Genetic Mapping and Characterization of the Pale Yellow Locus Responsible for Accelerated Senescence in Dark Tobacco

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Outline

- Background and Application of Pale Yellow (PY) trait
 - Reduced Curing Time
 - Improved Quality
- Mapping of the PY locus
- Genes identified within the Quantitative Trait Locus (QTL) region







Pale Yellow Trait

Found in accession TI1372

Accelerate chlorophyll breakdown

Trait noticeable usually by flowering and after topping

Traditional breeding using phenotypic selection*

Introduced into several dark lines

* Detached Leaf Ethephon Treatment Adapted from the LC Protocol https://www.uky.edu/Ag/Tobacco/Pdf/LC-Protocol.pdf

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



Application – Reduce Curing Time

Guidelines for Fire Curing Dark Tobacco*











Yellowing

- Ventilation as needed
- None or low heat
- Temp. not to exceed 100°F (37°C)
- 5 8 days

Color Setting

- Little or no ventilation
- Temp 100°F-115°F (37°C 46°C)
- 7 14 days

Stem Drying

- Full ventilation
- Temp not to exceed 130°F (54°C)
- 4 8 days

Finishing

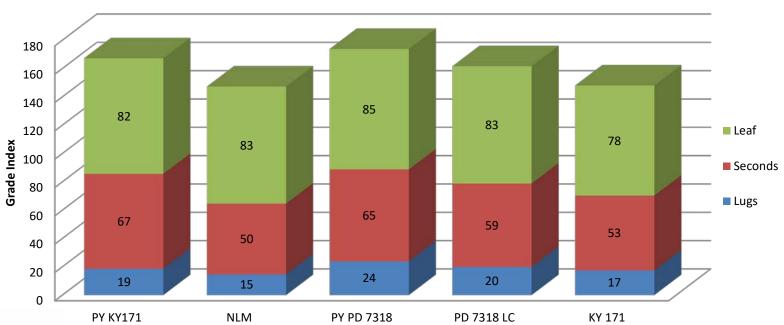
- No ventilation
- Temp not to exceed 120°F (48°C)
- 10 14 days

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



Application – Improved Quality

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





Mapping of Pale Yellow Locus

A 192 individual F2 population (NLM x TI1372)x

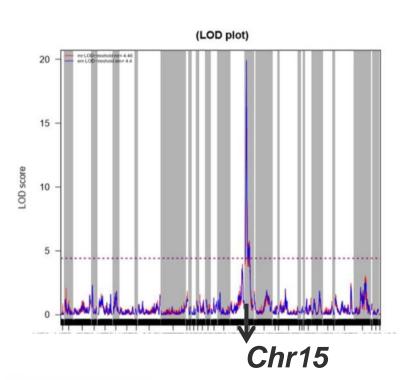
All individuals were scored on phenotype by

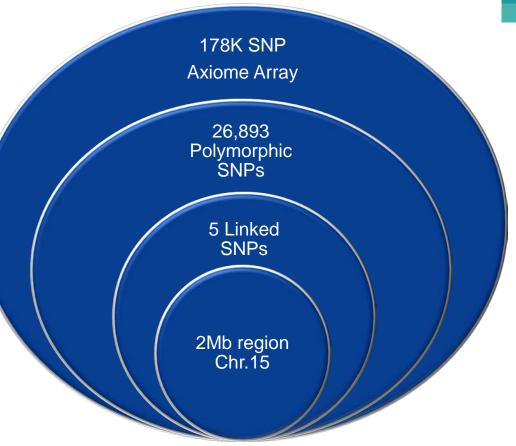
- Visual observation at 4 weeks post topping
- Ethephon screening of leaves at flowering leaves- 5 readings post treatment

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



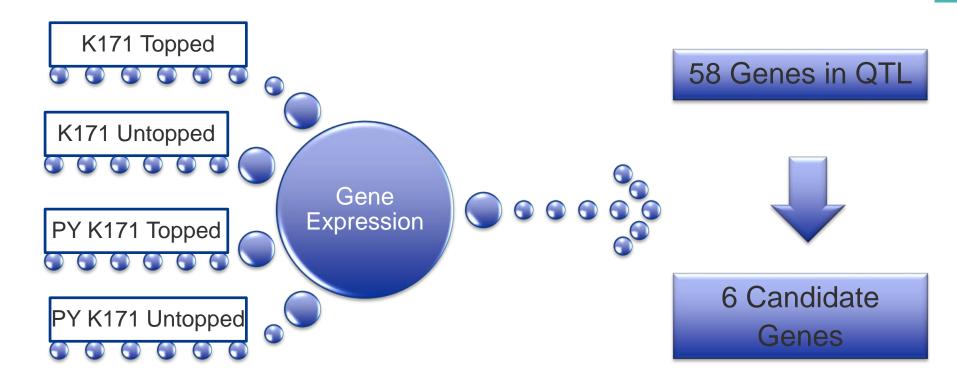
QTL Analysis





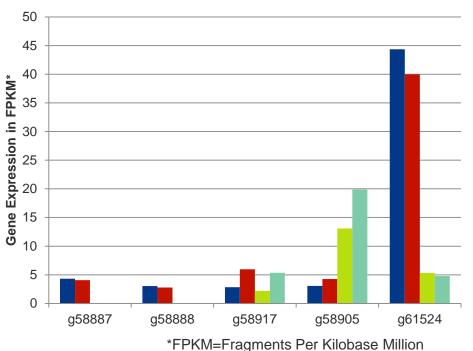


Identification of Gene Candidates





Expression Profile of Candidate Genes



300 250 Gene Expression in FPKM* 200 ■ KY171 (UT) ■ KY171 (T) 150 PYKY171 (UT) ■ PYKY171 (T) 100 50 0 q58899



Conclusions and Future Directions

Markers

- Five SNP markers for PY trait breeding
- Six functional gene candidates
- Further screening for functional gene marker

Gene Validation

- Allele sequencing
- Gene knockdown / overexpression
- Loss of function mutants from EMS populations





Thank You

QUESTIONS

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