

Genetic Strategy for Reducing Sucker Pressure in Tobacco

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Tobacco Suckers



What are Suckers?

Side branches that grow after apical meristem removal (topping). Suckers divert nutrition and impact leaf quality

Current Control Methods

- Manual removal (labor intensive)
- Chemical application



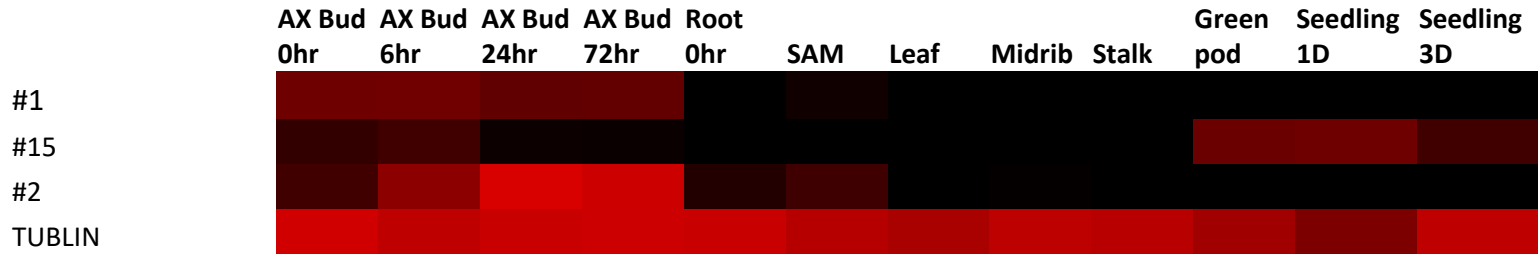
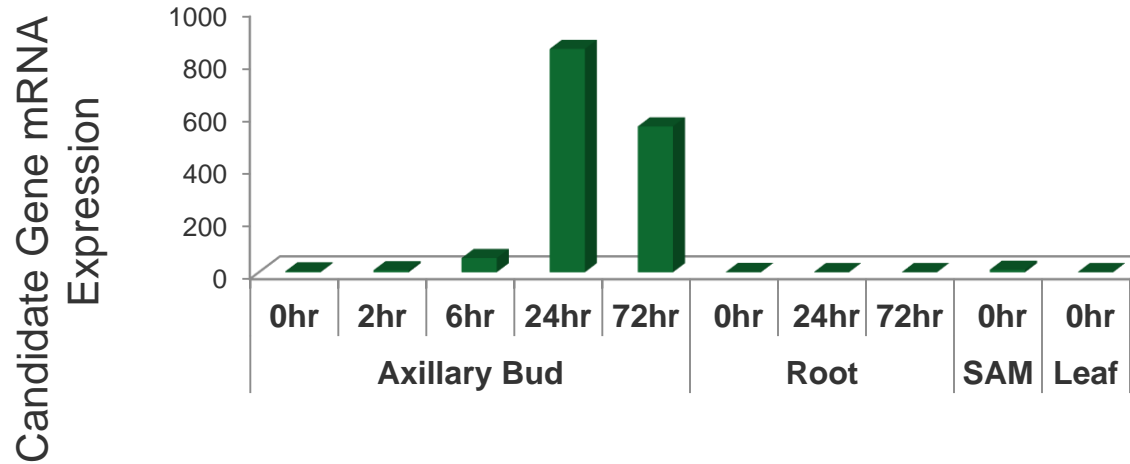
Objective

To develop plants with reduced suckering potential by delivering cell death genes driven by sucker specific promoters

To evaluate sucker control plants in a greenhouse and field setting



Axillary Bud Specific Genes Screening

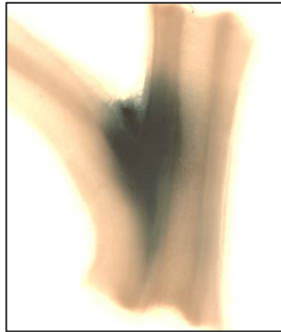


Three Genes Specially Expressed in Axillary Buds After Topping

Promoters from Axillary Bud Specific Genes



P1



P2



P15

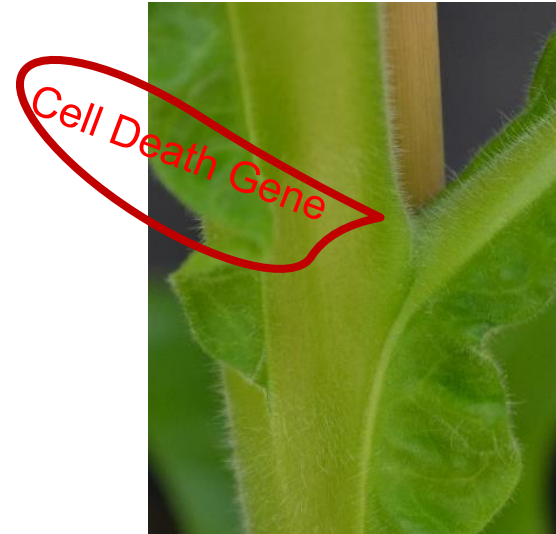
Varied Level of Expression and Specificity with GUS



Specific Expression of Cell Death (cd) Gene



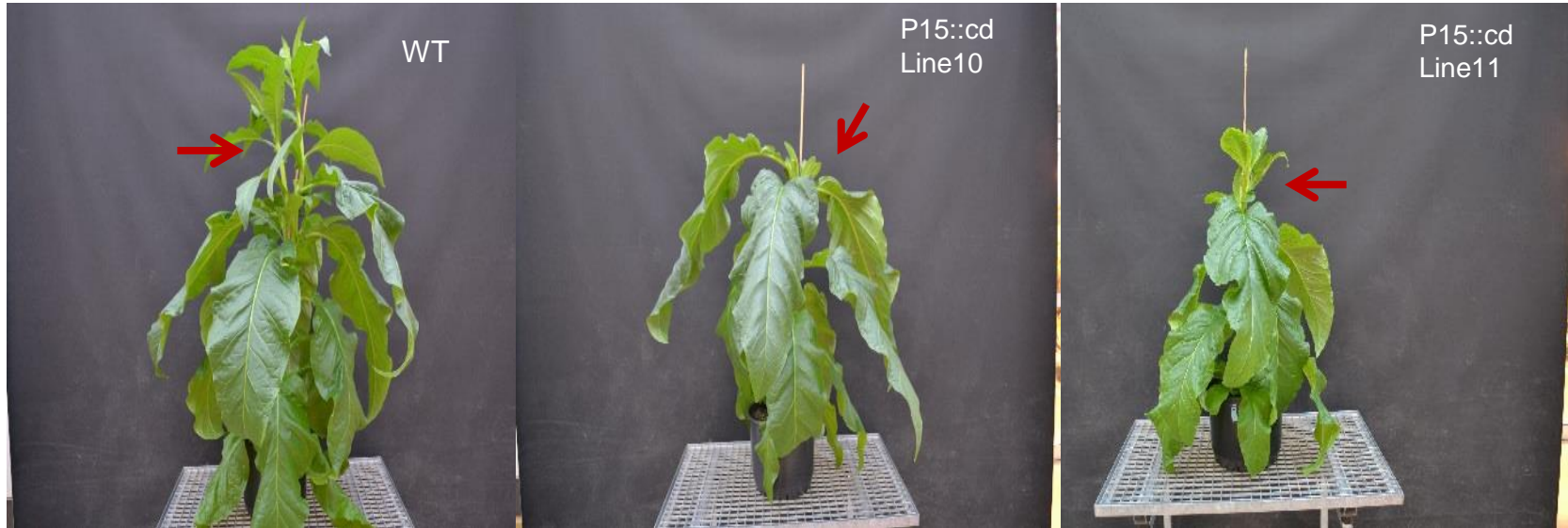
WT Control Plant



cd Gene Transgenic Plant



Three Weeks Post Topping Suckers

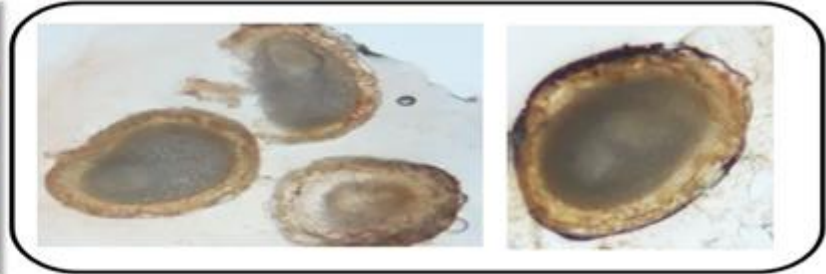
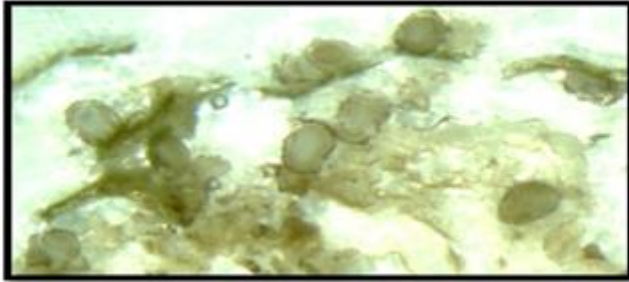


Sucker Growth Was Inhibited

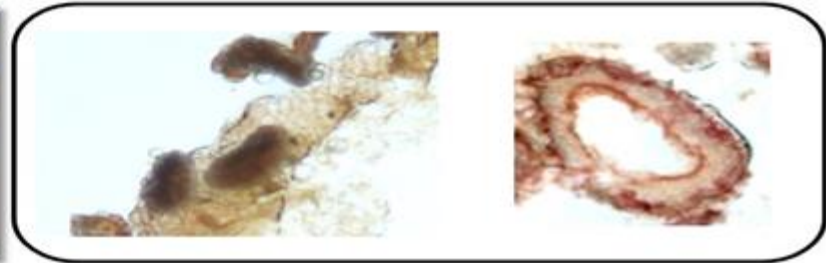


Cross Section of Non-viable Tobacco Seed

WT



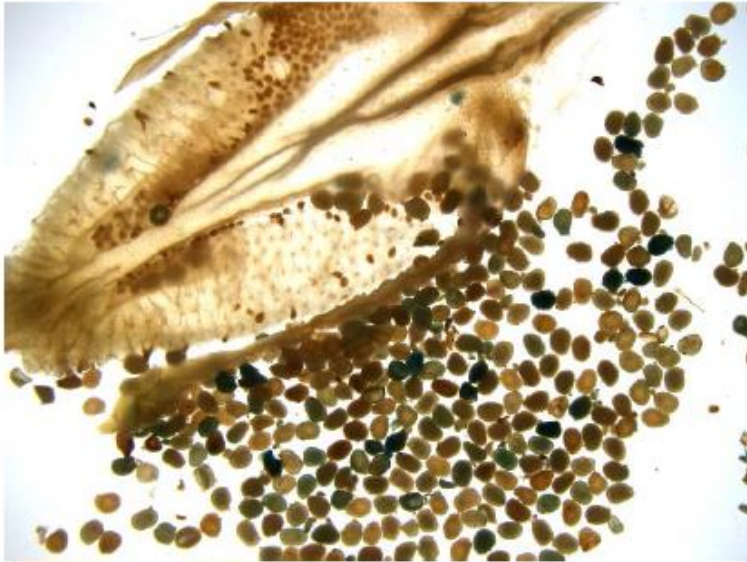
p15:cd



No Mature Embryo in Transgenic Lines With p15



Promoter Leakage Impacts Seed Development



P#15 ::GUS mature capsules and stigma/anthers after staining

Promoter Expressed in Seeds



P#1 2.5kb:: GUS Assay Shows No Leakage to Seeds



P#1 2.5kb::GUS plant: capsules and stigma/anthers after staining

Promoter Expressed in Seeds



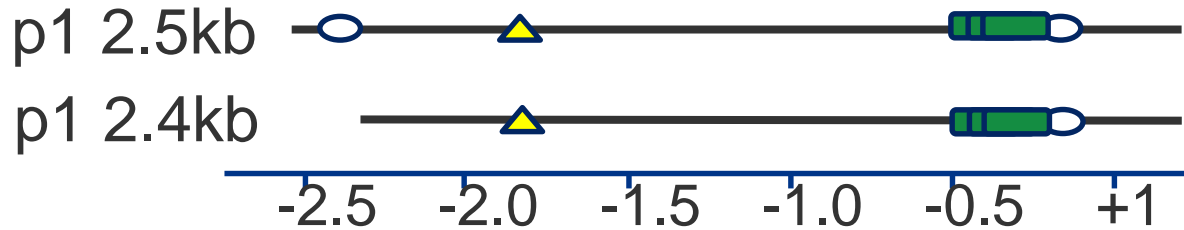
Promoter Leakage Impact Plant Development






P1-2.5 Promoter Cause Leaf Abnormality



Sugar Repressive Element was Removed to Increase Specificity



Cis-elements

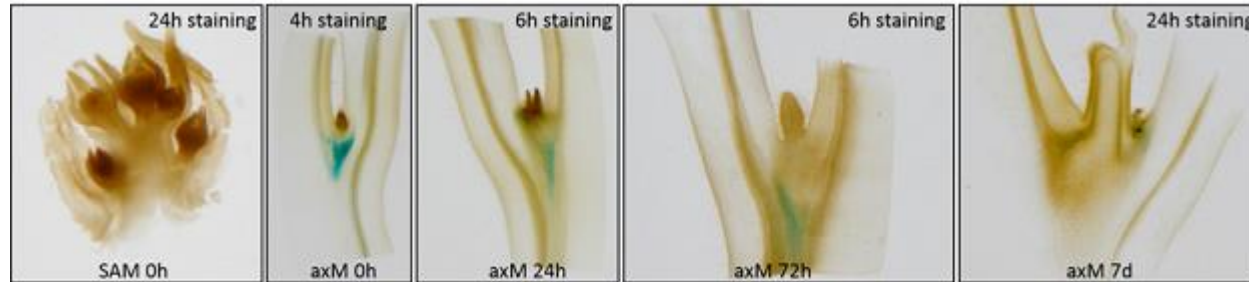
- BDE  Bud dormancy element (CACGTG)
- SRE  Sugar repressive element (TTATCC)
- UP2  Axillary bud up2 (AAACCCTA)

Reference:
Gonzales-Grandio et al., 2013
Tatematsu et al., 2005

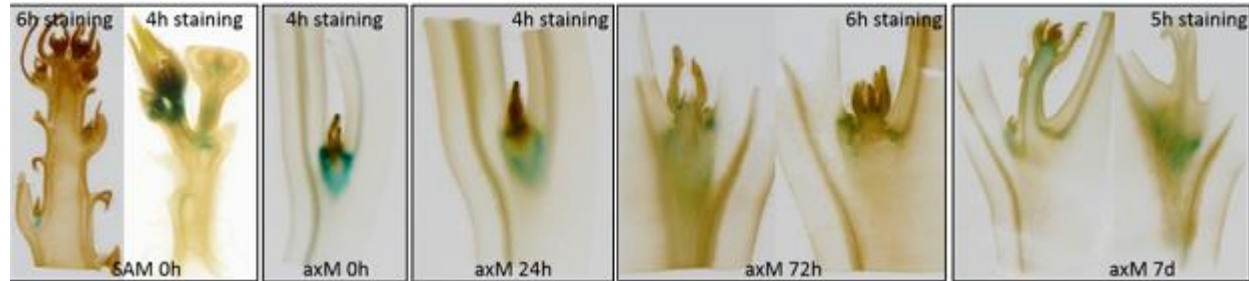


P1 Promoter After Topping GUS Activity

P#1-2.5::GUS



P#1-2.4::GUS



T1 Generation of P#1-2.5::GUS plant vs P#1-2.4::GUS plant

P1-2.4 Has Higher Specificity and Expression Level



T0 Generation With Modified Promoter



P#1-2.4::cd T0 plant Line-23 shows no bud initiate

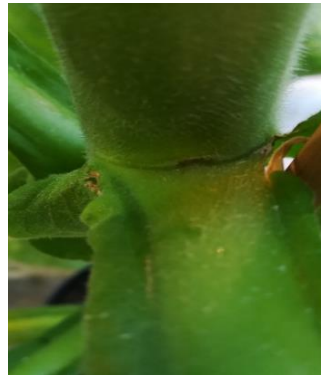


T1 Greenhouse Evaluation for Sucker Reduction

Wild type



Line 23-6



P#1-2.4::cd T1 generation 0h post topping



T1 Greenhouse Evaluation for Sucker Reduction

Wild type



Line 23-6



P#1-2.4::cd T1 generation 4 weeks post topping



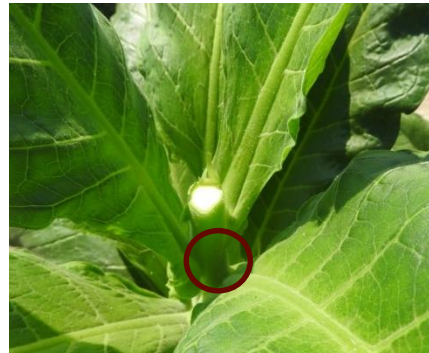
T1 Field Evaluation for P1-2.4 Plants

WT

L7

L23

0 WK



T1 Field Evaluation for Two Weeks

2 WK



WT



L7



L23



T1 Field Evaluation for Four Weeks

4 WK

**Topping
point**

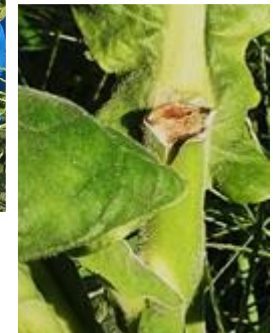
WT



L7



L23



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T2 P1-2.4 L7 Plants Show Diverse Phenotypes



Conclusion

- Expression of cell death gene with a sucker specific promoter is an effective strategy for sucker control
- In-depth promoter analysis is required to optimize the promoter specificity and activity
- The cell death gene in combination with a highly effective and efficiently modified promoter will lead to the development of sucker inhibited commercial lines



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