# Genetic and Biochemical Analysis of Very Low Nicotine Tobacco Leaf

## <u>Kudithipudi C</u>.; Govindarajulu R.; Bhuiyan N.; Shen Y. & Xu D



## Outline





# Background

Altria Client Services

- FDA issued Advance Notice of Proposed Rule Making (ANPRM) for nicotine
  - FDA is interested in levels such as 0.3 0.5 mg/g per cigarette filler
- Naturally occurring nic1nic2 deletions and ALCS experimental lines have a 85-97% nicotine reduction and a negative impact on leaf quality





## **Investigate Genetic and Biochemical Differences of Very Low Nicotine Lines**



Altria Client Services | Plant Genetics | Coresta AP 2019 | 4

# **Very Low Nicotine Tobacco Leaf Phenotype**

**Rigid (Cell wall)** 

Thin body (Developmental)

**Texture is grainy** (Cell Wall)

Stay-green (many factors) (many factors) (many factors)

Leaf quality is regulated by multiple factors/genes and VLN tobacco typically has poor quality





# Senescence delayed

# **Susceptible to mold attack**



## **Experimental Lines and Data Collection**

- Burley 21 Series: Bu21, HiBu21, LiBu21, LABu21
- TN90 Series: TN90, TN90 PMTRNAi, TN90 PR50RNAi
- Flue Cured Series: K326, K326 PMTRNAi, LAFC53, Low nic B&W







# **Correlation of Nicotine Levels and Grade Index on Burley Tobacco**



- Tobacco leaf grade index is associated with nicotine levels
- nic1 nic2 deleted lines have poor lines

## None of these lines achieve nicotine levels of 0.3-0.5 mg/g





# leaf quality compared to Altria VLN



# **Correlation of Nicotine Levels and Grade Index on Flue-Cured Tobacco**



- VLN leaf grade index is better compared to Burley VLN lines

None of these lines achieve nicotine levels of 0.3-0.5 mg/g





## Grade index in Altria VLN lines are improved but sensorially they are similar to conventional VLN lines



## Data Analysis Design

**Expression Data** 





## Specific comparisons

)	TN90 PMT RNAi	
)	TN90 PR50 RNAi	
II Bu21	LI Bu21	LA Bu21
RNAi	LI Bu21 and	
) RNAi	LA Bu21	

# **Leaf Metabolite Profiling**



# Down Regulated Up Regulated



# **Venn Diagrams Showing the Overlap in Differentially Expressed Genes**





TN90 vs TN90 PMT RNAi

TN90 vs TN90 PR50 RNAi

BU21 vs LI BU21 vs LA BU21

TN90 PMT RNAi and TN90 PR50 RNAi vs LI BU21 and LA B21

## **Potential Leaf Quality Correlated Gene Expression**







Altria Client Services | Plant Genetics | Coresta AP 2019 | 12

# **Confirmation of Differentially Expressed Genes by RTPCR**







## **Differential Expression of Candidate Genes**



Altria Client Services



## rpkm values

High Mid

Low

## **Class 1: Cell wall Related Gene Expression**





TN90C TN90PMT TN90PR

## **Class 3: Senescence Related Gene Expression**



Altria Altria Client Services

senescence is delayed in VLN leaf



## Conclusions

- Cured leaf quality of transgenic VLN lines is better compared to conventional VLN controls
- Targeted gene reduction of nicotine biosynthetic pathway has minimal impact on genes which affect leaf quality
- Frequency of negatively correlated genes to leaf quality increased from flowering to harvest in VLN lines
- Both positive and negatively correlated candidate genes are being studied to further improve the leaf quality



Altria Client Services | Plant Genetics | Coresta AP 2019 |

# Altria's Center for Research & Technology (CRT)



