IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE PALE YELLOW GENE IN TOBACCO

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The Pale Yellow Trait

- ✓ Found in accession TI1372
  - ➢ accelerated chlorophyll breakdown[1]
- ✓ trait noticeable usually when plants flower and after topping[1]
- ✓ introduced into several dark lines
- ✓ traditional breeding using phenotypic selection[2]
  - ➢ detached leaf ethephon treatment

[1] Chaplin James F.; 1969; *Inheritance and Possible Use of Pale Yellow Character in Tobacco 1;* Crop Science; 9 (2) p. 169

[2] Adapted from the LC Protocol; https://www.uky.edu/Ag/Tobacco/Pdf/LC-Protocol.pdf

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The Pale Yellow Trait – Pros and Cons

- Pros
  - reduced curing time
  - improved overall quality
  - balance genetic impact affecting senescence and quality

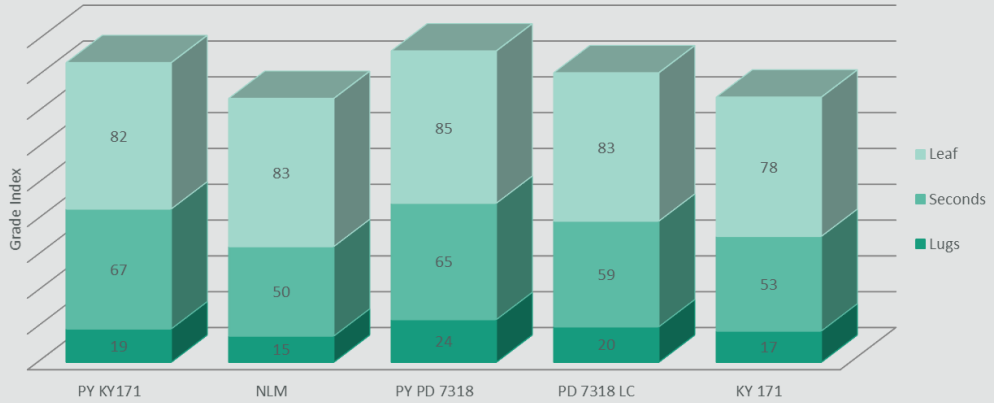
- Cons
  - reduced field harvest time window

Guidelines for Fire Curing Dark Tobacco*

Yellowing	Color Setting	Stem Drying	Finishing
• Ventilation as needed • None or low heat • Temp. not to exceed 100˚F (37˚C) • 5 – 8 days	• Little or no ventilation • Temp 100˚F-115˚F (37˚C - 46˚C) • 7 – 14 days	• Full ventilation • Temp not to exceed 130˚F (54˚C) • 4 – 8 days	• No ventilation • Temp not to exceed 120˚F (48˚C) • 10 – 14 days

*Bailey, A., 2006. Harvesting, Curing, and Preparing Dark Fire-Cured Tobacco for Market. University of Kentucky – College of Agriculture, AGR152.

Grade Index (GI) Average of Two Locations in 2009: Blackstone, VA and Princeton, KY



Lusso M., Hayes A., Lion K., Davis G., Hart F., Morris J., 2014; *Methods of Reducing Tobacco-Specific Nitrosamines (TSNAs) And/Or Improving Leaf Quality in Tobacco;* US 2014/0076339 A1

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Mapping the Pale Yellow Locus

F2 mapping population was grown in 2018

more information

https://www.coresta.org/sites/default/files/abstracts/2019_AP35_PramodAdams.pdf

all individuals were phenotyped by

a) visual observation at 4 wpt

b) ethephon screening of leaves at flowering

PY locus
2Mb region, Chr 15
5 linked SNPs

93 F2 individuals and parents genotyped on the ~178K SNP tobacco Axiom array

F2: (NLMxTI1372)xF2
wpt: weeks post topping
SNP: Single Nucleotide Polymorphism

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QTL Analysis – Phenotypic Evaluation versus Genetic Analysis

PY locus
2Mb region, Chr 15
5 linked SNPs

KASP® assay design

TI 1372 Phenotype: 27 plants

Heterozygous Phenotype: 45 plants

NLM Phenotype: 27 plants

100% correlation between genetic analysis and observed phenotype

KASP: Kompetitive Allele Specific PCR

Mapping the Pale Yellow Locus and QTL analysis

F2 mapping population was grown in 2018

NLM (X) TI1372

more information

https://www.coresta.org/sites/default/files/abstracts/2019_AP35_PramodAdams.pdf

all individuals were phenotyped by

a) visual observation at 4 wpt

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93 F2 individuals and parents genotyped on the ~178K SNP tobacco Axiom array

1 : 2 : 1

PY locus
2Mb region, Chr 15
5 linked SNPs

58 genes in QTL

RNAseq

5 candidate genes

F2: (NLMxTI1372)xF2
wpt: weeks post topping
SNP: Single Nucleotide Polymorphism
QTL: Quantitative Trait Locus
PY: Pale Yellow

Verification of Identified Candidate Genes in (NLMxTI1372)xF2

- by quantitative real-time PCR
  - relative to NLM untopped (UT)
- g58899 expression is drastically reduced in TI1372 and F2 plants genotyped as homozygous for Pale Yellow (PY)

NLM: Narrow Leaf Madole – Dark variety

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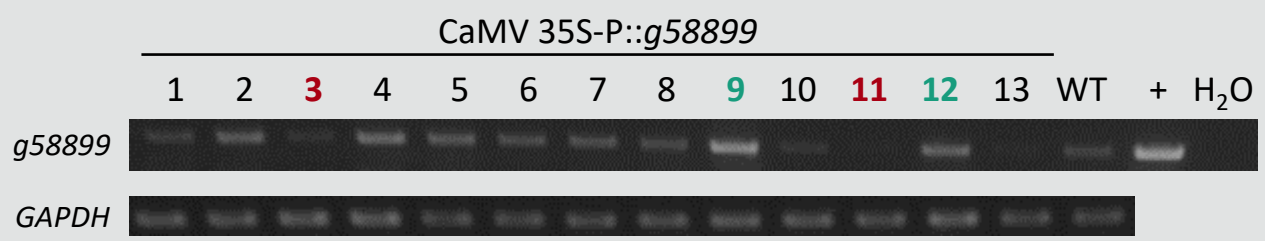
Characterization of g58899 overexpression lines – T_0

L3 L9 L11 L12



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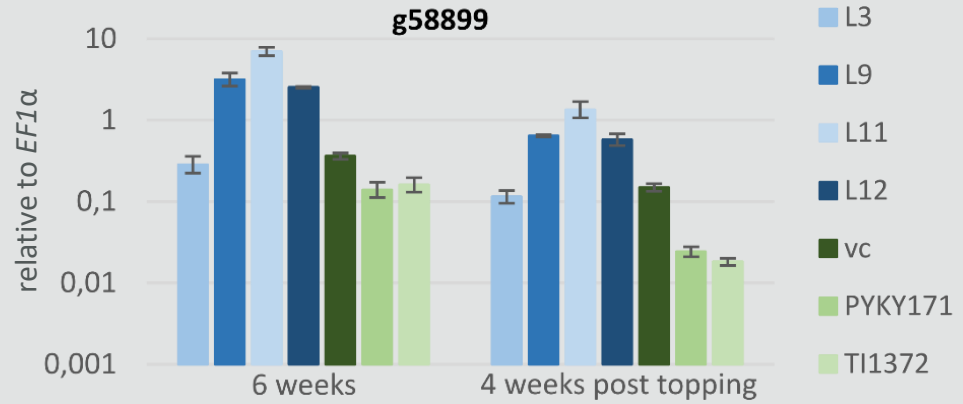
Characterization of g58899 overexpression lines – T_1

pictures taken 4 weeks post topping

- gene expression in medial leaves



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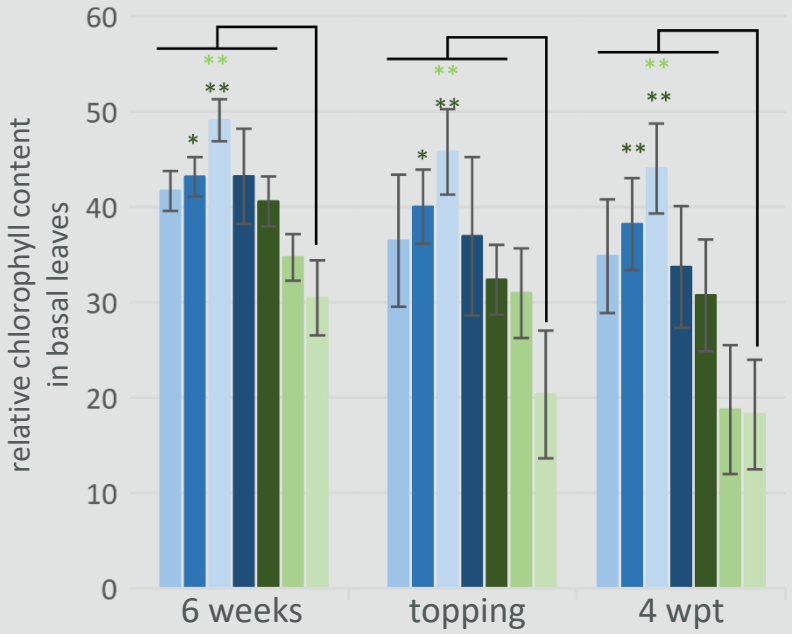
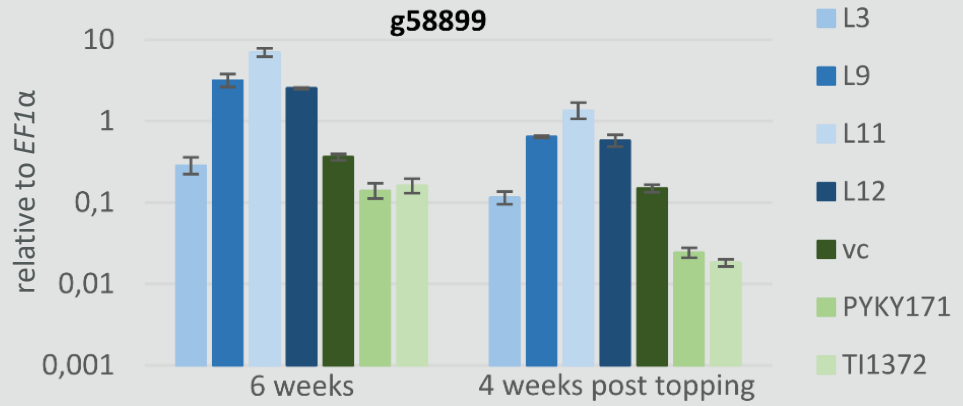
Characterization of g58899 overexpression lines – T_1

- leaves of transgenic lines stay green

pictures taken 4 weeks post topping



- gene expression in medial leaves



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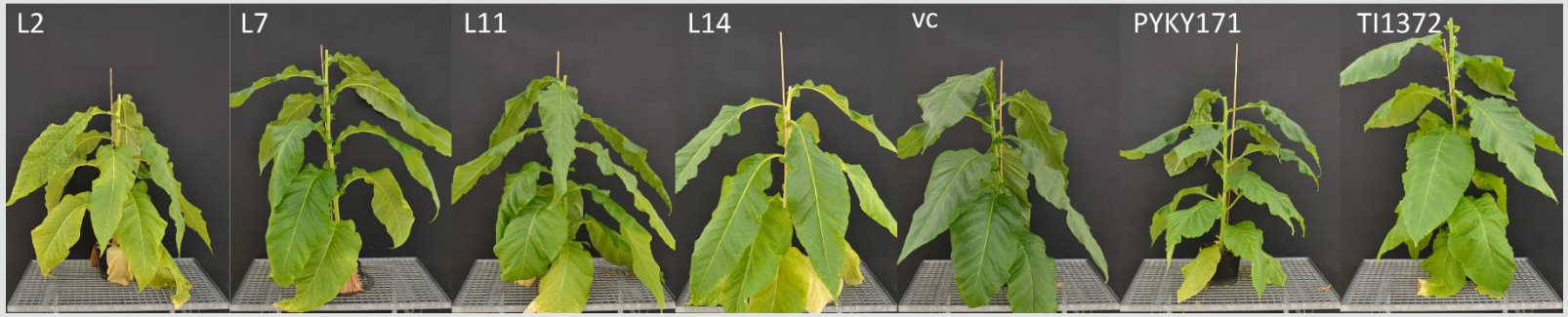
Characterization of g58899 RNAi lines – T_0

- pale yellow and whitish leaves even in tissue culture

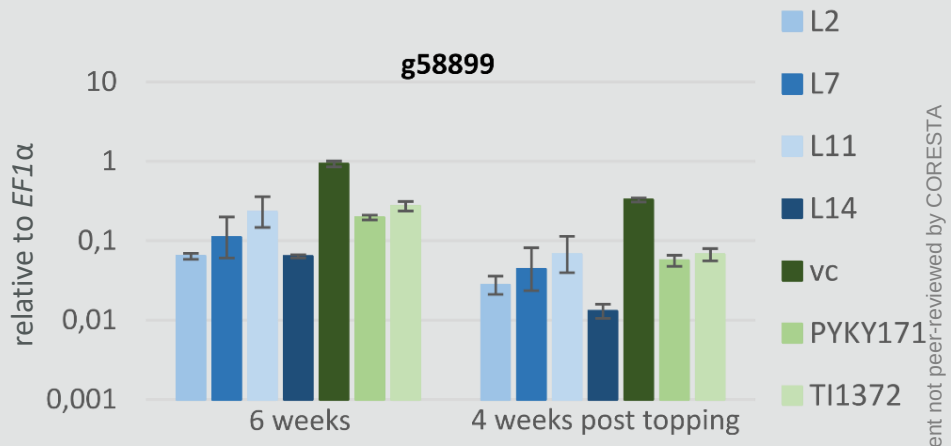
Characterization of g58899 RNAi lines – T_1

- pale yellow and whitish leaves even in tissue culture



pictures taken 4 weeks post topping

- gene expression in medial leaves



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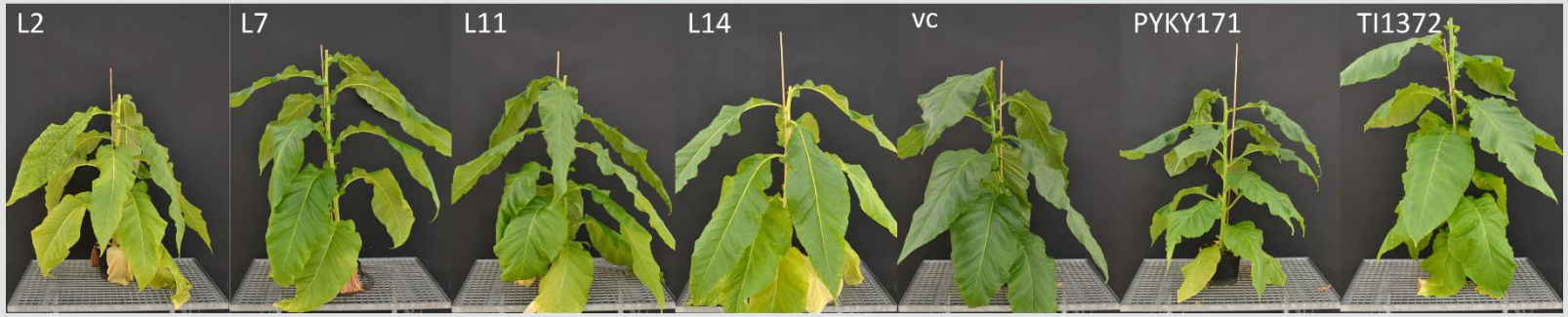
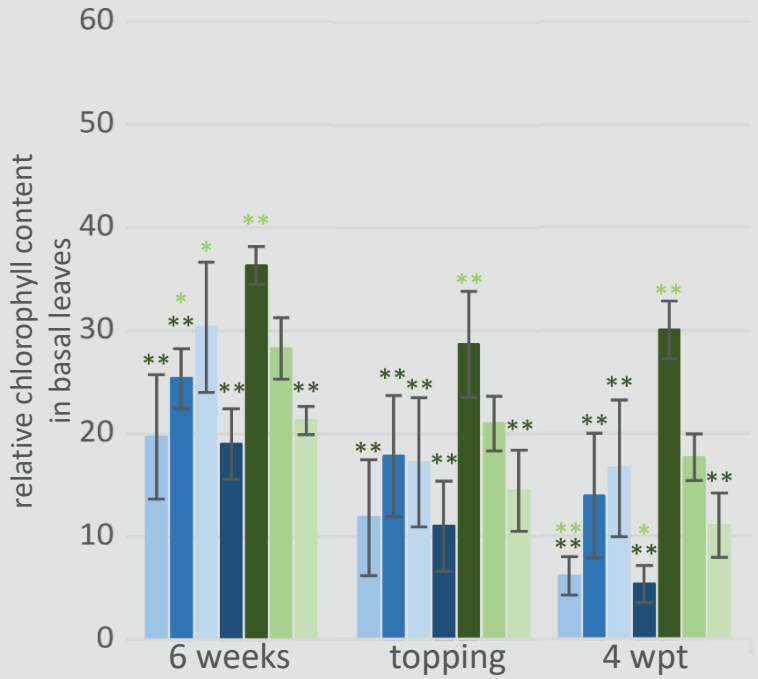
Characterization of g58899 RNAi lines – T_1

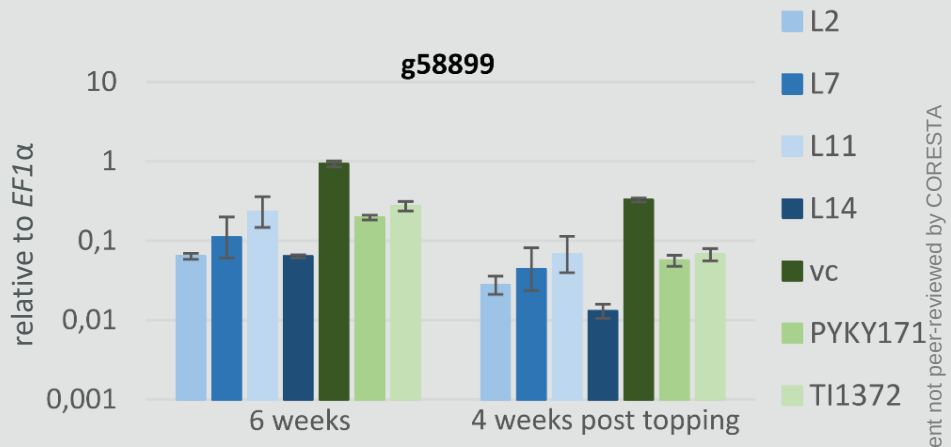
- pale yellow and whitish leaves even in tissue culture
- chlorophyll content is reduced



pictures taken 4 weeks post topping



- gene expression in medial leaves



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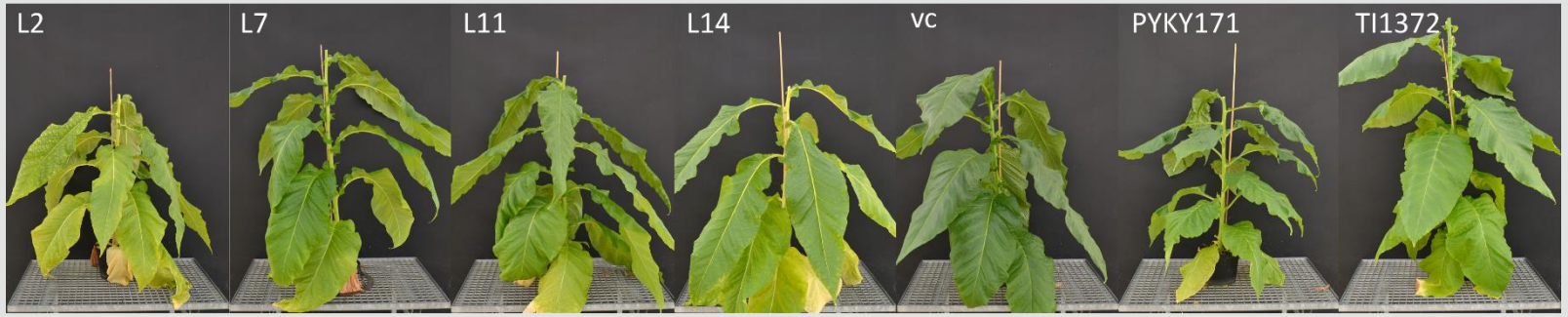
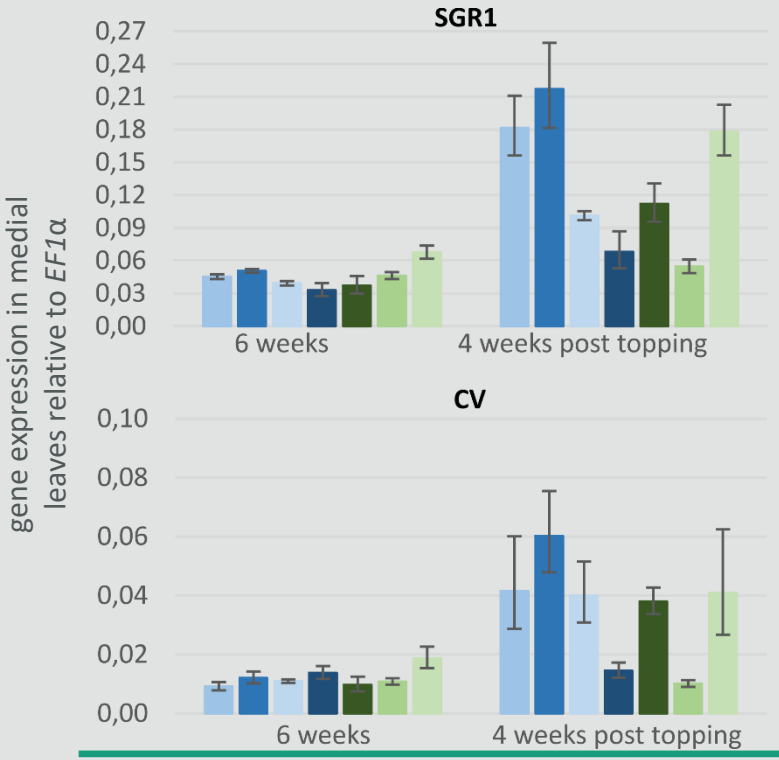
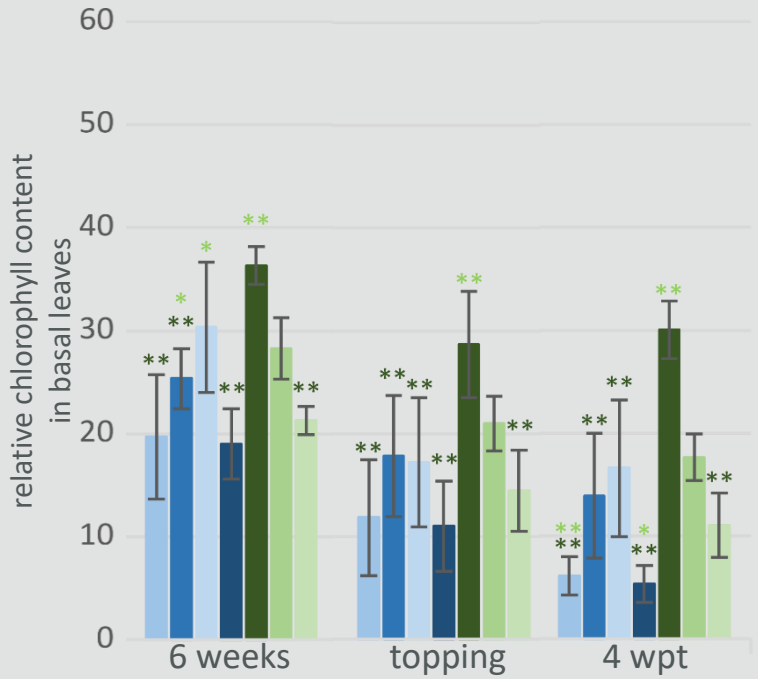
Characterization of g58899 RNAi lines – T_1

- leaf senescence-related gene expression is increased

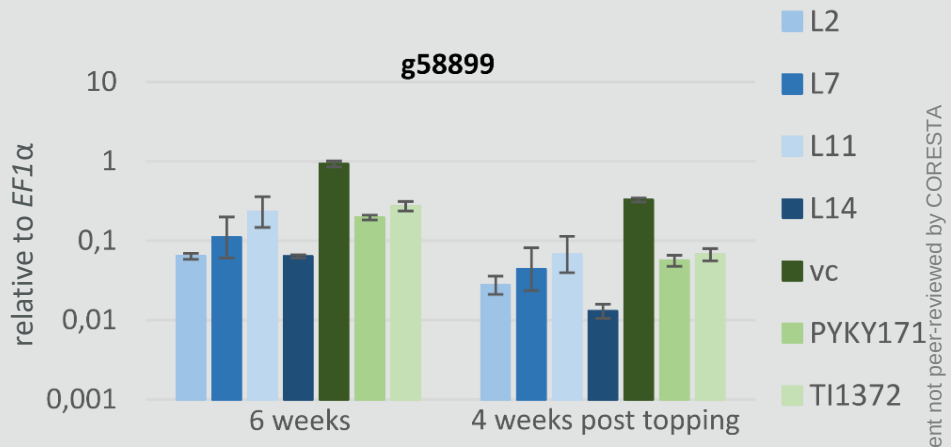


pictures taken 4 weeks post topping





- gene expression in medial leaves



Mayta *et al., 2018,* Expression of a Plastid-Targeted Flavodoxin Decreases Chloroplast Reactive Oxygen Species Accumulation and Delays Senescence in Aging Tobacco Leaves, doi: 10.3389/fpls.2018.01039

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Characterization of g58899 in tobacco

- ✓ overexpression ➡ stay green
- ✓ RNAi ➡ pale yellow

- ➢ g58899 shares ~64 %/58 % identity with AtBCM1/2

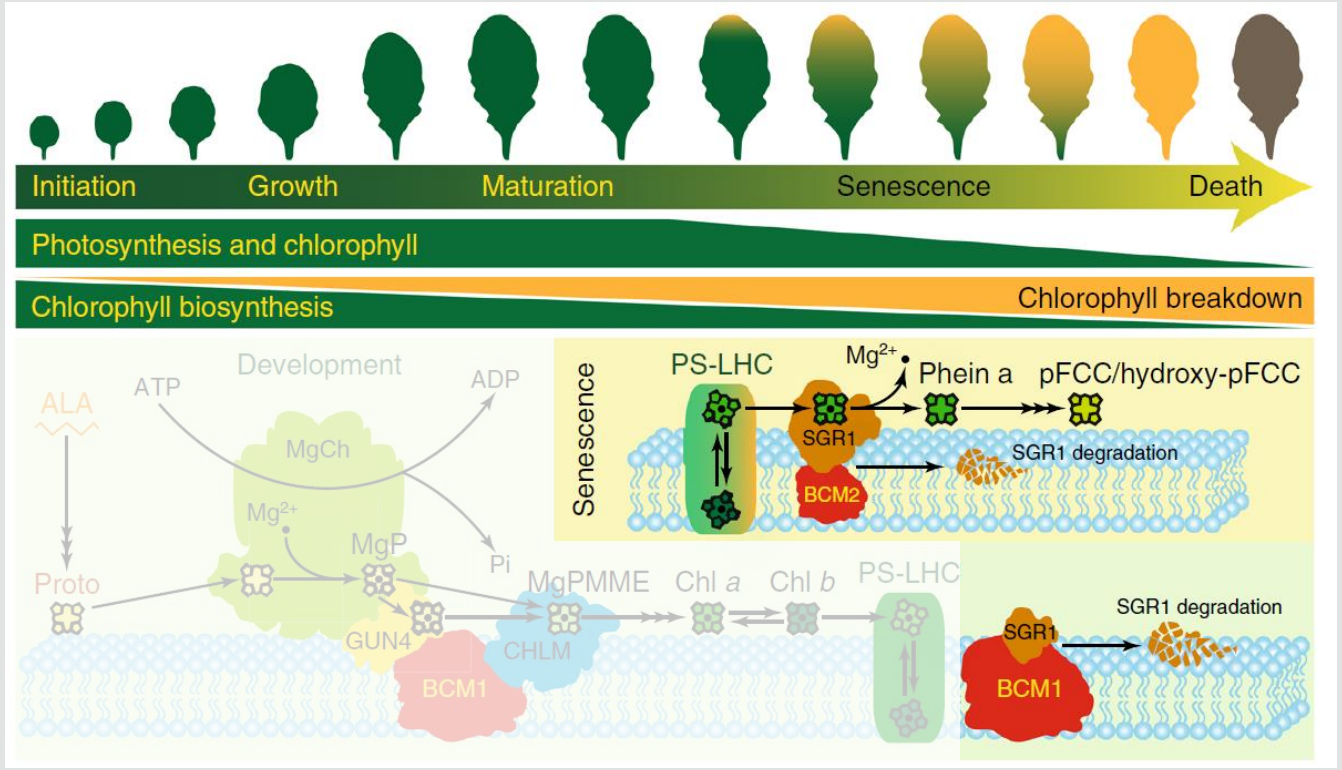

```
BCM2        MGLPLLSCSSTRVTLSSSSSSSWCSSGSGGFRSSSKLFDSPACSRSDLKKRSGKRNSRLN       60
BCM1        MELPLLSYASSASF----SRTGLCSSSSS---SSTSIYEFPERRRSLKLRFNGGE-----       48
g58899      MEVPVLARCTNTPT----T------SFLG---CKVSLFDFPIRRKLNKRNYKAKF-----       42
            * :*:*: .:.       :      *  .   .. .::: *   :    . ..

BCM2        GLSLEKLRSIKASSSSAGQSSSEVIDDGDAAARGLAVTSGDVTSVGSFSSGEFVGAGSGG       120
BCM1        -----RSRSVIA--------------SAERSSEGIEKTT--------DTVGGGGGGGAGR       81
g58899      -----SVLRVKA--------------MAERTST--------------EASADARERESGG       69
                     : *               .: ::               : .     :*

BCM2        LAGPSGEVTSVG-EFVGGSGGDFKDWDKIGAIVRLSYGIGIYCGMAVAGRFICEVAGIDY       179
BCM1        FAGTAMEVTTLDRGFANSTTVDFPIWDKIGAVVRLTYGIGIYGAMAVAGRFICSVTGIDS       141
g58899      YTGTTMEVTTFNQSFSD---AQLPVWEKIGAVVRLSYGIGIYGAMALAGKFICSMTGIDC       126
             :* : ***:..  * .    ::  *:****:***:****** .**:**:***.::***

BCM2        TGGFNASLDTIIAGLGYASPPIMALLFILDDEVVKLSPHARAIRDVEDDELRGFFQGMSA       239
BCM1        SGGFDPSLDALLAGLGYATPPIMALLFILDDEVVKLSPHARAIRDVEDEELRSFFFGMSP       201
g58899      TGGFSPSLDAIVEGLGYAAPPIMALLFILDDEVVKLSPHARAIRDVEDEELRNFFYGMSP       186
            :***. ***::: *****:****************************:***.** ***

BCM2        WQFILVVTASSVGEELFYRAAFQGALADIFLRGTDLISDSRGMVALTGLLPPFVPFAQVF       299
BCM1        WQFILIVAASSIGEELFYRVAVQGALSDIFLKGTQLMTDSRGMASLTGVFPPFVPFAEVF       261
g58899      WQFILIVAASSVGEELFYRAAVQGALADIFLRGSGFVTDARGMASLTGVLPPYVPFAQAF       246
            *****:*:***:*******.*.****:****:*: :::*:***.:***::**:****:.*

BCM2        AATITAALTGSLYYIAASPKDPTYIMAPVLKTRSARDELKKLFAAWYERRQMKKIYSPLL       359
BCM1        AAVITATLTGSLYFLAASPKDPTYIVAPVLRS--RRDDFKKLLSAWYEKRQMKKIYSPLL       319
g58899      AAVITAALTGSLYYMAASPKDPTYVVAPVLKSHSGREDLKKLFAAWYERRQMKKIYSPLL       306
            **.***:******::*********::****::   *:::***::****:**********

BCM2        EGLLGLYLGFEWIQTNNLLAPIITHGIYSAVVLGNGLWKLHHHQQRLRLRVQKLETEGDN       419
BCM1        EGLLALYLGIEWVQTDNILAPMMTHGIYSAVILGHGLWKIHDHRRRLRRRIEHIRSEATD       379
g58899      EAMLALYLGFEWIQTNNIFAPIITHGIYSAVILGHGLWKIHDHRRRLHQRIQQLKQEGNN       366
            *.:*.****:**:**:*::**::********:**:****:*.*::**: *::::. *. :

BCM2        NSR*-       422
BCM1        KLI*-       382
g58899      SRNL*       370
            .
```

Wang *et al.,* 2020, Post-translational coordination of chlorophyll biosynthesis and breakdown by BCMs maintains chlorophyll homeostasis during leaf development. https://doi.org/10.1038/s41467-020-14992-9

Model for the concurrent regulation of Chl biosynthesis / catabolism by BCMs

- ✓ AtBCMs target SGR1 (involved in chlorophyll breakdown) for degradation

- ➢ down-regulation of BCMs (and probably g58899) prevents degradation of SGR1
- ➢ chlorophyll breakdown is induced earlier



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Conclusion and Outlook

- ✓ low expression of g58899 = PY phenotype ➡ identifying a key target gene for breeding
- ✓ PY KASP® assay ➡ demonstrating the utility of a high-density SNP array for mapping of traits

- ➢ more precise and faster breeding of the PY trait in commercial tobacco cultivars

  - ❖ loss of function mutants from EMS populations will be analyzed
  - ❖ development of PY lines and agronomic evaluations in progress

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THANK YOU

QUESTIONS?

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