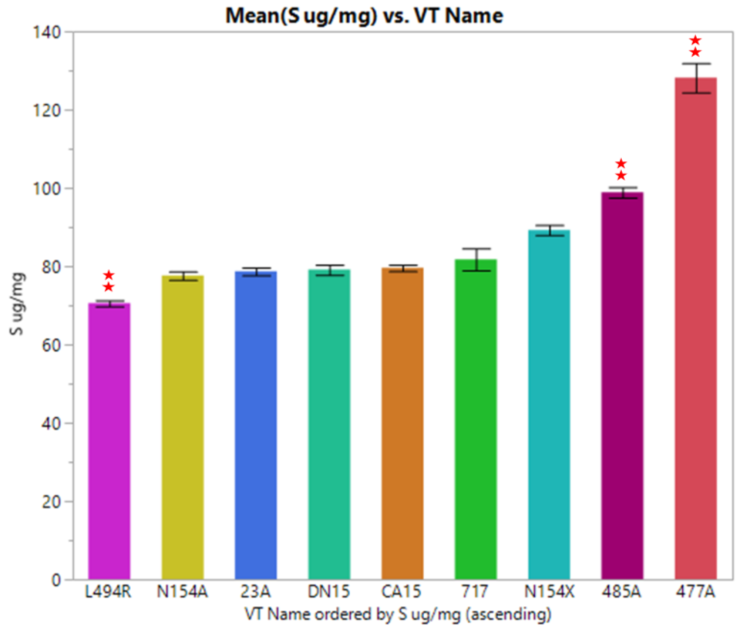


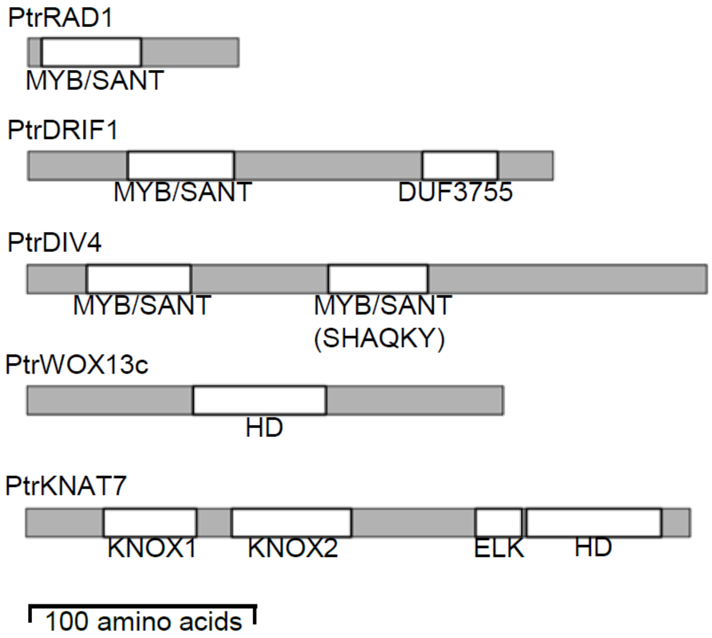


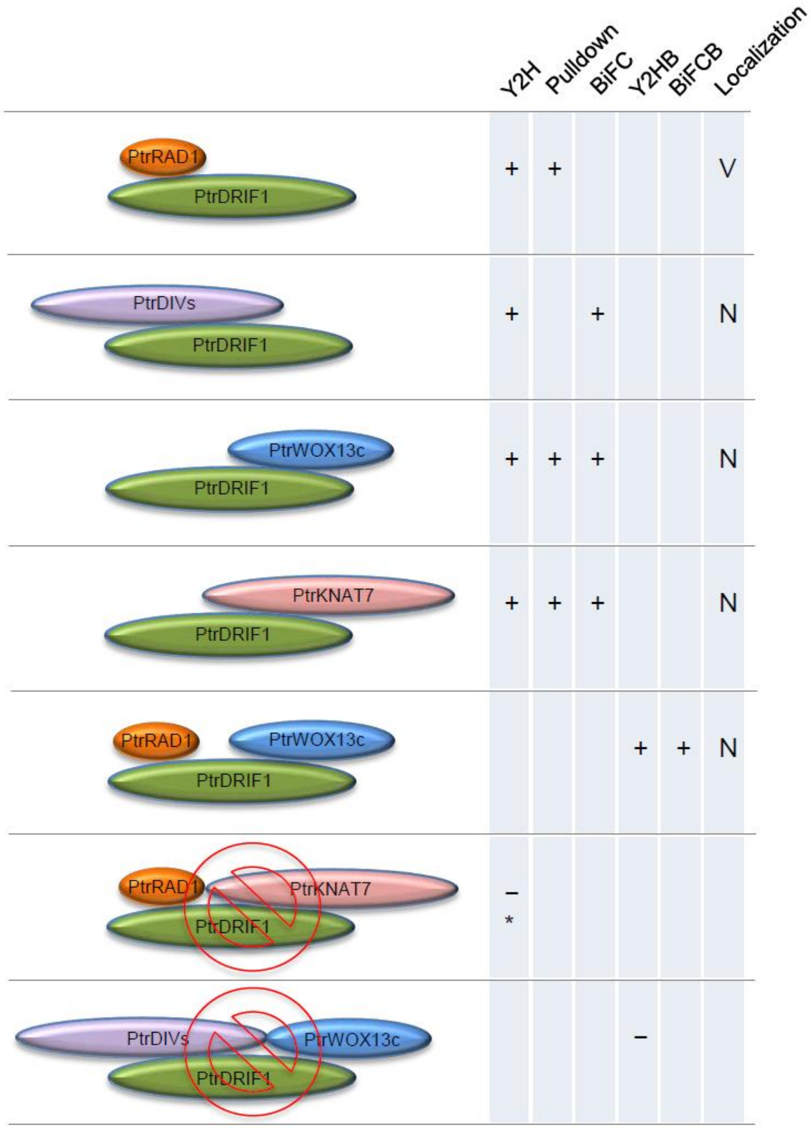

Mean(Xyl ug/mg) vs. VT Name
Xyl ug/mg
200
150
100
50
0
DN15
717
CA15
L494R
N154A
N154X
485A
23A
477A
VT Name ordered by Xyl ug/mg (ascending)


Mean(Cellulose ug/mg) vs. VT Name
Cellulose ug/mg
500
400
300
200
100
0
717
23A
L494R
N154X
DN15
485A
CA15
477A
N154A
VT Name ordered by Cellulose ug/mg (ascending)


Mean(Rha ug/mg) vs. VT Name
Rha ug/mg
5
4
3
2
1
0
DN15
CA15
L494R
N154A
717
485A
N154X
477A
23A
VT Name ordered by Rha ug/mg (ascending)


Mean(S ug/mg) vs. VT Name
S ug/mg
140
120
100
80
60
40
20
0
L494R
N154A
23A
DN15
CA15
717
N154X
485A
477A
VT Name ordered by S ug/mg (ascending)



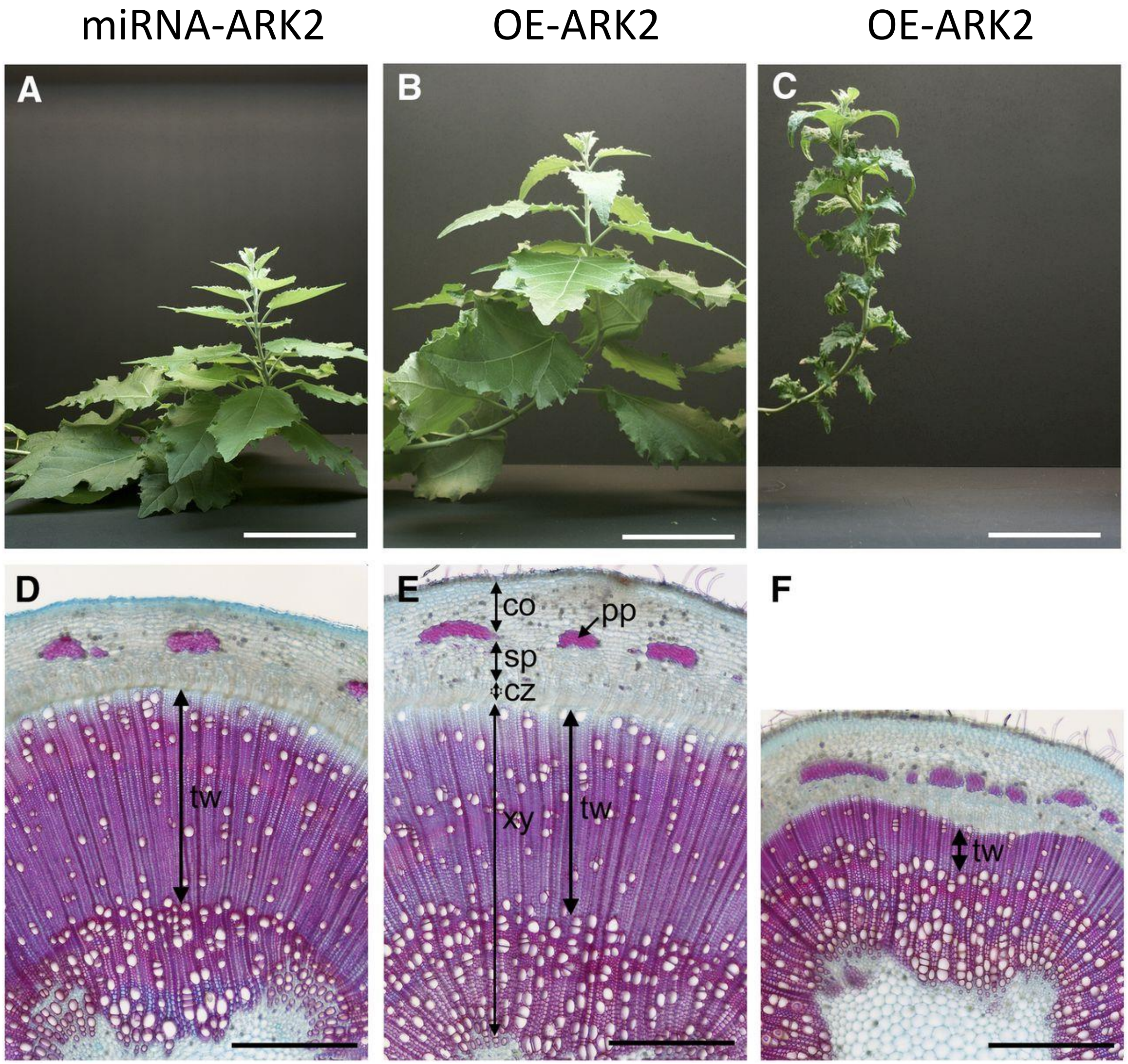


	Y2H	Pulldown	BiFC	Y2HB	BiFCB	Localization
PtrRAD1 PtrDRIF1	+	+				V
PtrDIVs PtrDRIF1	+		+			N
PtrWOX13c PtrDRIF1	+	+	+			N
PtrKNAT7 PtrDRIF1	+	+	+			N
PtrRAD1 PtrWOX13c PtrDRIF1				+	+	N
PtrRAD1 PtrKNAT7 PtrDRIF1	– *					
PtrDIVs PtrWOX13c PtrDRIF1				–		

Petzold et al. 2018

LMX5::DIV1-SRDX phenotypes

LMX5::DIV1-SRDX WT



Suzanne Gerttula et al. Plant Cell 2015;27:2800-2813

Summary comments

- GRNs and integration with GWAS shows potential
  - As less biased approach to advance understanding of these complex processes in trees
  - For improving precision in identifying genes with key roles in phenology and growth
- SCW genes can have important roles in growth not related to wood formation
- Protein-Protein networks can identify novel regulators/regulatory complexes

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