#### 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





#### 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**

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## Promoters from Axillary Bud Specific Genes





## Specific Expression of Cell Death (cd) Gene

#### Promoter

#### Cell Death Gene







#### cd Gene Transgenic Plant



## Three Weeks Post Topping Suckers



Sucker Growth Was Inhibited



#### Cross Section of Non-viable Tobacco Seed



No Mature Embryo in Transgenic Lines With p15



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### 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



#### P15-2.5 Promoter Cause Leaf Abnormality



### Sugar Repressive Element was Removed to Increase Specificity



**Cis-elements** 

- BDE Bud dormancy element (CACGTG)
- SRE O 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





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



#### **TO** Generation With Modified Promoter



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



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## **T1** Greenhouse Evaluation for Sucker Reduction



Line 23-6

Wild type









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

## **T1** Greenhouse Evaluation for Sucker Reduction

#### Wild type







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



#### T1 Field Evaluation for P1-2.4 Plants













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#### T1 Field Evaluation for Two Weeks



WT



**L7** 



L23



2 WK

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### **T1** Field Evaluation for Four Weeks



#### Topping point



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## 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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