

Institute for Agricultural Biosciences



3210 Sam Noble Parkway, Ardmore, OK73401

<http://www.oces.okstate.edu/institute-for-agricultural-biosciences>

Tadege's Lab Research Focus

Biomass Functional Genomics

Our ultimate aim is to understand the molecular basis of biomass accumulation in switchgrass and sorghum by dissecting plant developmental programs.

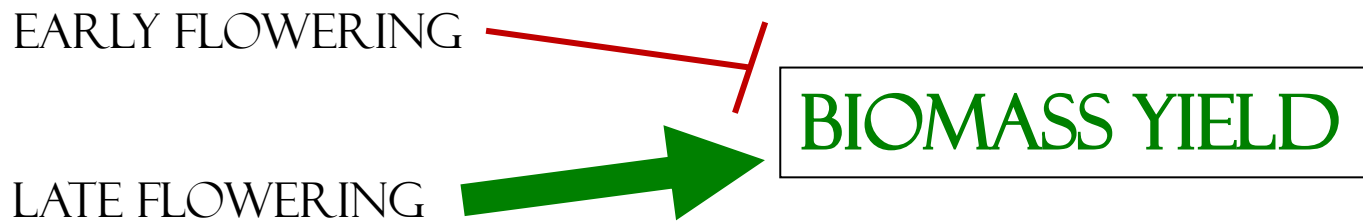
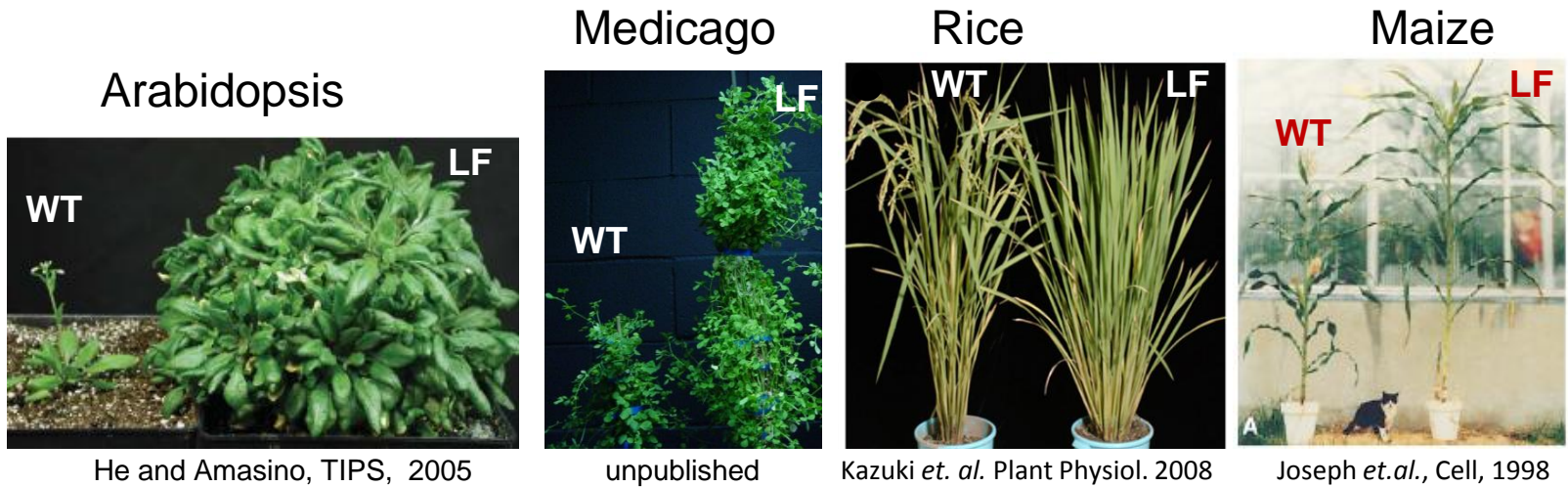
We focus on two developmental programs for this talk.

- Control of flowering time.
- and
- Regulation of leaf blade development.

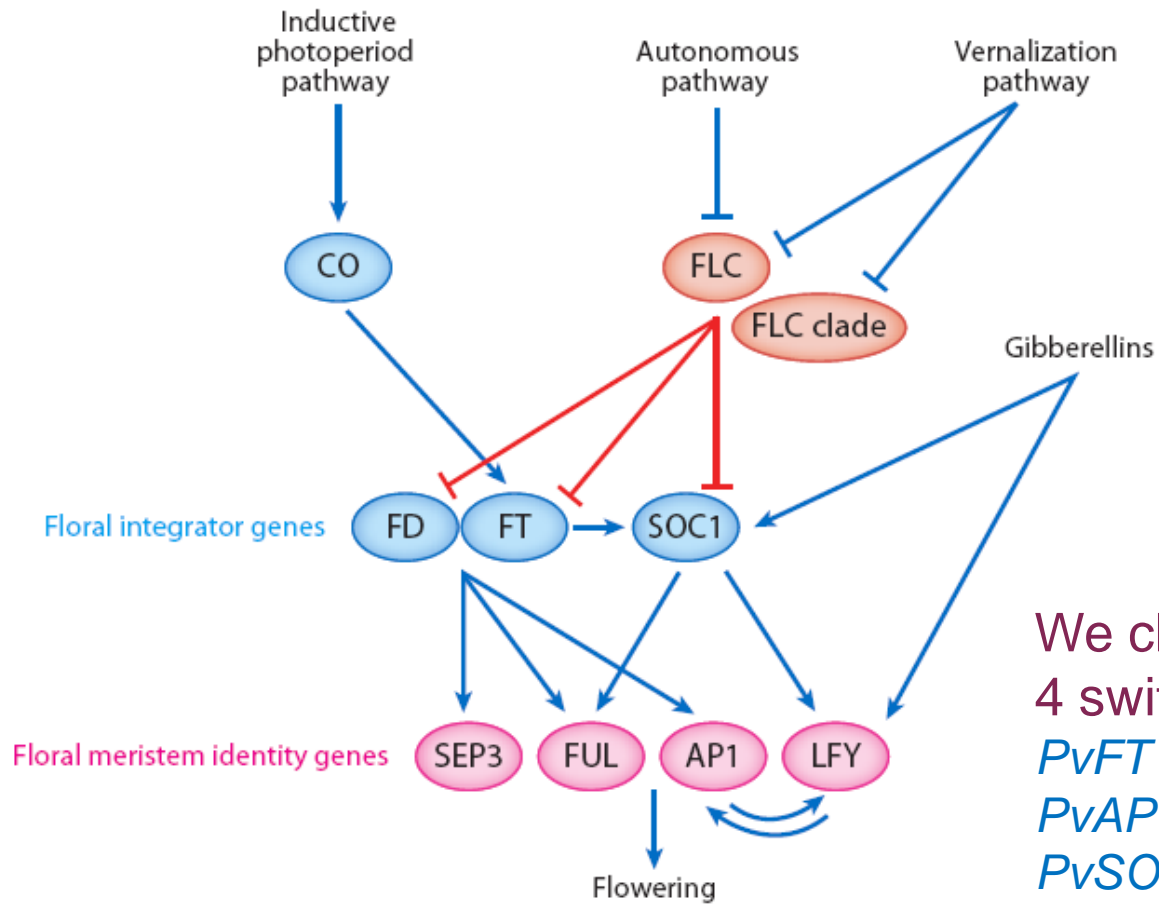
We use models to facilitate our study

CONTROL OF FLOWERING TIME

Delaying the onset of flowering leads to increase in total vegetative biomass accumulation in several species provided that the delaying is not caused by other developmental defects. Consider the following examples:



Flowering pathways in Arabidopsis



We chose to focus on 4 switchgrass genes:

PvFT

PvAP1

PvSOC1

PvID1 – homolog of maize *ID1*

Kim et al. *Annu. Rev. Cell Dev. Biol.* 2009.

Strategy of cloning target genes from switchgrass

Step 1

```

SbSLR1 TGACCGCTTACCGAGTCCCTGCACTACTACTCCACCATGTTGATTCCTCTCGAGGGCG 1537
ZnSLR1 CGACCGCTTACCGAGTCCCTGCACTACTACTCCACCATGTTGATTCCTCTCGAGGGCGC 1532
CsSLR1 CGACCGGTTACCGAGTCCCTGCACTACTACTCCACCATGTTGATTCCTCTCGAGGGCGG 1526
TaSLR1 GGACCGCTTACCGAGTCTCTGCACTACTACTCCACCATGTTGATTCCTCTCGAGGGCGG 1508
*****

SbSLR1 -----CCGGCTCCGGCCAAATCCACCGATGCCTCTCCGGCCCGCGC-----CCG 1580
ZnSLR1 CCGCGCCCGGCTCCGGCCAGTCCACCGACGCTCCCGCGCCCGCGC-----CCG 1580
CsSLR1 CAGCTCCGGCCAGS-----CCGACTCTCTCCCGCCGCTCCCGGGG-----CCGGCG 1574
TaSLR1 CAGCTCCGGCCCGCCCATCCGAGTCTCATCCGGGCTGCTGCTGCTCCTCCCGCCCGC 1568
*****

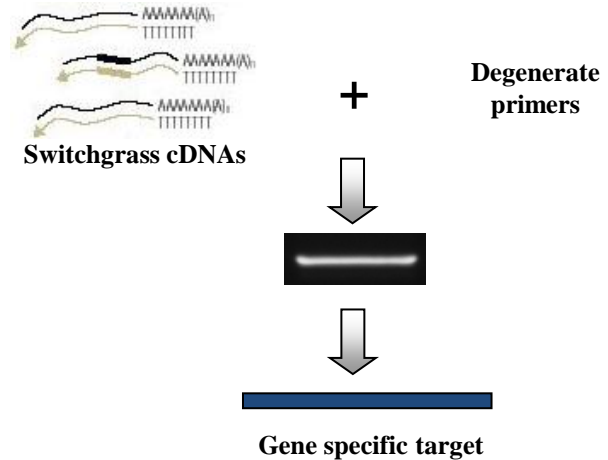
SbSLR1 CCGCACCGAACCGGTCATGTCGAGGTTGTAOCTGGCCCGCCAGATCTGCAACGTTGTGEC 1640
ZnSLR1 CCGCACCGAACCGGTCATGTCGAGGTTGTAOCTCGCCCGCCAGATCTGCAACGTTGTGEC 1640
CsSLR1 TGCCACCGAACCGGTCATGTCGAGGTTGTAOCTCGCCCGCCAGATCTGCAACGTTGTGEC 1634
TaSLR1 CCGCACCGAACCGGTCATGTCGAGGTTGTAOCTCGCCCGCCAGATCTGCAACGTTGTGEC 1628
*****

SbSLR1 ATGTGAGGGCCCGGAGCCGACCGAGCCGACCGAGACGCTGAGTCAGTGGCCCGCCCGCCCT 1700
ZnSLR1 GTGCGAGGGCCCGGAGCCGACCGAGCCGACCGAGACGCTGAGTCAGTGGCCCGCCCGCCCT 1700
CsSLR1 GTGCGAGGGCCCGGAGCCGACCGAGCCGACCGAGACGCTGAGTCAGTGGCCCGCCCGCCCT 1694
TaSLR1 CTGCGAGGGCCCGGAGCCGACCGAGCCGACCGAGACGCTGAGTCAGTGGCCCGCCCGCCCT 1688
*****

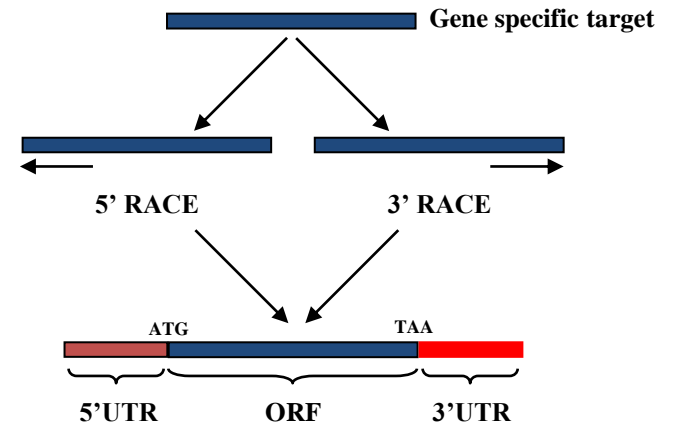
SbSLR1 CGTCCGCTCCGGGTTGAGCCCGTGCACCTGGGCTCCCAATGCTACAAGCAGCGGAGC 1760
ZnSLR1 CCGCCGCTCCGGGTTGAGCCCGTGCACCTGGGCTCCCAATGCTACAAGCAGCGGAGC 1760
CsSLR1 CCGCCCGCCCGGCTTGGAGCCCGTGCACCTGGGCTCCCAATGCTACAAGCAGCGGAGC 1754
TaSLR1 GGGCAACCGCCGGTGGAGCCCGTGCACCTGGGCTCCCAATGCTACAAGCAGCGGAGC 1748
*****

SbSLR1 GCTGCTGCACTCTTCAACCGCCGACCGGTTACAGGGTGGAGGAGGACCGGGTGCCT 1820
ZnSLR1 GCTGCTGCGCTCTTCCCGCCCGGACCGGTTACAGGGTGGAGGAGGACCGGGTGCCT 1820
CsSLR1 GCTCCTCGGCTTTTCCCGCCCGGACCGGTTACCGGGTGGAGGAGGAGGGCTGCCT 1814
TaSLR1 GCTGCTGCGCTCTTCCCGCCCGGACCGGTTACAGGGTGGAGGAGGAGGCTGCCT 1808
*****
    
```

Step 2



Step 3



Phylogenetic analysis of orthologs of the FT, AP1, SOC1 and ID1 proteins

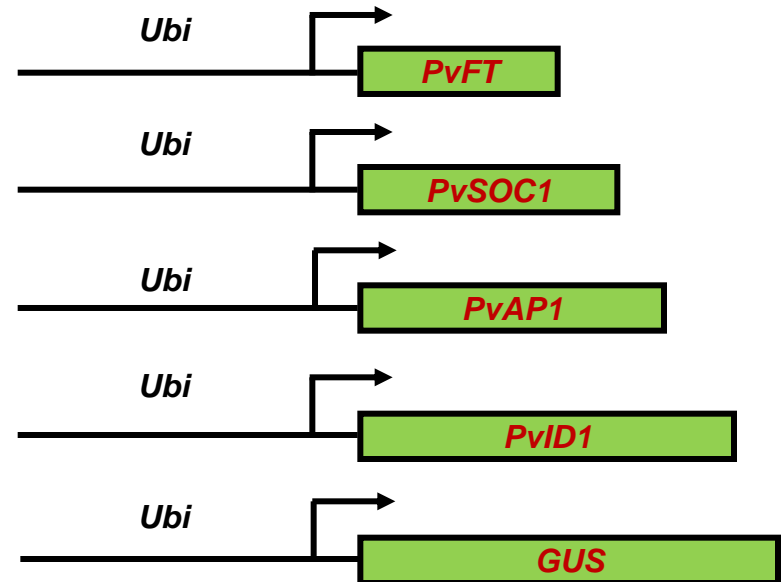
AP1

SOC1

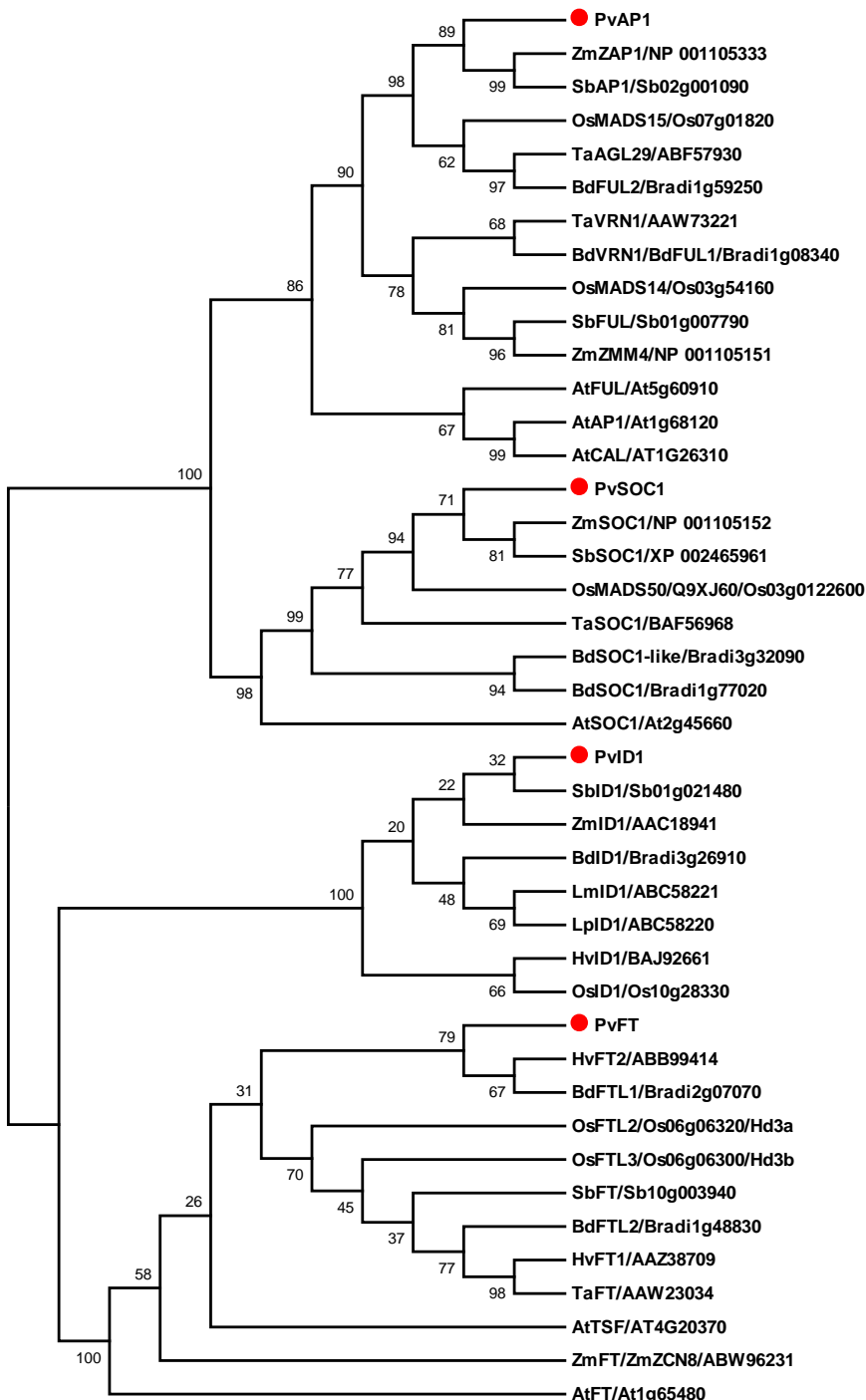
ID1

FT

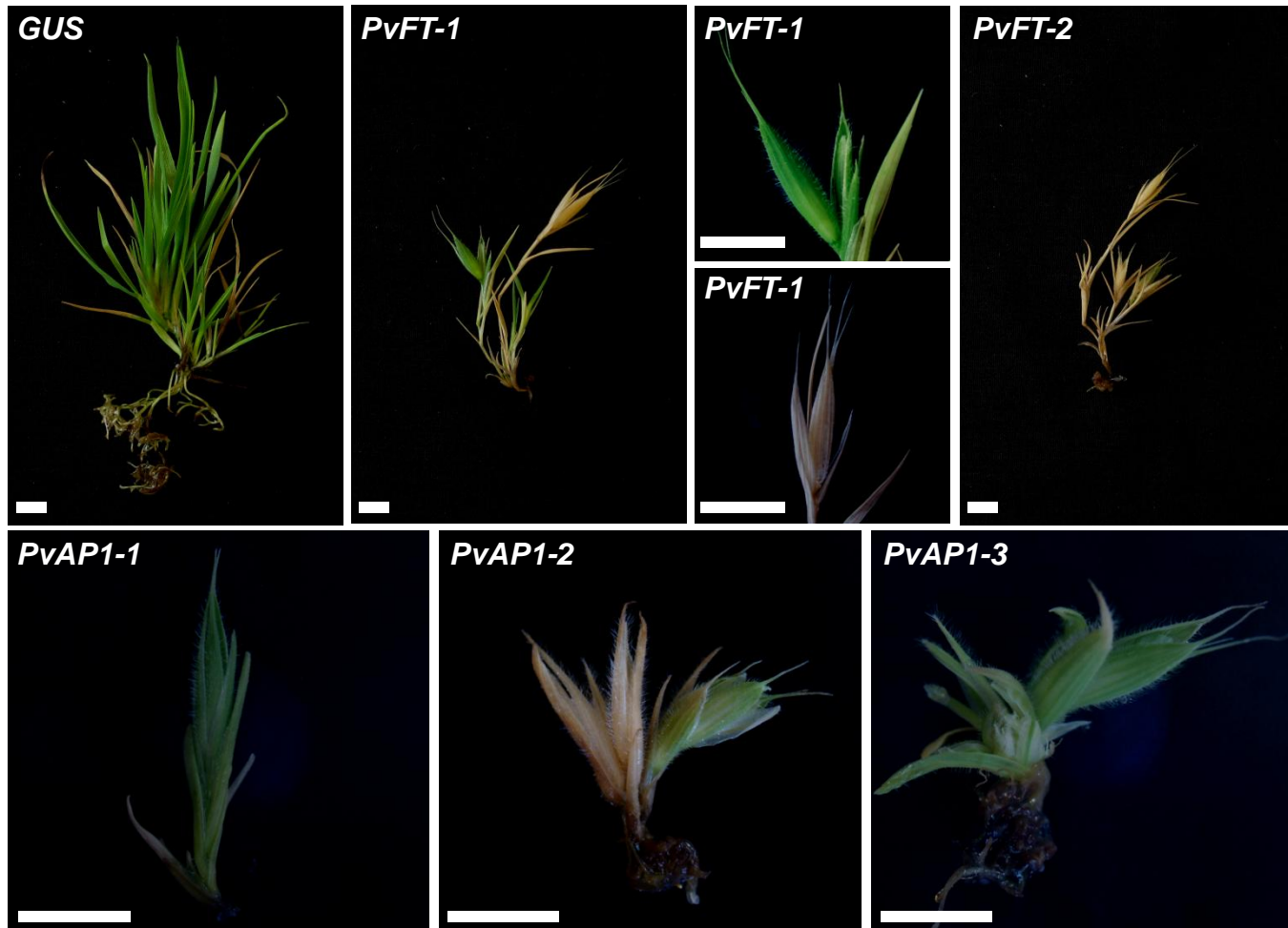
Constructs introduced into *Brachypodium*



Brachypodium distachyon



Ectopic expression of *PvFT* and *PvAP1* cause extremely early flowering in *Brachypodium*



Bar=0.5cm

Planning for the next step

- Complement Arabidopsis late flowering *ft* and *ap1* mutants
- Silence *PvFT* and *PvAP1* by RNAi in switchgrass
 - Zeng Yu Wang.
- Analyze total biomass, cell wall composition and digestibility in transgenic plants.
- Map *PvFT*, *PvAP1*, *PvSOC1* and *PvID1* genes in switchgrass mapping population and use as molecular markers to select for late flowering and high biomass yielding lines.
 - Yanqi Wu.

STF mediated regulation of leaf blade expansion

Why bother?

Leaves are the major organs of photosynthesis and gaseous exchange in plants

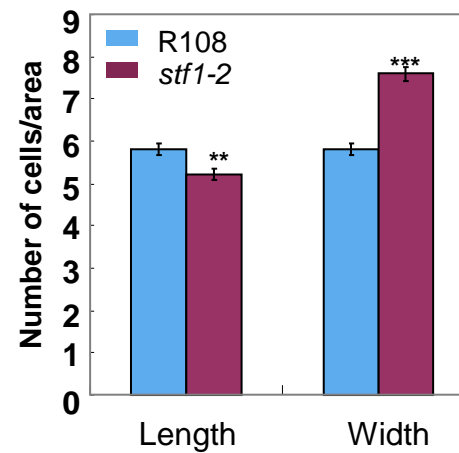
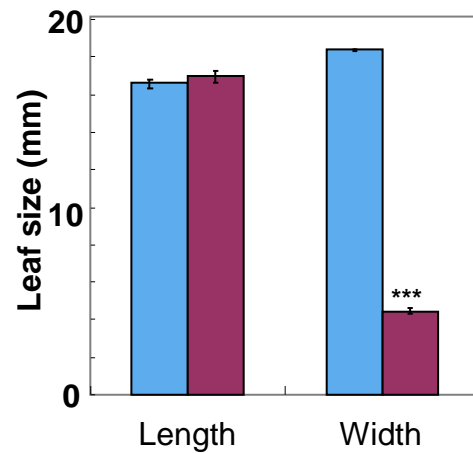
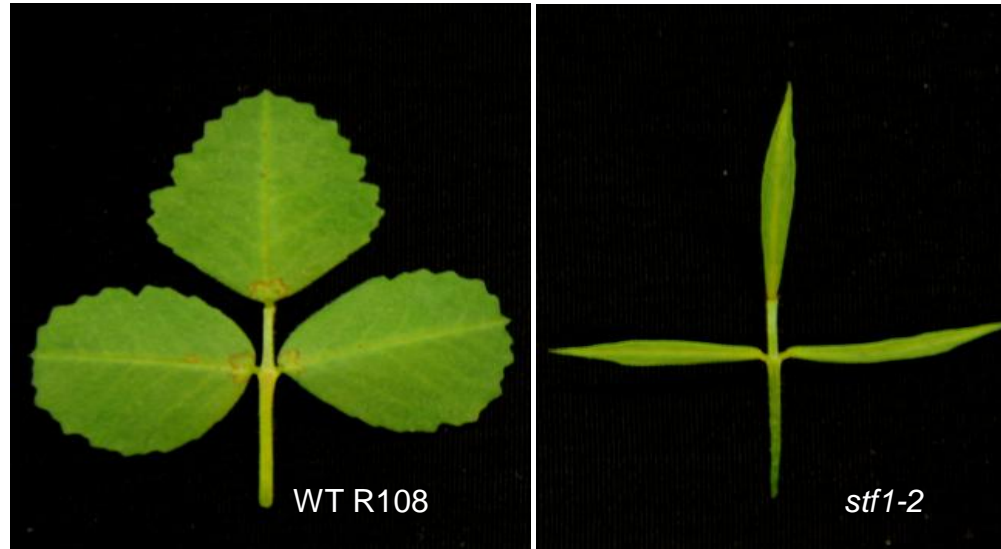


Carbon dioxide + light energy \longrightarrow chemical energy + oxygen

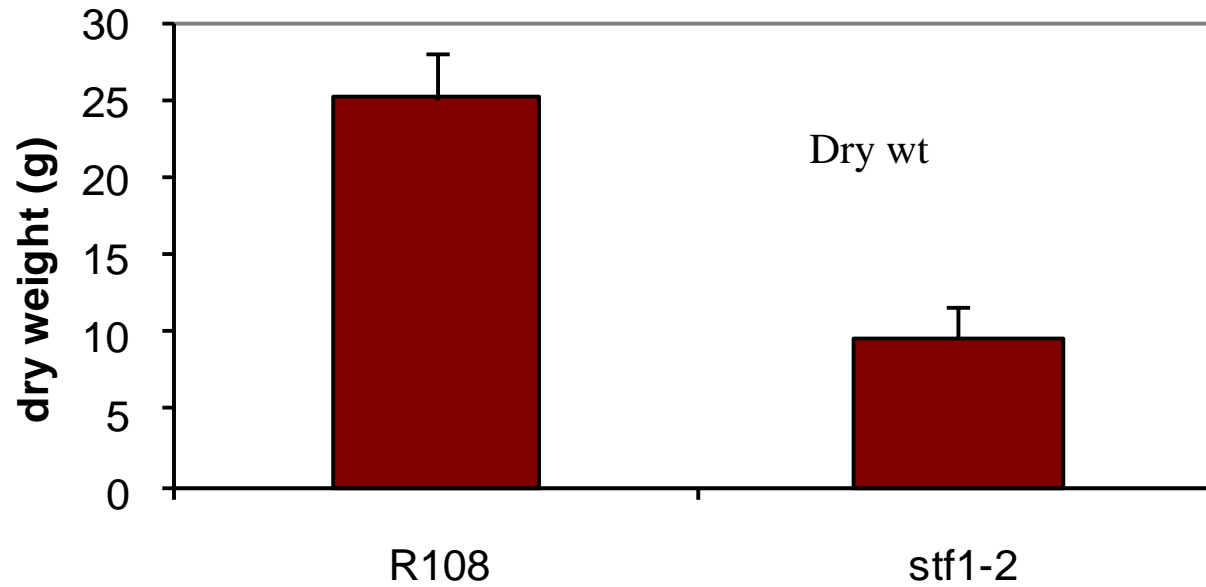
- Solar panel – chemical energy for making biomass.
- CO₂ sink – reduces environmental pollution.
- O₂ release – fresh air for aerobic life.

Leaf blade is the biomass synthesis and transpiration storehouse.

The *stf* mutation in *Medicago* deletes most of the lamina

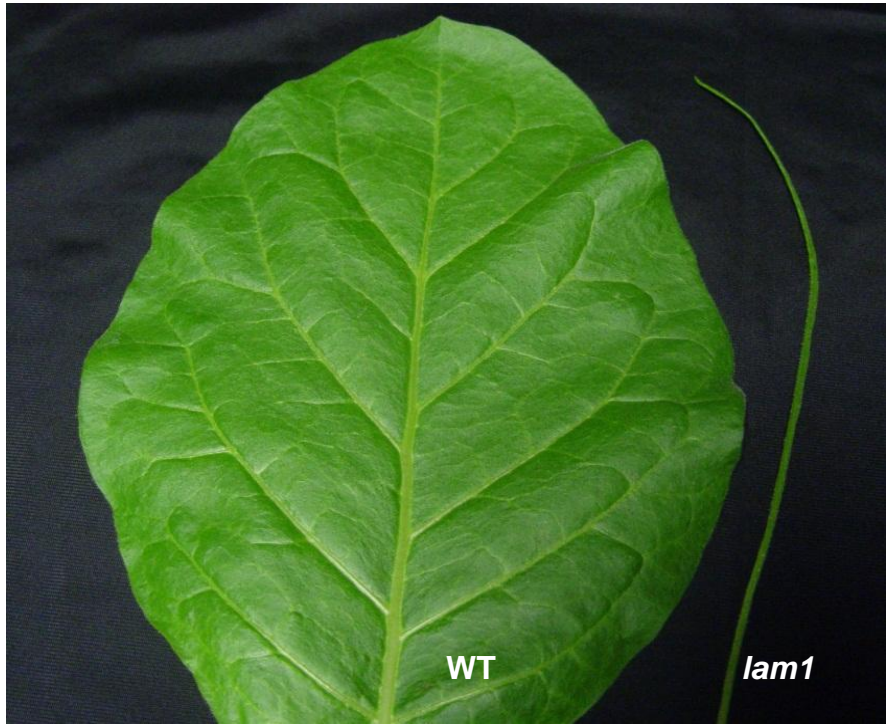


Effect of the *stf* mutation on total plant biomass



Stem
thickness

The effect on biomass is even worse in the *lam1* mutant of tobacco



leaves



Adult plant

How does *STF/LAM1* regulate blade outgrowth?

STF-like proteins have a conserved motif at the C-terminus



| | | | |
|---------------|---|---|-----|
| STF | 1 | MWMVGYNEGGEFNMADYFSG-RKLRPLIPR--PVPVPTTSPNNTS--TITPSLNRIHGNDLFSQYHNLQQAASVGDHHSKRSELNNNNNPAAVVVSSRWNPTEQLRALEELYRRGTR | 116 |
| VvSTL1 | 1 | MWMMGYNDGGEFNM-PDSFNG-RKLRPLIPR-----PATSPSGTATTSSCLSRING-TDLFCNLNHLLE-----QSNIR-EFNTPFV-----IVSSRWNPTEQLRLEELYRRGTR | 98 |
| GmSTL1 | 1 | MWMVGYNEGGEFNMADYFSG-RKLRPLMPR-----PVTSPNNTSNTNSPCLSRIRHGGNDFFSQYHNLVADQ-----GKR-EFNPP-----PVSRRWNPTEQLRALEELYRRGTR | 101 |
| MsSTL1 | 1 | MWMVGYNEGGEFNMADYFSG-RKLRPLMPRPVVPVPTTSPNNTS--TITPSLNRIHGNDLFSQYHNLQQAASVGDHHSKRSELNNN--PAAVVVSSRWNPTEQLRALEELYRRGTR | 112 |
| NsSTF1 | 1 | MWMMGYNDGGDFNM-ODSFNG-RKLRPLMPR-----VPHLETANISTNPTCLRSIHG-ENFVALNHHQLAMS-----EONKR-DFNTQQL-----VSSRWNPTEQLQTEELYRRGTR | 101 |
| RcSTL1 | 1 | MWMMGYSDGGEFNMTADSFNG-RKLRPLIPR-----PGPNSNTNNTSNSSPPLSRILHG-TDFSLNHHLATMA-----DOSKR-DFHTQPV-----VSSRWNPTEQLRALEELYRRGTR | 104 |
| PxhMAW | 1 | MWMMGYNDGGDFNM-PDSFNG-RKLRPLMPR-----IPHVPTATSSTN--CLRSIHG-DNFIALNHHQLGMS-----EPNKR-DYNTQQL-----VSSRWNPTEQLQTEELYRRGTR | 99 |
| MeSTL1 | 1 | MWMMGYNDGGEFNM-ADSFNG-RKLRPLVPR-----PPVPSPTTSSSSPPRLSRIRHG-NDFSLNHHLATMA-----DOSKR-EFHTQPV-----VSSRWNPTEQLRLEELYRRGTR | 102 |
| AtWOX1 | 1 | MWTMGYNEGG-----ADSFNGRKLRPLIPR-----LTSCPTAAVNTNSDHRFNMAVVTMTAEQNKRELMLL-----NSEPQHPPV-----MVSSRWNPTEPDQLRVLEELYRGT | 96 |

| | | | |
|---------------|-----|--|-----|
| STF | 117 | TPSAEQIQITTAQLRKFQKIEGKNVYWFQNHKARERQKRRROMESA-----AAEFDSAIEKKDL--CASRTVFEVEHTKNWLPSTNSSTSTLPLAEESVSIQRAAAA | 217 |
| VvSTL1 | 99 | TPSAEQIQIHTAQLRRYKIEGKNVYWFQNHKARERQKRRROLE-----PDE-----QNRDVESTERKES--GGRTGFE-EOTKNWALSTNCSEI-----LAEESLSIQRAAKA | 195 |
| GmSTL1 | 102 | TPSAEQIQITTAQLRKFQKIEGKNVYWFQNHKARERQKRRROMESA-----EGH-----HTRDFDSTIEKKDL--CASRTVFEVDOTKNWAPSTNCST-----LAEESVSIQRAAKA | 203 |
| MsSTL1 | 113 | TPSAVQIQIHTAQLRKFQKIEGKNVYWFQNHKARERQKRRROMESA-----AAEFDSALEKKDL--CASRTVFEVEHTKNWLPSTNSSTSTLPLAGEESVSIQRAAAA | 212 |
| NsSTF1 | 102 | TPSAEQIQIHTAQLRRYKIEGKNVYWFQNHKARERQKRRROLESAAAGGGAANAAGGGDDQSRSNCPNENTERKES--CANRTGFEETEOTKNWPSFTNCSTLA-----EKTVAATKAAA | 215 |
| RcSTL1 | 105 | TPTTEQIEHITKQLRRYKIEGKNVYWFQNHKARERQKRRROMESA-----PDHDQQQQQHHDIEIIEKES--EANRTGVEGEOTKNWPPSTNCST-----LPEESISIHRAAKA | 211 |
| PxhMAW | 100 | TPSAEQIQIHTAQLRRYKIEGKNVYWFQNHKARERQKRRROLESNA-----ANDGGGGDEQSRNNCNVENAERKDSGACANRTGFEIEOTKNWPSFTNCSTLSP--EKTVAATKAAA-- | 211 |
| MeSTL1 | 103 | TPSAEQIQITTAQLRKFQKIEGKNVYWFQNHKARERQKRRROMESA-----PEH--QHQQQNRDIEIHERKES--CANRTAYEVEOTKNWAPSTNCST-----LEEDMTIQREAKG | 207 |
| AtWOX1 | 97 | TPSADIIQIHTAQLRKFQKIEGKNVYWFQNHKARERQKRRROMETIGH-----EETVLSTASLVSNHGFDDKDPGKYKVEQVKNWICSVGCDTQ-----PEKPSRDYHLEEP | 198 |

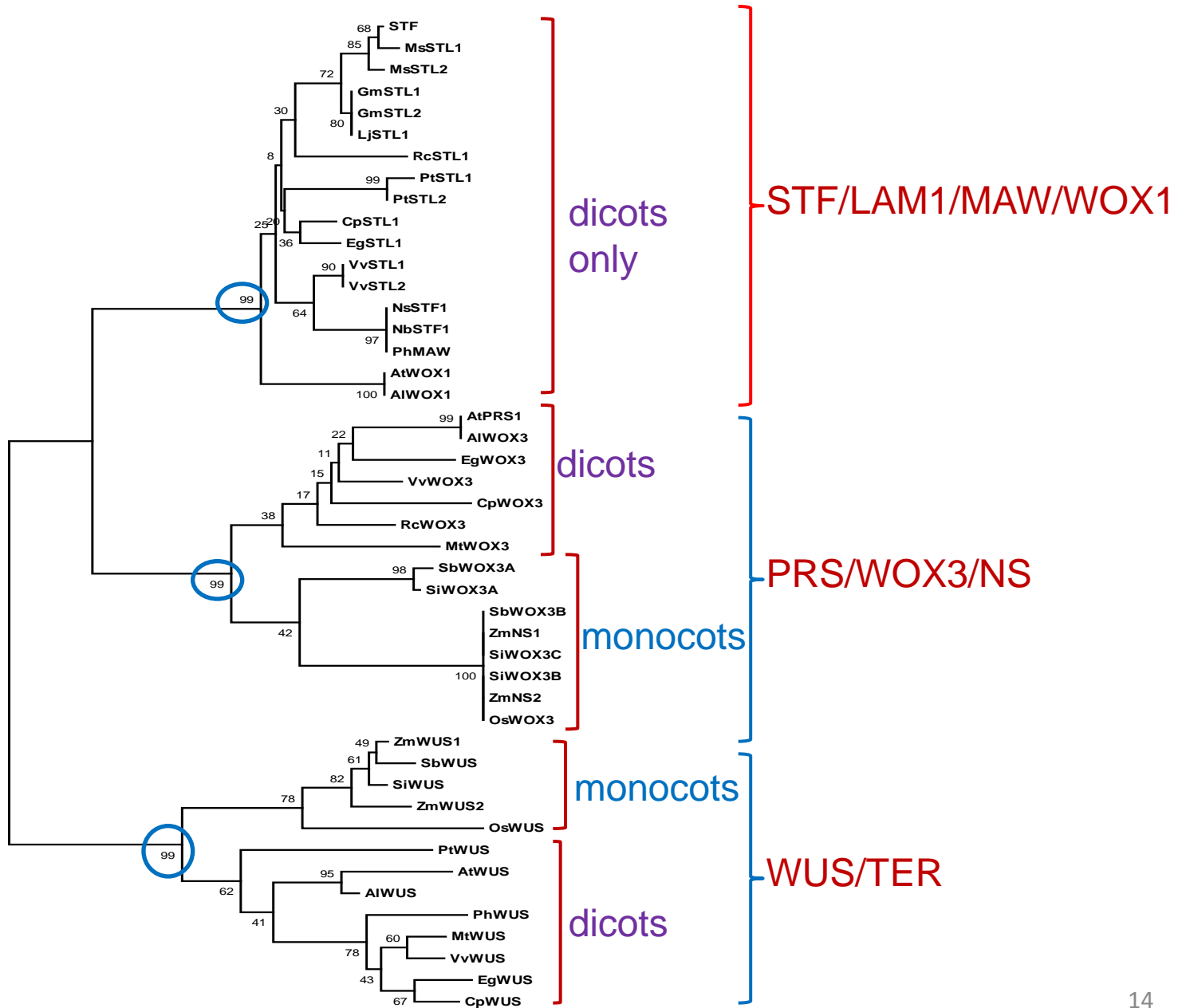
| | | | |
|---------------|-----|---|-----|
| STF | 217 | -----KADGWLOFD-EAELQ-QRR--NEMERNATWHMMQLTSSCP-----TASMSTTT-----TVTTRLMDP--KLIKTH-EL | 278 |
| VvSTL1 | 196 | TAAAAECR-TDGIWLOFD-EGELQ-HRR--SIVERNATWQMMQLSCPS-----TTLINTTTTTRT-----KEAAAVRMDP--KLIKTR-DL | 270 |
| GmSTL1 | 204 | A--IAECRTDGIWLOFD-EGELQ-HRR--NEMERNATWHMMQLSCPPPTVSPHLINTSPITSTTSMATAT-----TVTARLMDP--KLIKTH-DL | 284 |
| MsSTL1 | 212 | -----KADGWLOFD-EAELQ-QRR--NEMERNATWHMMQLTSSCP-----TASMSTTT-----TVTTRLMDP--RLIKTH-EL | 273 |
| NsSTF1 | 216 | GGVAECRVAARWIPFD-EGE--QRRSLLADORNATWQMMHLSCSPPST-----TTPHHLMNINSSSTAASNTISGTTSPICSSNT-----PSTPRTTMEPK-QEFKTKDHL | 316 |
| RcSTL1 | 211 | --VAEACRAGDGLIOPD-EVEFQ-HRR--SIVERNATWQMMQLSCPS-----PHLINTSSSTSNNTTSTATTTLTAAATSTATTTSTAPTAAATRRRTMDP--KLIKTHDL | 311 |
| PxhMAW | 211 | --VAEYR-SAEWRWIPFD-EGATEMQRRSLLA-ERNATWQMMHLSCSFP-----TPHHMN--NSST--SNTIT-TTTTISS-----PSTFR-TMEPK-HFEKVKDHL | 298 |
| MeSTL1 | 207 | --VVAECR-GEGWTOYENEVEELQ-HRRN--YIMERNATWQMMQLSCPS-----TTLINTSSGTCIT-ITTTTATILAEAEATTST-----VAETIRRTMDPNRQLIKTHGTL | 303 |
| AtWOX1 | 198 | -----ANIRVEHNARCAGD-ERR--SFLGINATWQMMQLPPSFYSS-----SHHHQRNLIILNSPVSNNMSNNAVS-----ASKDTVTVSE--VELRTREAT | 283 |

| | | | |
|---------------|-----|---|-----|
| STF | 279 | NLFISPHTYKERENAFIHLNNTSSTH-----QNES--DOTLQLFPIRNGDHGCTDHHHHHHNIIKETQIS--ASAINA--FNQFTEFLPLKN | 358 |
| VvSTL1 | 271 | NIFIAPYRSEGHNHLGAVGADHSNE-----EKGES--OTLQFLPLRSGDGN--ENINE--KEGEIS--VAAMNNTL-PHQFFEFLPLKN | 347 |
| GmSTL1 | 285 | SEFTSPN--RENGIHLSSISTQ-----DDNSVE--OTLQFLPFRNADR--SSDNIHQ--KETEVS--VSAMNA--PSOFFEFLPLKN | 357 |
| MsSTL1 | 274 | NLFISPHTYKERENAFIHLNNTSSTH-----QNES--DOTLQLFPIRNGDHGCTDHHHHHHNIIKETQIS--ASAINA--FNQFTEFLPLKN | 353 |
| NsSTF1 | 317 | NIFIAPFRTDNRKHENMENVGDEG-----QEBES--OTLELFPLRSNDNDDNFSEK--DEVEISGADANSNFSGSHYQFFEFLPLKN | 399 |
| RcSTL1 | 312 | NIFIAPY-YRENG--NGLIYNNNFNNSHVINEQDNGCGES--OTLQFLPLRSGGGGGDGNESIND--KETEIS-AVAAAMNANFT--HYOFFEFLPLKN | 401 |
| PxhMAW | 299 | NIFIAPFRTDHSHKSLSEIHGD-----SOTLELFPLRSNNEINDENNISEK--DDIEISGAVATSNTNFSGSNYQFFEFLPLKN | 376 |
| MeSTL1 | 304 | NIFIAPY-IENNIGQHGLIHHSNE-----EDHNGCGDSHOTLQFLPLRNGGGG--ECIHD--KETEISSAVAAAMNANFATCQFFEFLPLKN | 387 |
| AtWOX1 | 284 | NTECHRNGDDNKDQEQHEDCSNGE-----LDHQE--OTLELFPLR--KEGFCS--DGEKDKNISGHCYFFEFLPLKN | 350 |

WUS box

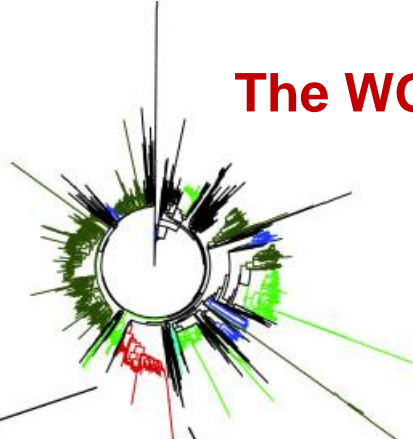
STF box

STF encodes a WOX domain protein



The WOX family is evolutionarily dynamic.

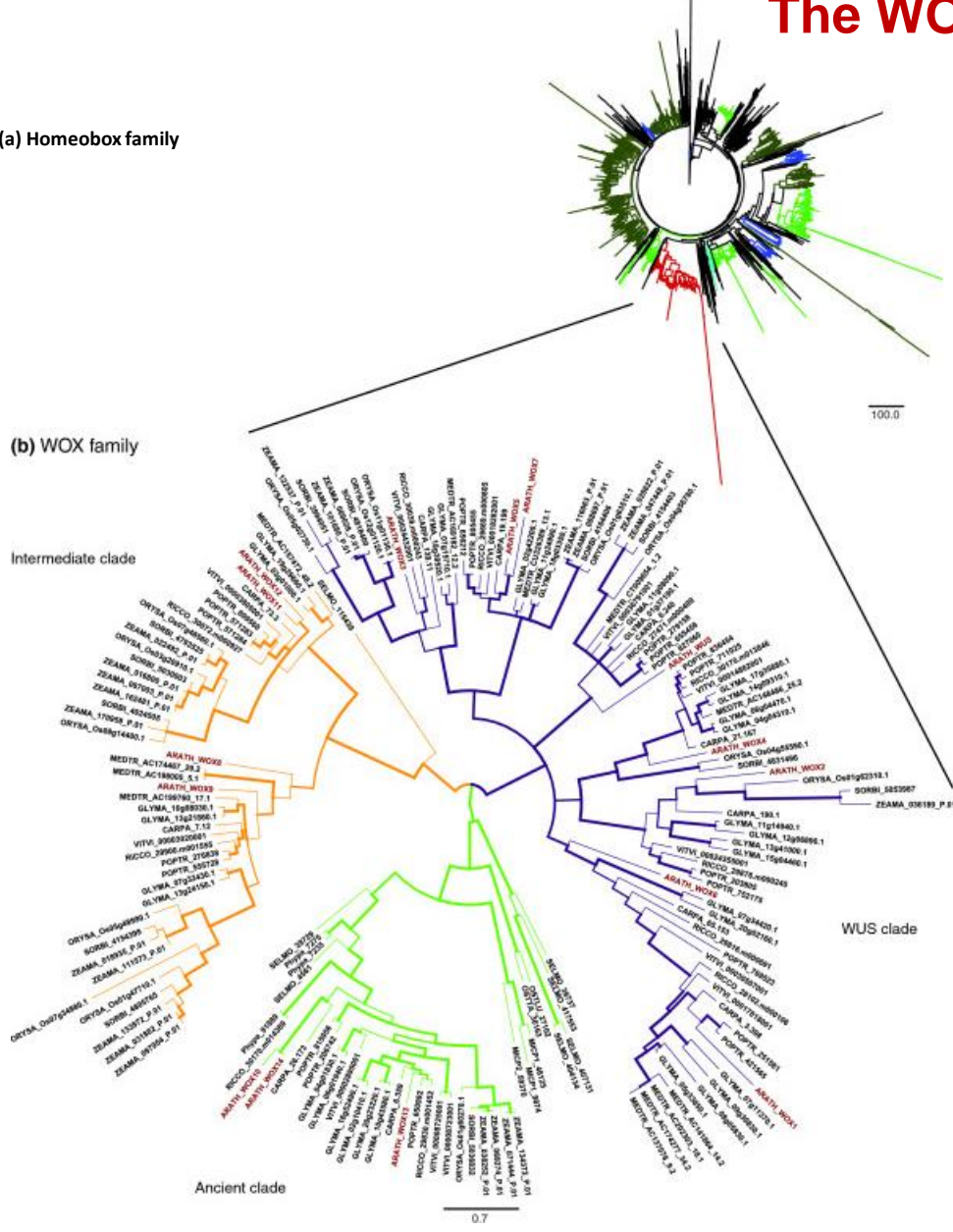
(a) Homeobox family



(b) WOX family

Intermediate clade

Ancient clade



3 clades of the WOX family

Ancient clade: green algae & moss
WOX10, 13 and 14.

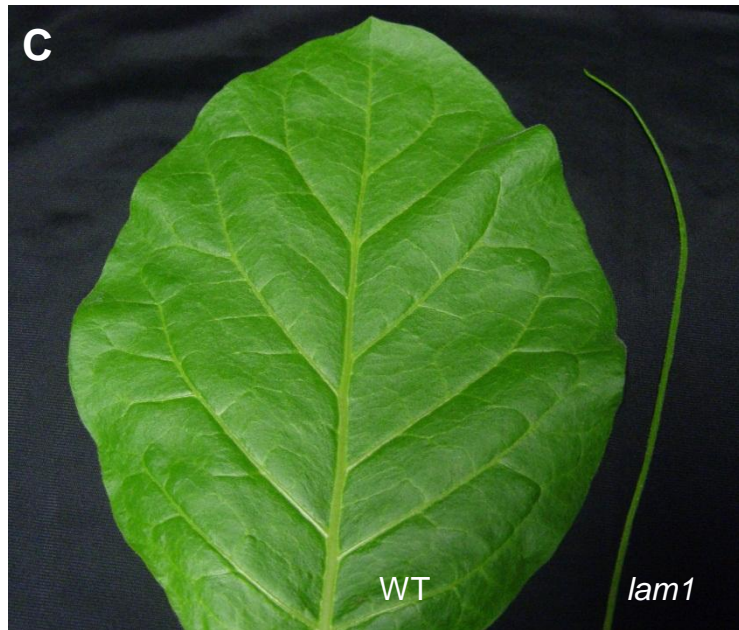
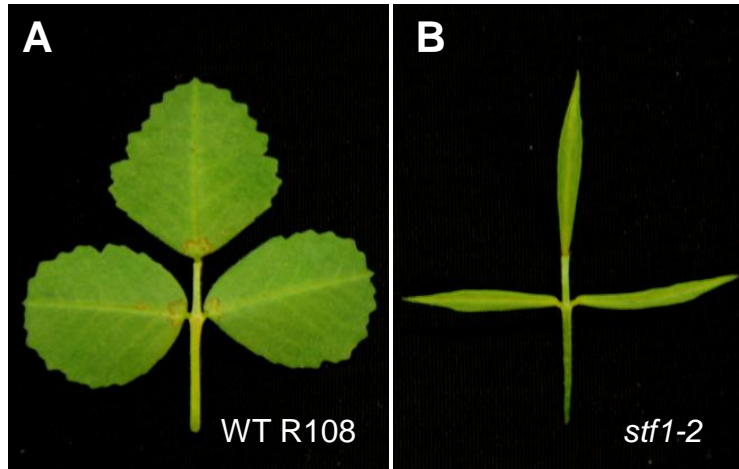
Intermediate clade: vascular plants
WOX8, 9, 11 and 12.

WUS/modern clade: seed plants
WUS, WOX1-7.

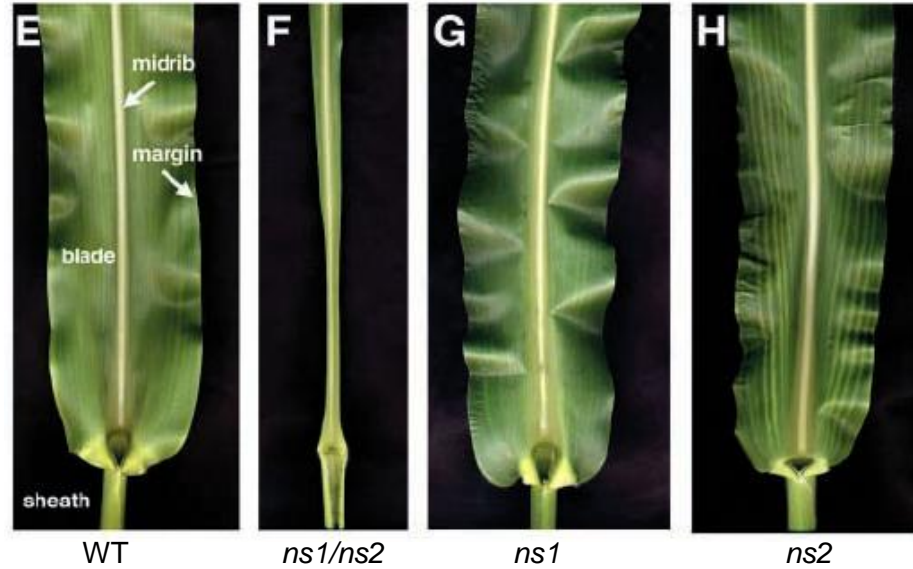
Distinct WUS and WOX5 - Angiosperms

Distinct STF and WOX3 – dicots?

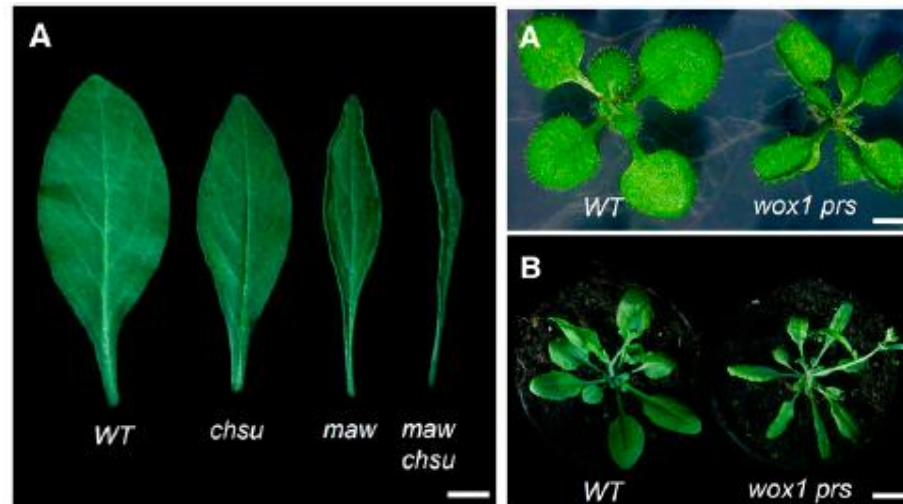
WOX proteins in lateral organ development



Tadege et al., 2011, Plant Cell.



Nardmann et al., 2004, Development.



Vandenbussche et al., 2009, Plant Cell.

Summary

Combined analysis of transcript and metabolite profiling suggests that *STF* is a “master switch”.

Auxin

Cytokinin

ABA

GA

SA

Others?

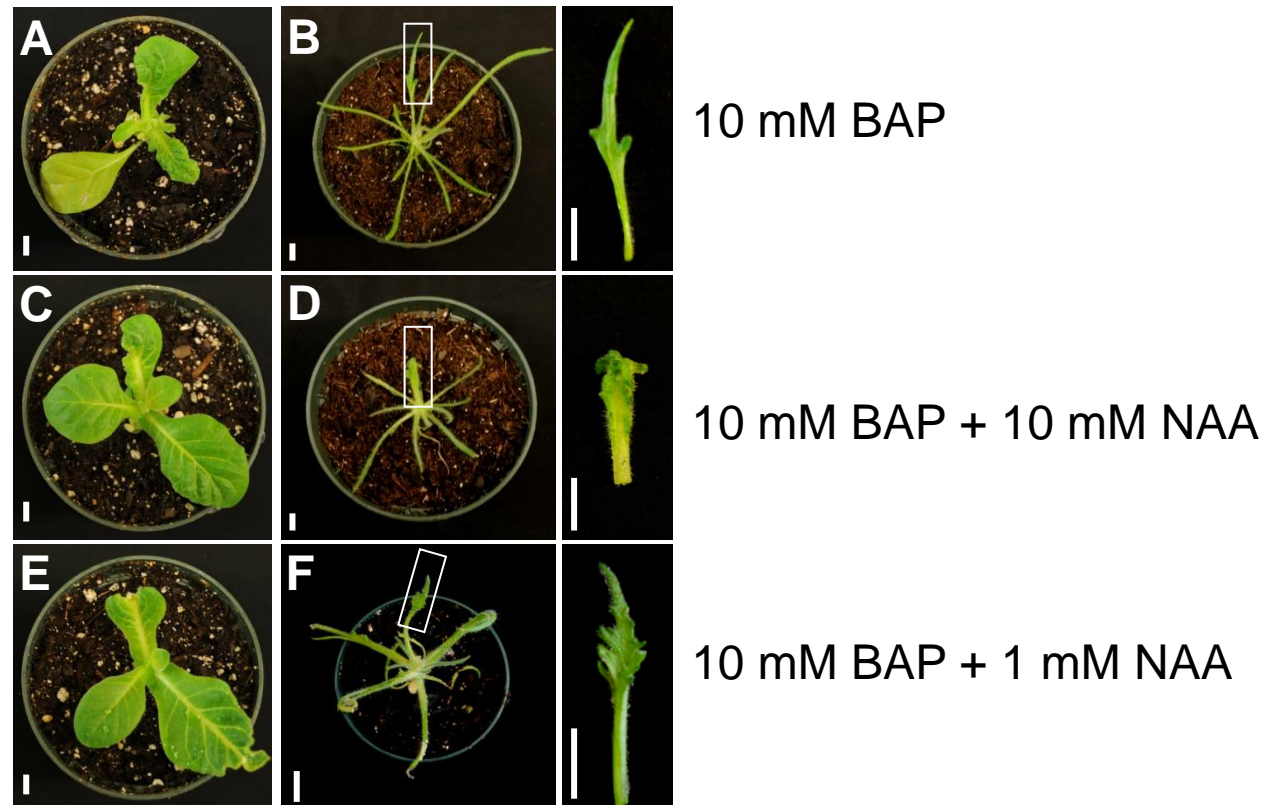
- Sugar metabolism
- Shikimate pathway
- flavonoids – lignin
- Matrix polysaccharides, cellulose
- Fatty acids and phospholipids
- Amino acids
- Polyamines
- Alkaloids

- Stress response
- Membrane transport

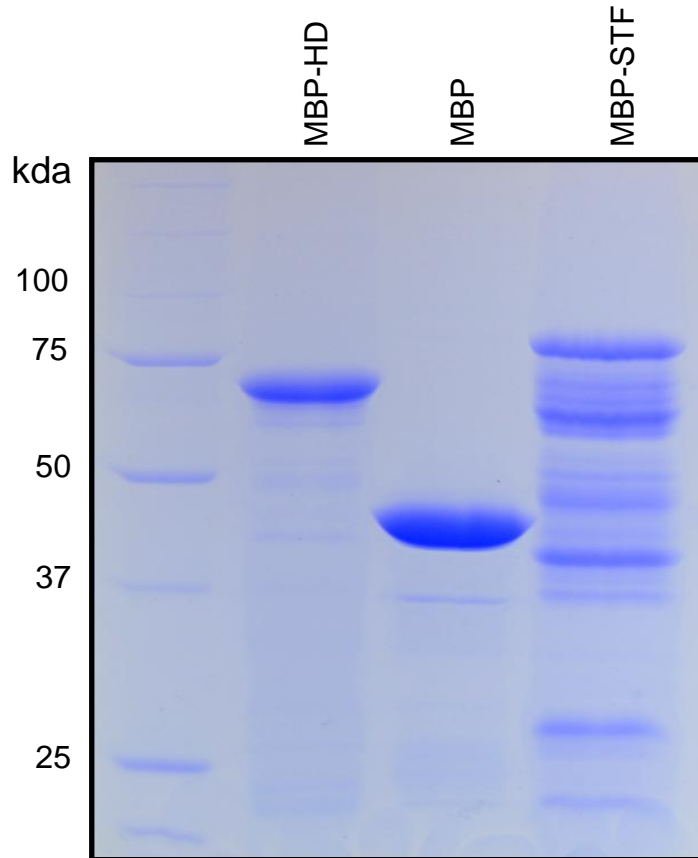
Ectopic expression of *STF* in tobacco leads to auxin and cytokinin overproduction phenotypes



Application of auxin and cytokinin together partially rescues the *lam1* blade



Purification of STF protein for EMSA



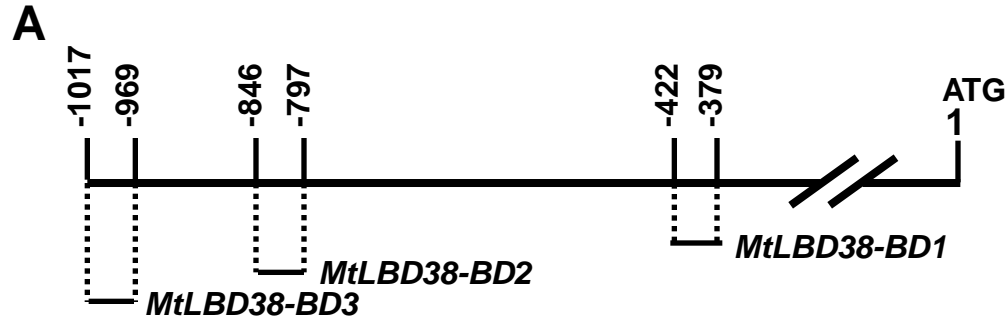
STF binding site



consensus

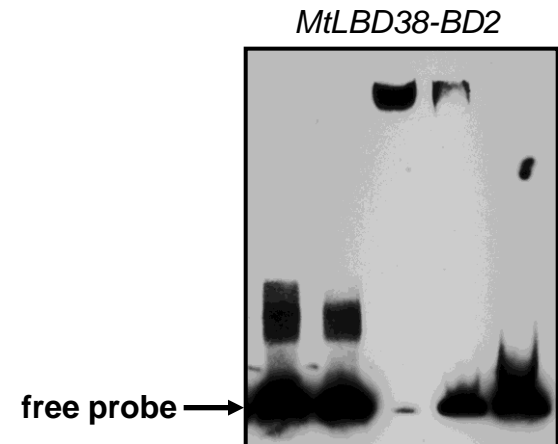
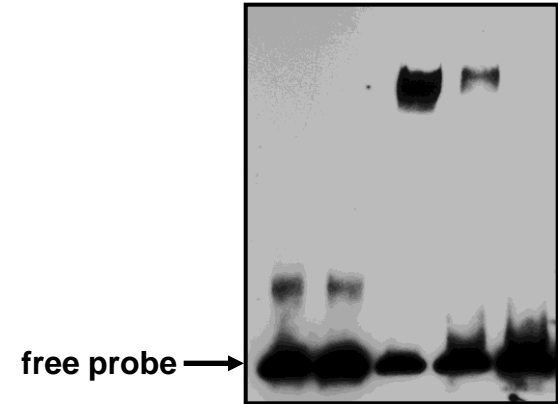
[CT][TG][TAG]T[CT]AAT[TA]

STF binds to the promoter of *MtLOB38* (*MtLBD38*)



C

| | | | | | |
|------------|--------------------|---|---|---|----|
| DNA probe | <i>MtLBD38-BD1</i> | | | | |
| MBP | - | + | - | - | - |
| MBP-HD | - | - | + | + | + |
| Competitor | - | - | - | + | ++ |

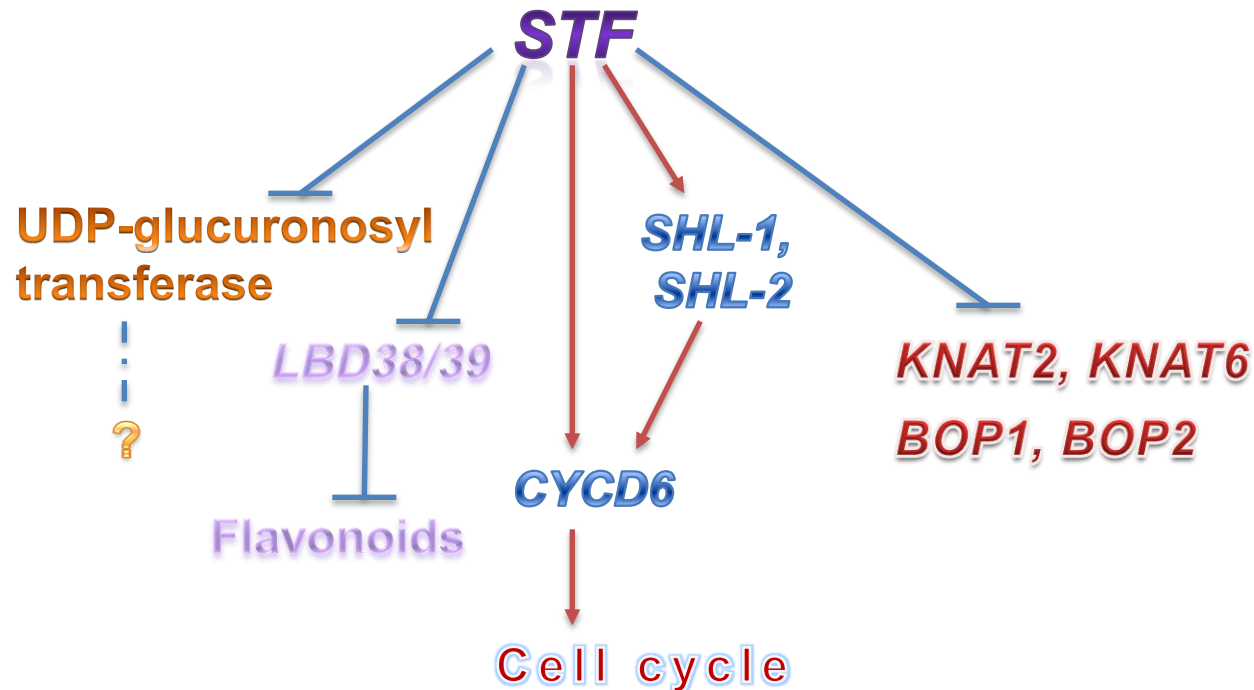


B

BD1 AAAAAAAAAATTATATATTAATAGTAATAAATAATAAAAAATAAT
BD2 TCAAATTTCAAATAGAATAAATTTGAAAGTGATAAATGATTAATTATTTT
BD3 AAGGATGAGCCTCAATAGATGAGCCATCAAATTTTATCTTGATCACATT



STF regulates cell division in leaf margins through GRAS and Cyclin genes.



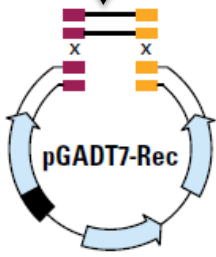
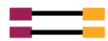
How is the cell cycle affected only in the mediolateral axis?

Yeast Two-hybrid screening using Matchmaker Gold Y2H System

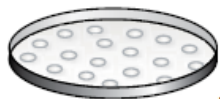
Library construction



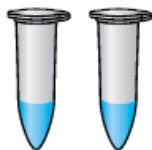
mRNA-cDNA-dscDNA



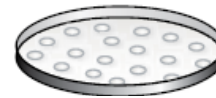
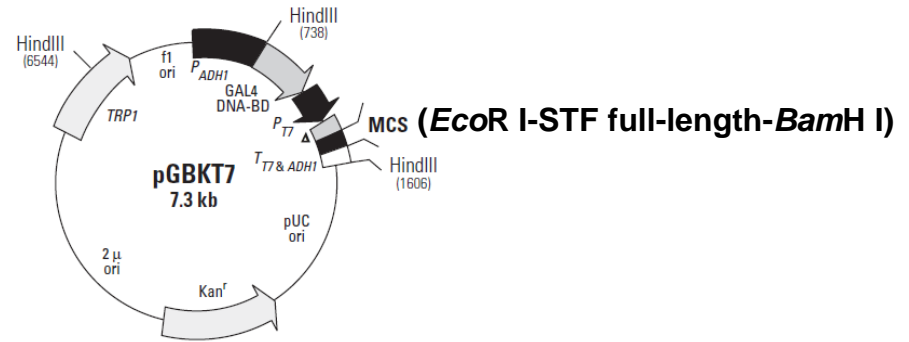
In-vivo recombination in
Yeast strain Y187



Pool colonies and aliquot
into 1 ml vials



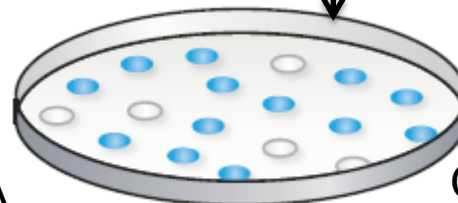
Bait preparation



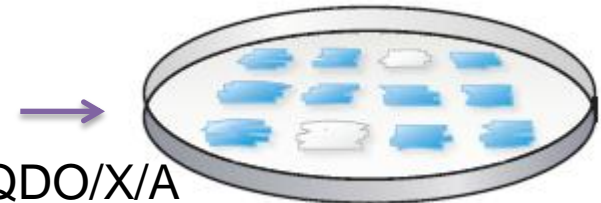
Yeast strain Y2H gold transformation



Mating



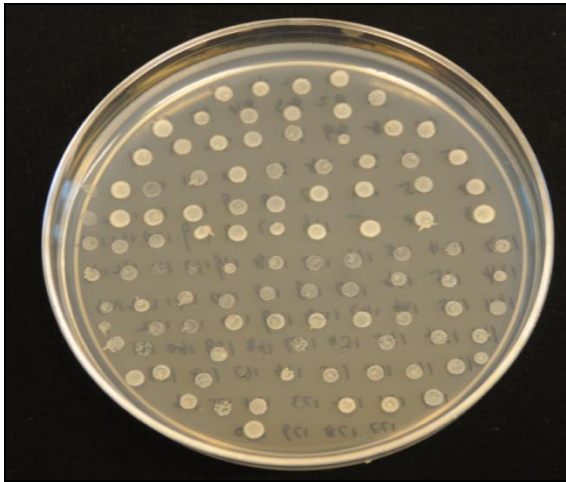
DDO/X/A



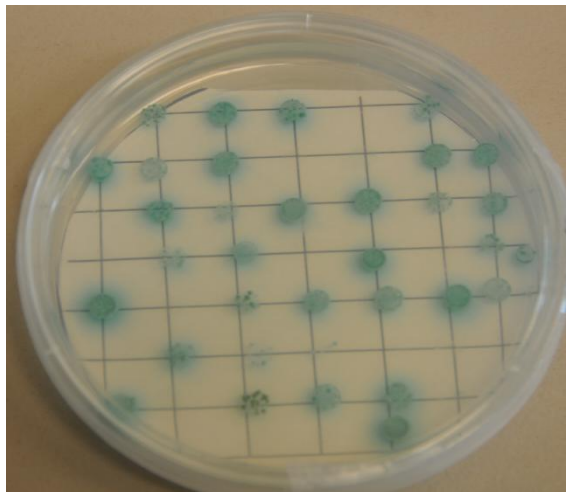
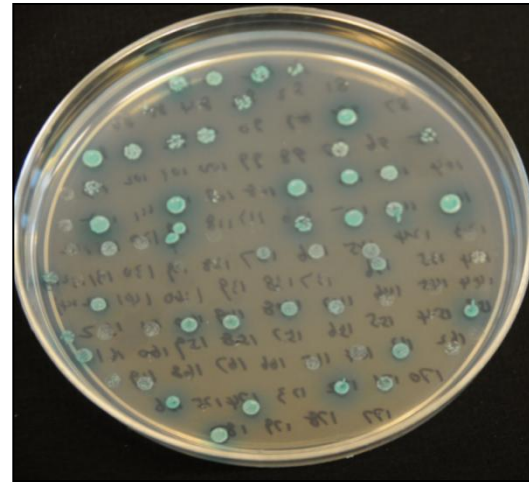
QDO/X/A

Confirmation and sequencing of positive colonies

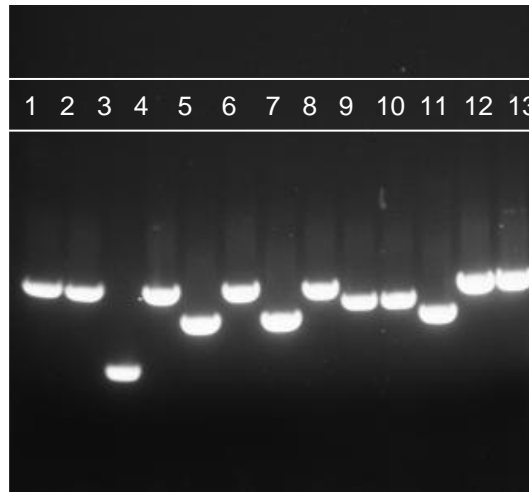
Growth on DDO



Growth on QDO/X/A

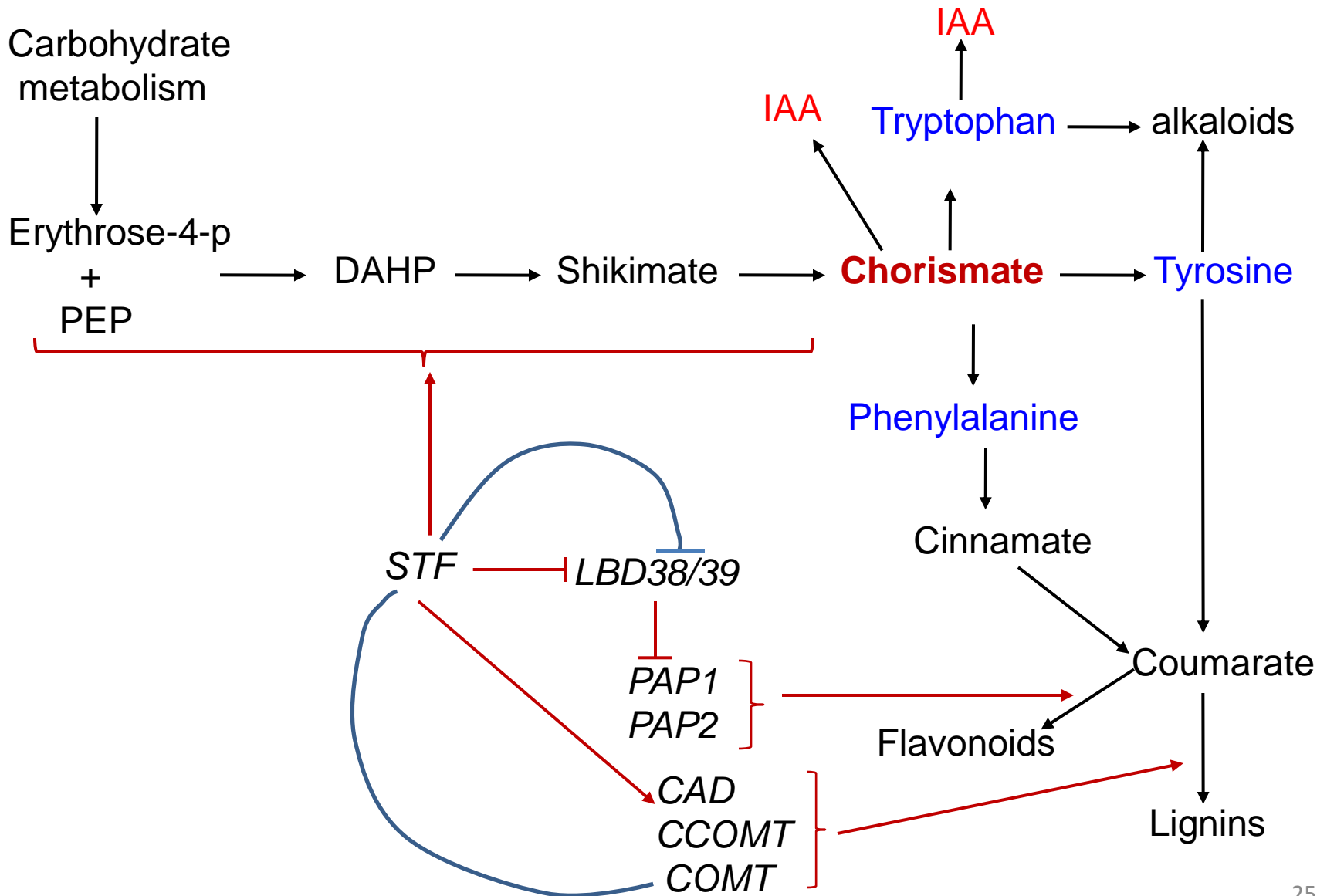


Re-confirmation

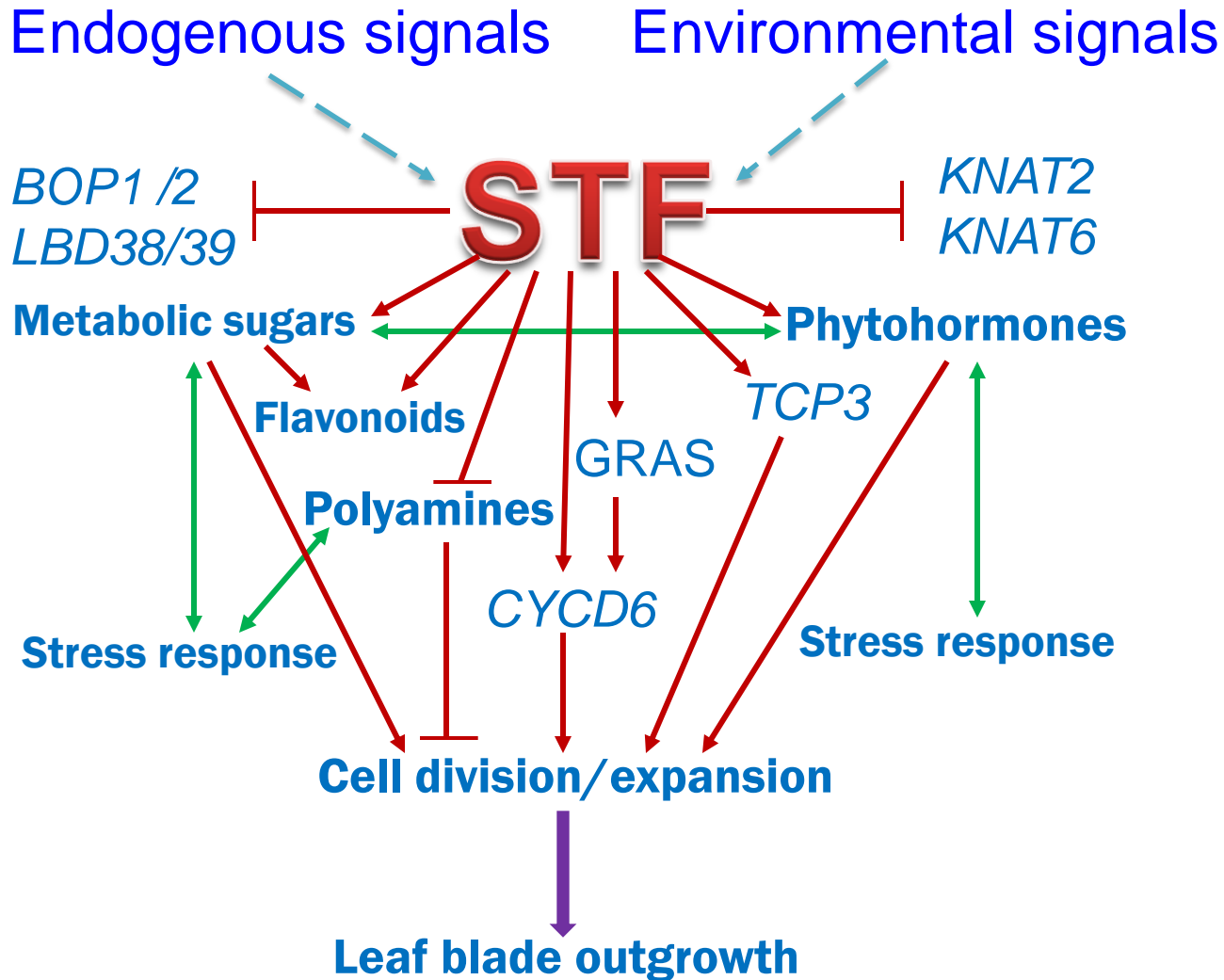


PCR confirmed colonies

Multilevel regulation of phenylpropanoids by *STF*



Working Model





Acknowledgements

Lab members

Hao Lin

Lifang Niu

Fei Zhang

Tezera Walebu

Hui Ling Yeang

Collaborators

Kirankumar Mysore

Paco Madueño

Neil McHale

Pascal Ratet

Mohamed Bedair

Yuhong Tang

Jiangqi Wen

