Genetic and Biochemical Analysis of Very Low Nicotine Tobacco Leaf

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Outline

- Background
- Leaf transcriptome and metabolite analysis
- Integration of differential gene expression, metabolites and leaf grade index
- Identification of candidate genes correlated with leaf quality
- Evaluation of candidate genes

Background

- 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

Normal Tobacco

VLN Tobacco

Leaf quality is an important trait for high quality tobacco products

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Objective

Investigate Genetic and Biochemical Differences of Very Low Nicotine Lines

Very Low Nicotine Tobacco Leaf Phenotype

Rigid (Cell wall)

Thin body (Developmental)

Texture is grainy (Cell Wall)

Stay-green (many factors)

Senescence delayed (many factors)

Susceptible to mold attack (many factors)

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

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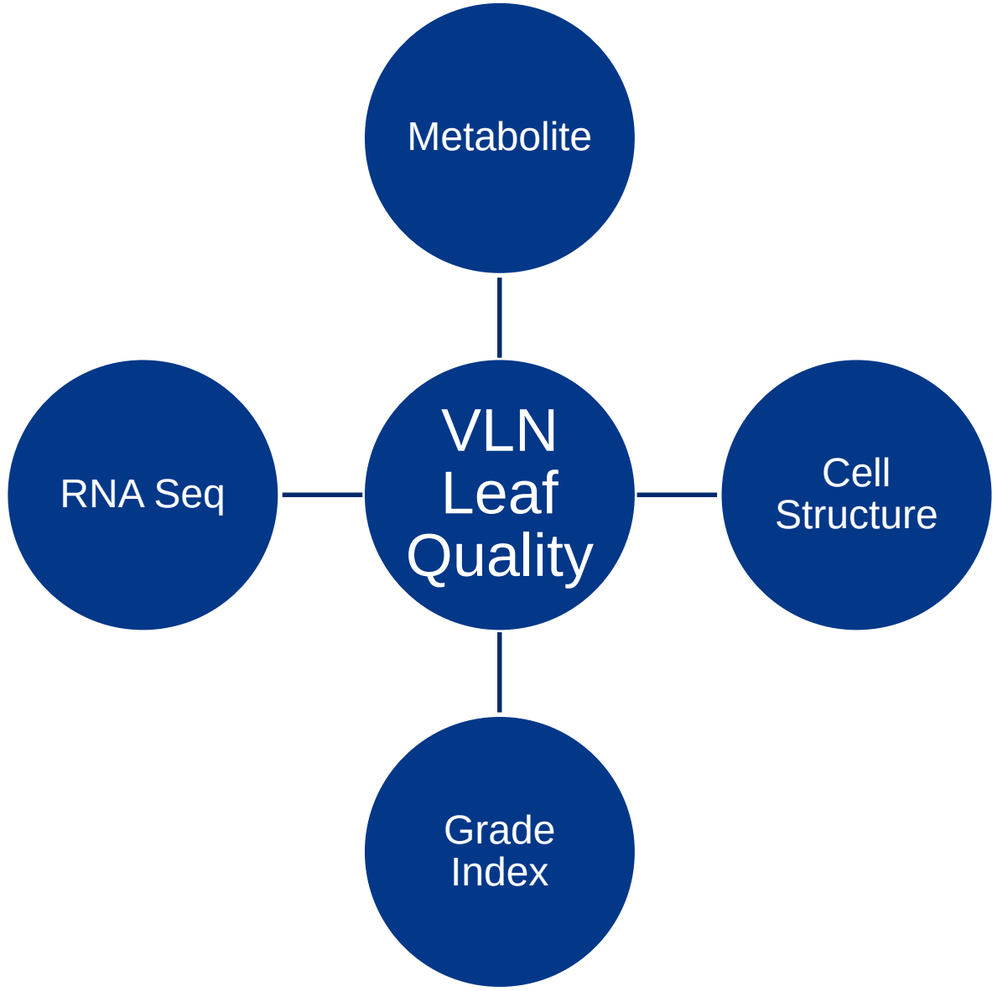
Experimental Lines and Data Collection

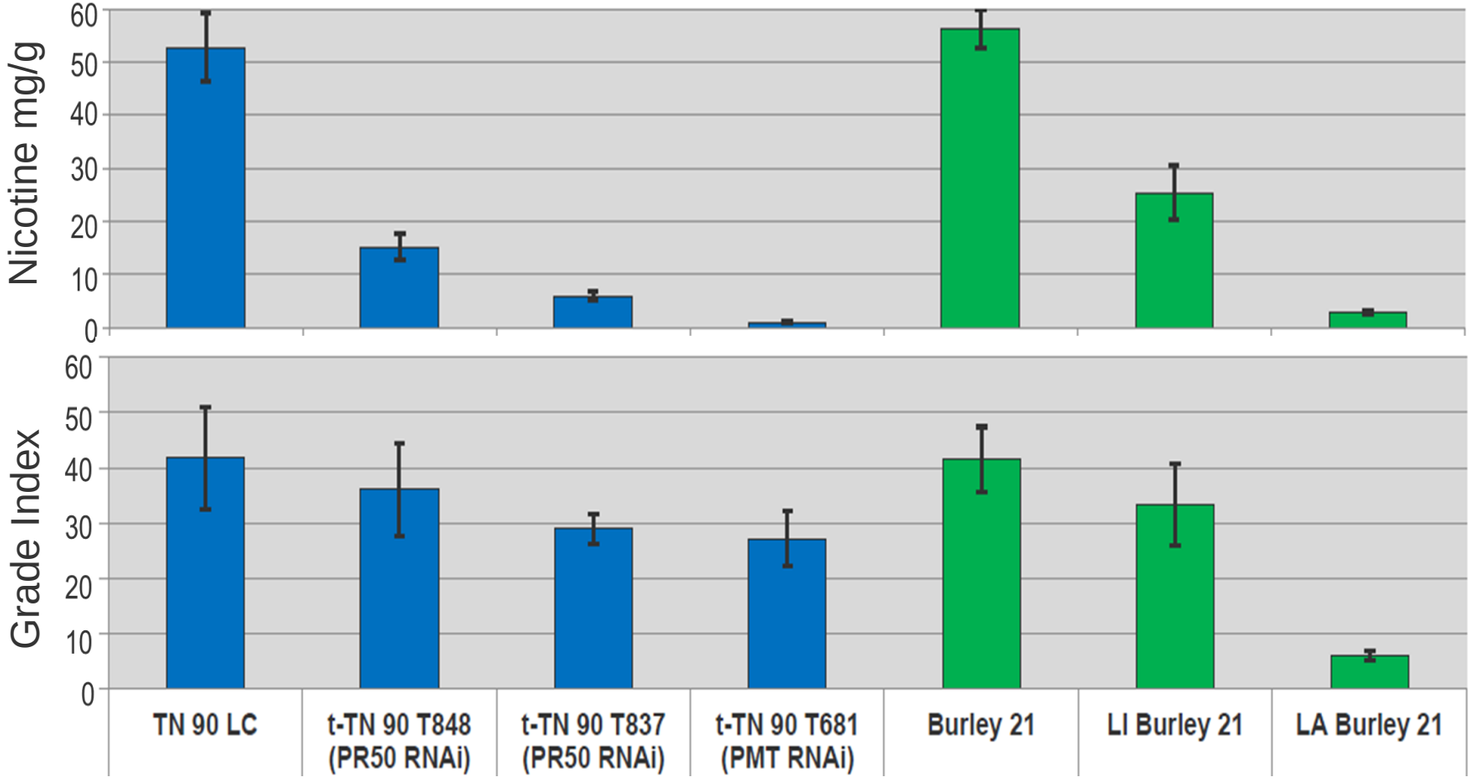
- Burley 21 Series: Bu21, HiBu21, LiBu21, LABu21
- TN90 Series: TN90, TN90 PMTRNAi, TN90 PR50RNAi
- Flue Cured Series: K326, K326 PMTRNAi, LAFC53, Low nic B&W

Experimental lines



Data types

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 leaf quality compared to Altria VLN lines

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

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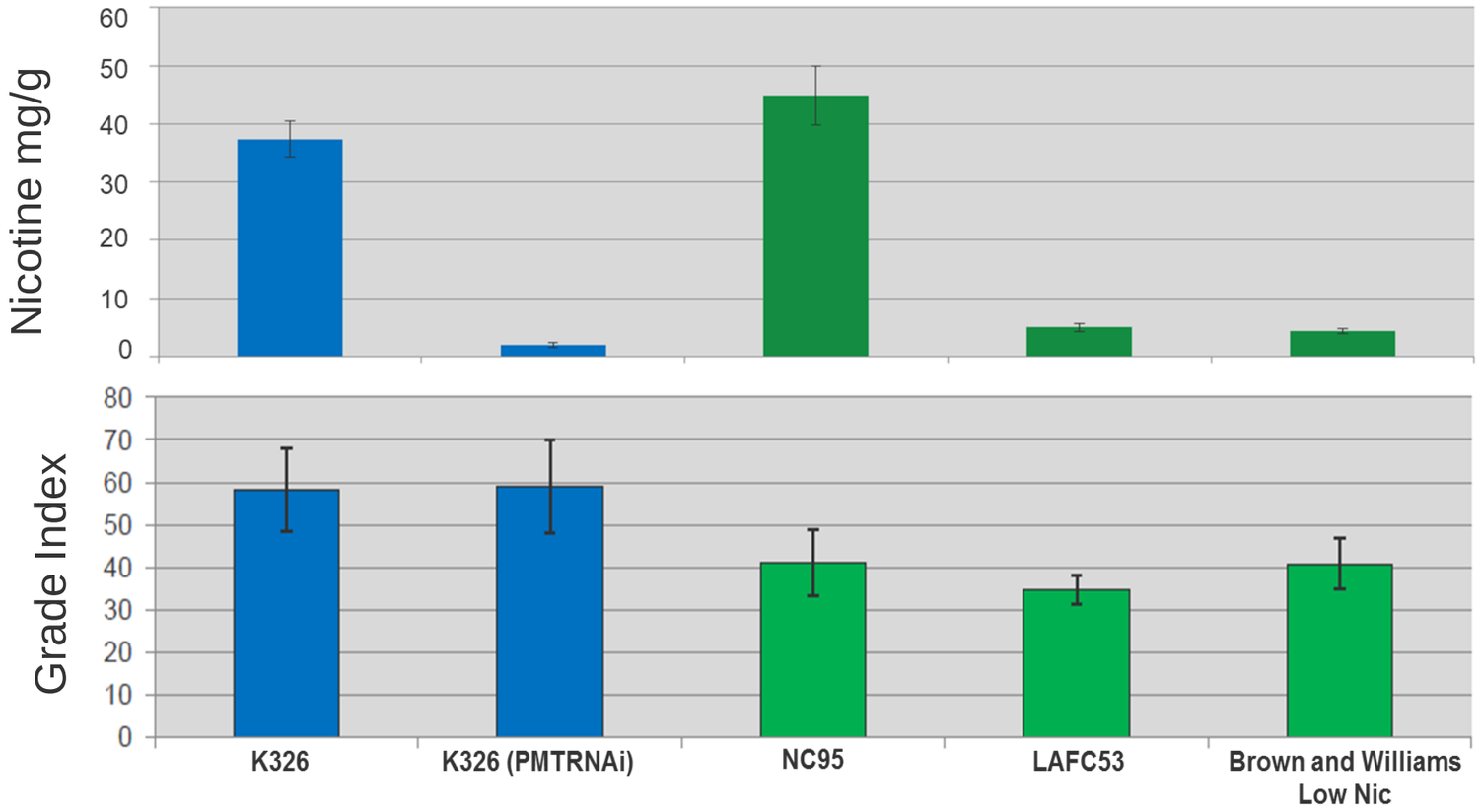
Correlation of Nicotine Levels and Grade Index on Flue-Cured Tobacco



- VLN leaf grade index is better compared to Burley VLN lines
- Grade index in Altria VLN lines are improved but sensorially they are similar to conventional VLN lines

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

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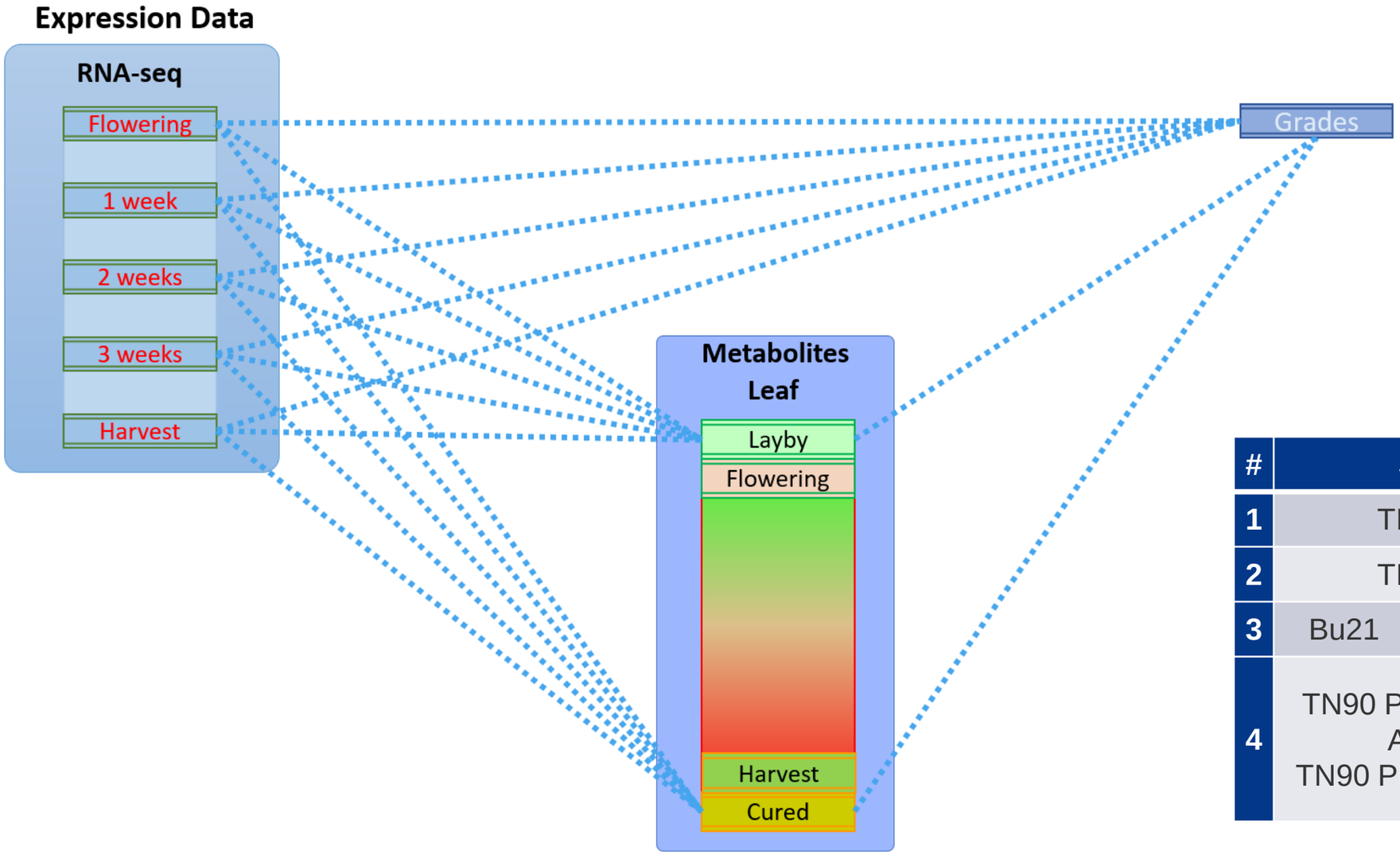
Data Analysis Design



#	Specific comparisons			
1	TN90		TN90 PMT RNAi	
2	TN90		TN90 PR50 RNAi	
3	Bu21	HI Bu21	LI Bu21	LA Bu21
4	TN90 PMT RNAi And TN90 PR50 RNAi		LI Bu21 and LA Bu21	

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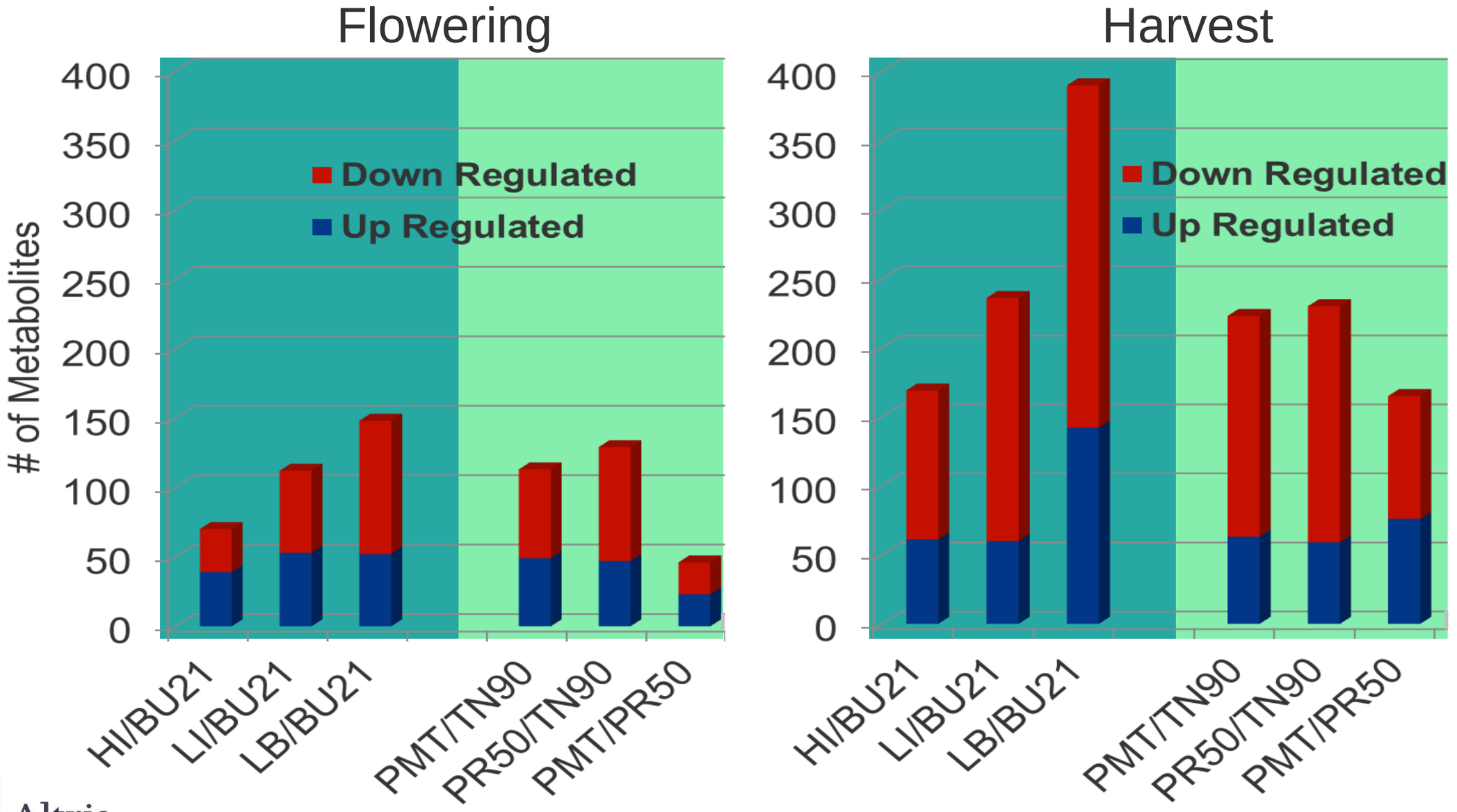
Leaf Metabolite Profiling



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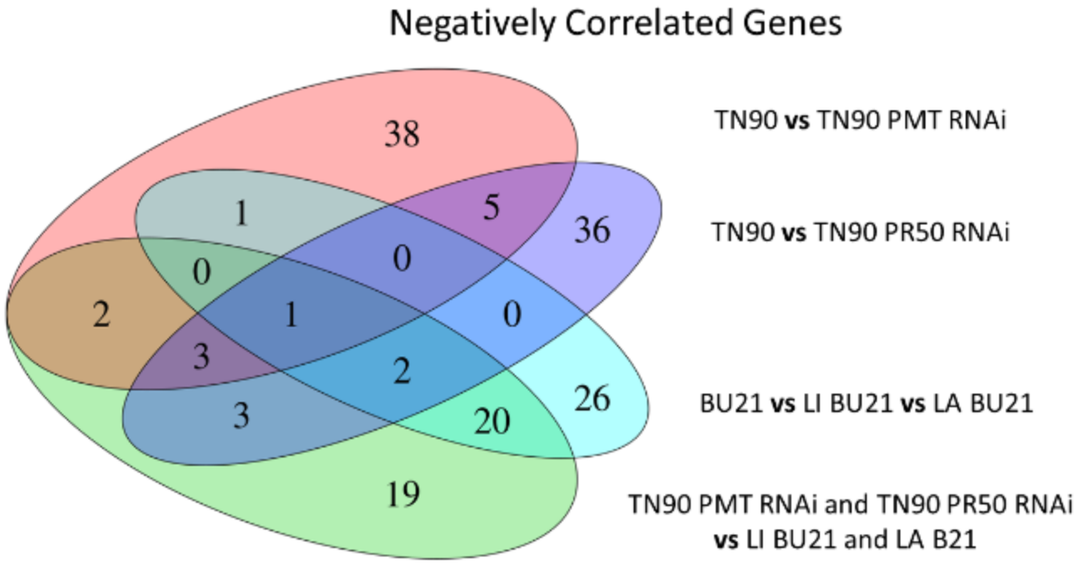
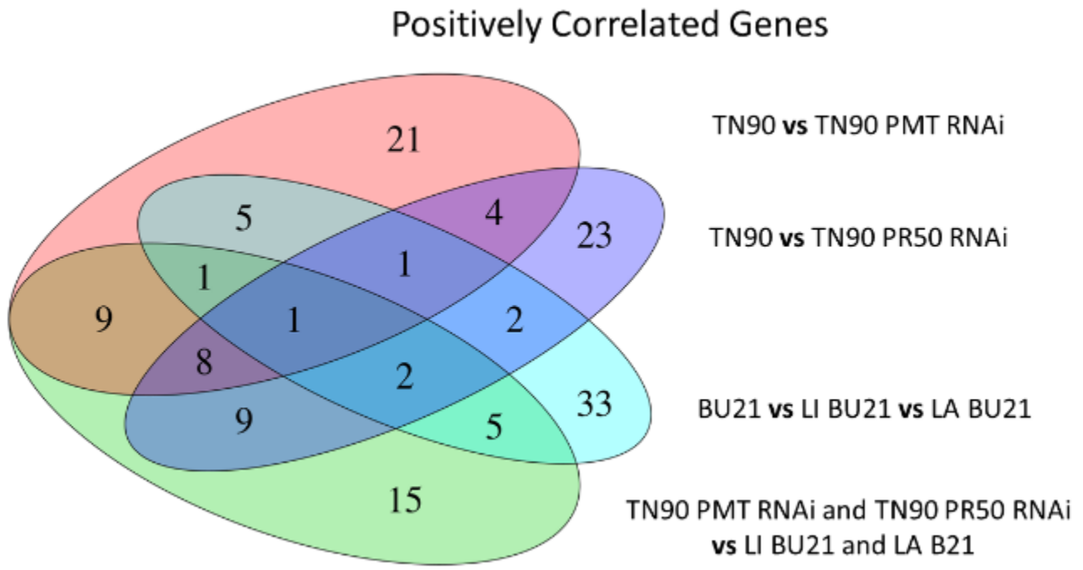
Venn Diagrams Showing the Overlap in Differentially Expressed Genes





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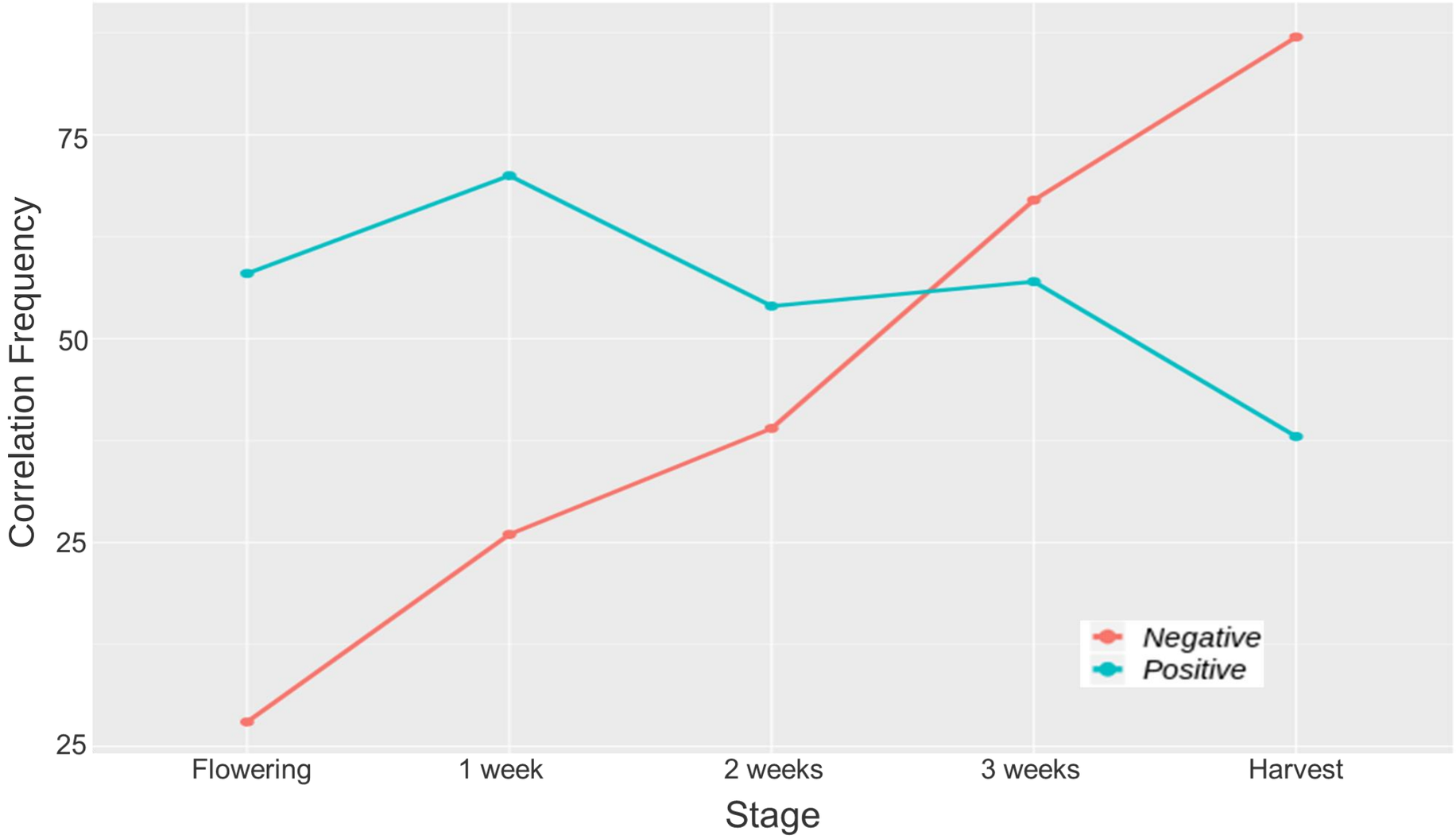
Potential Leaf Quality Correlated Gene Expression



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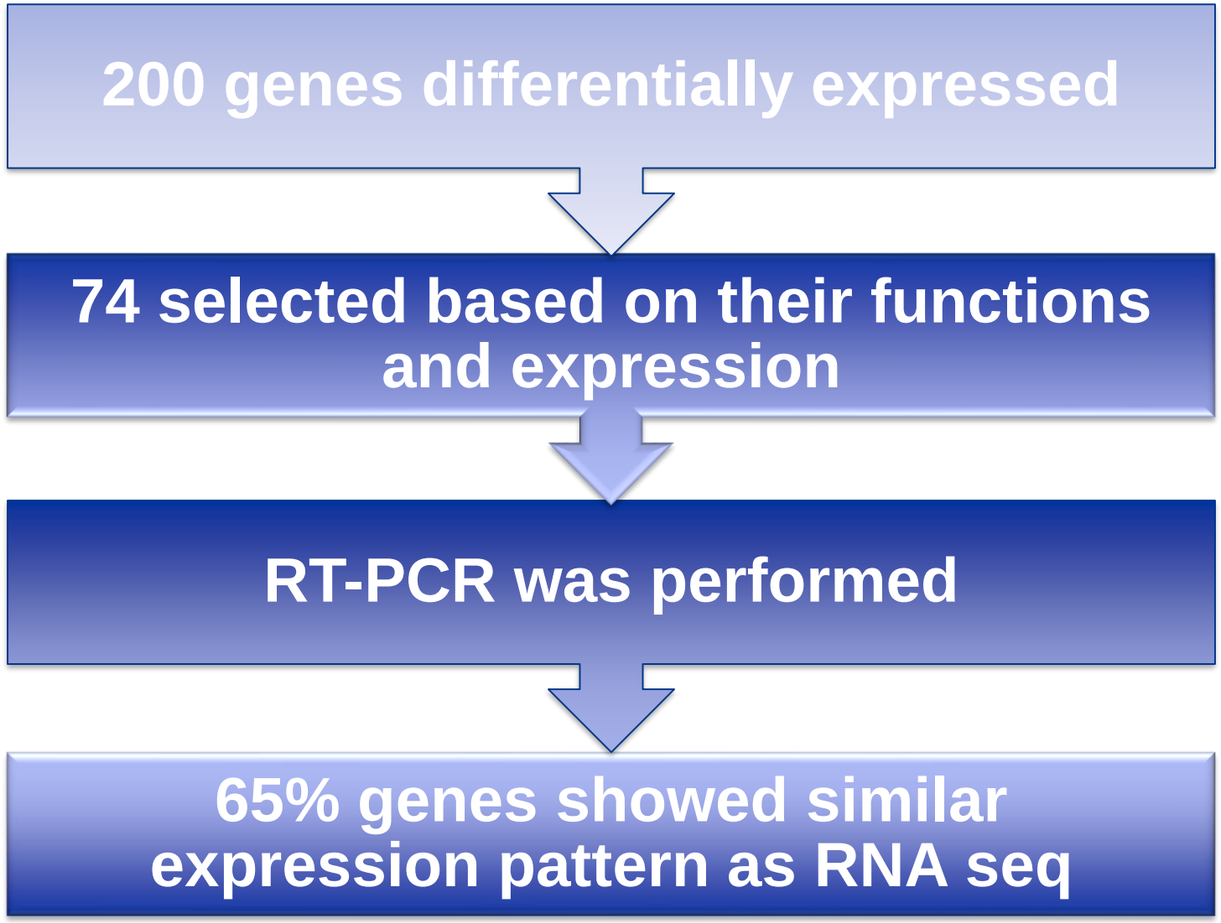
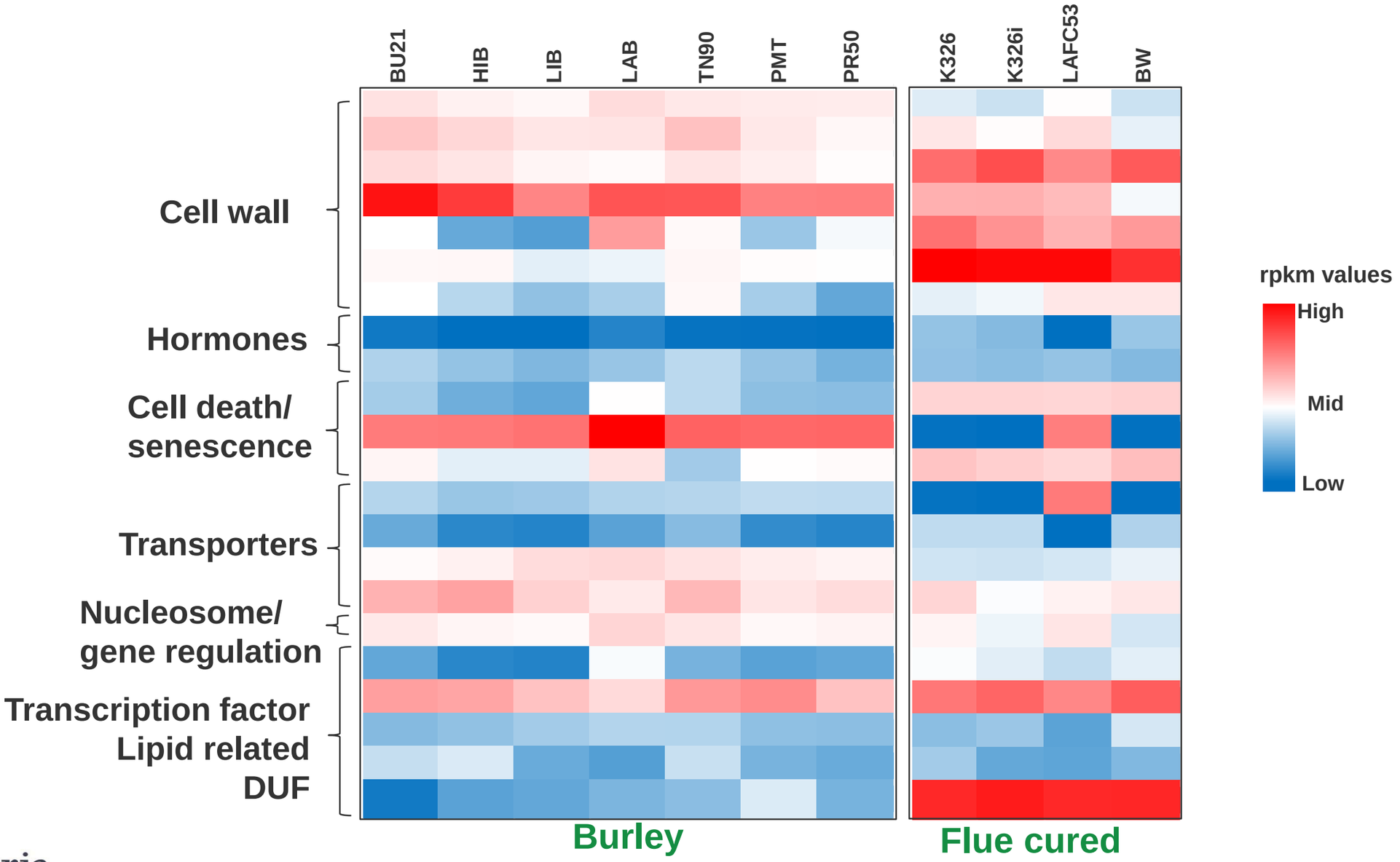
Confirmation of Differentially Expressed Genes by RTPCR



Differential Expression of Candidate Genes



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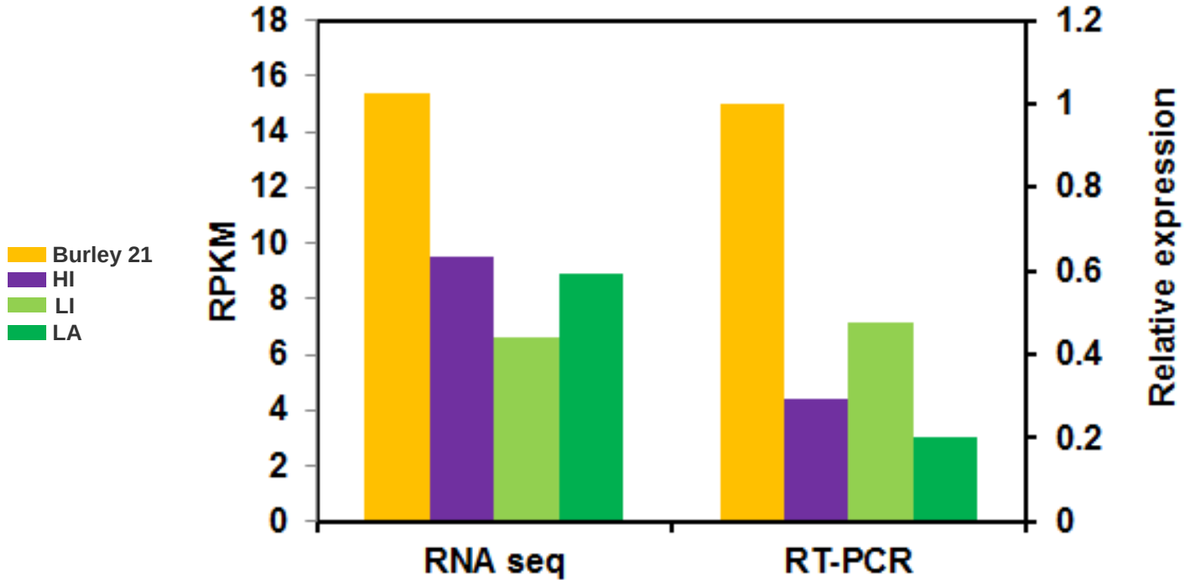
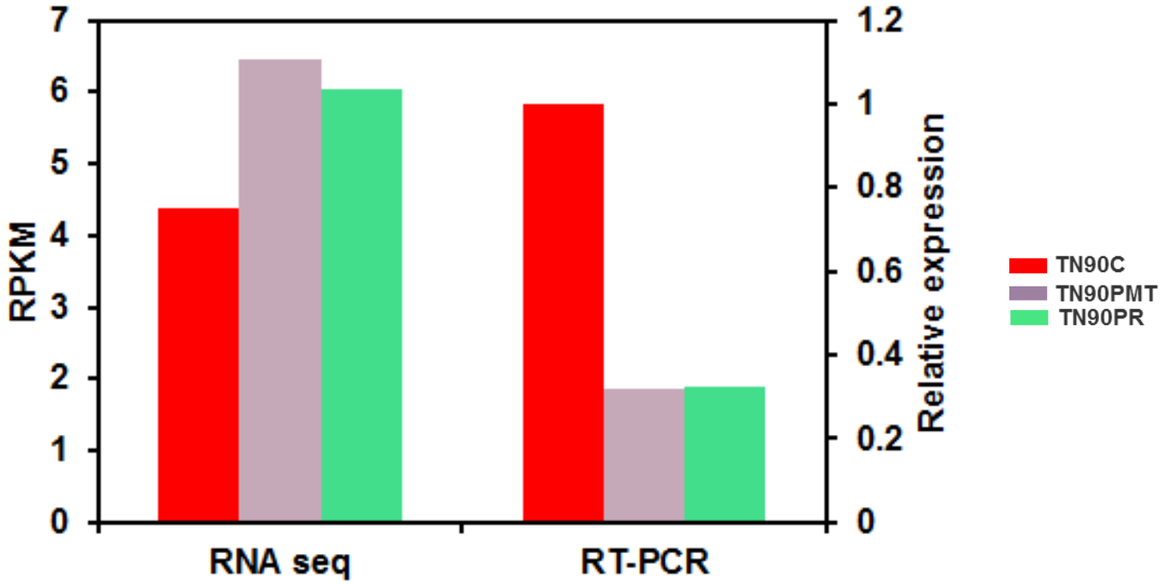
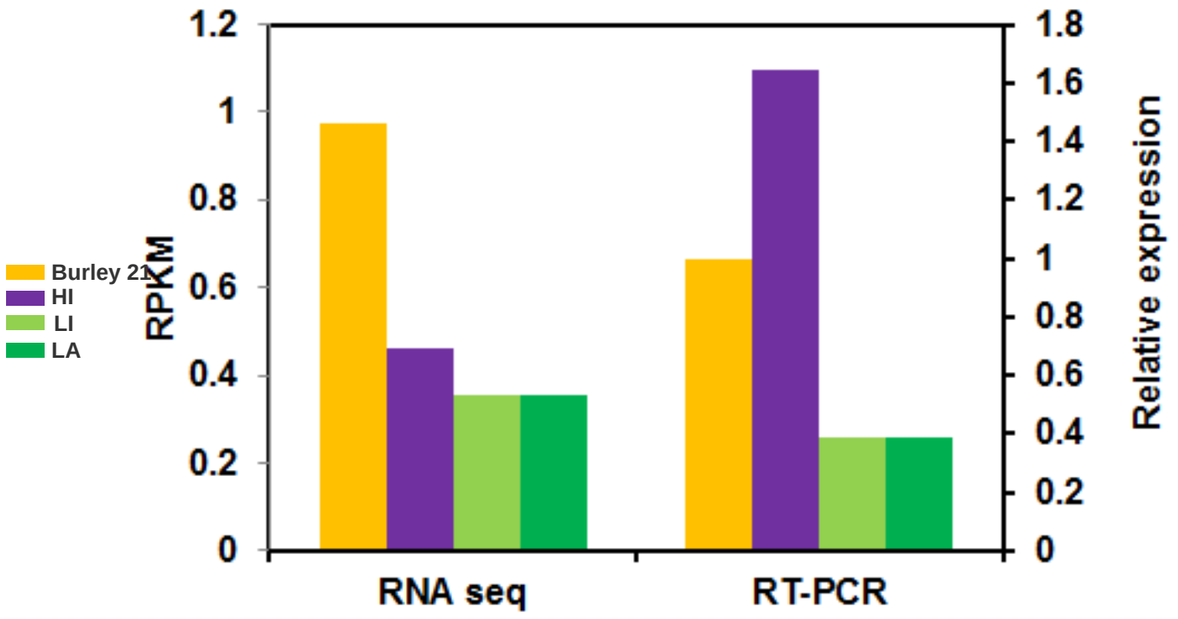
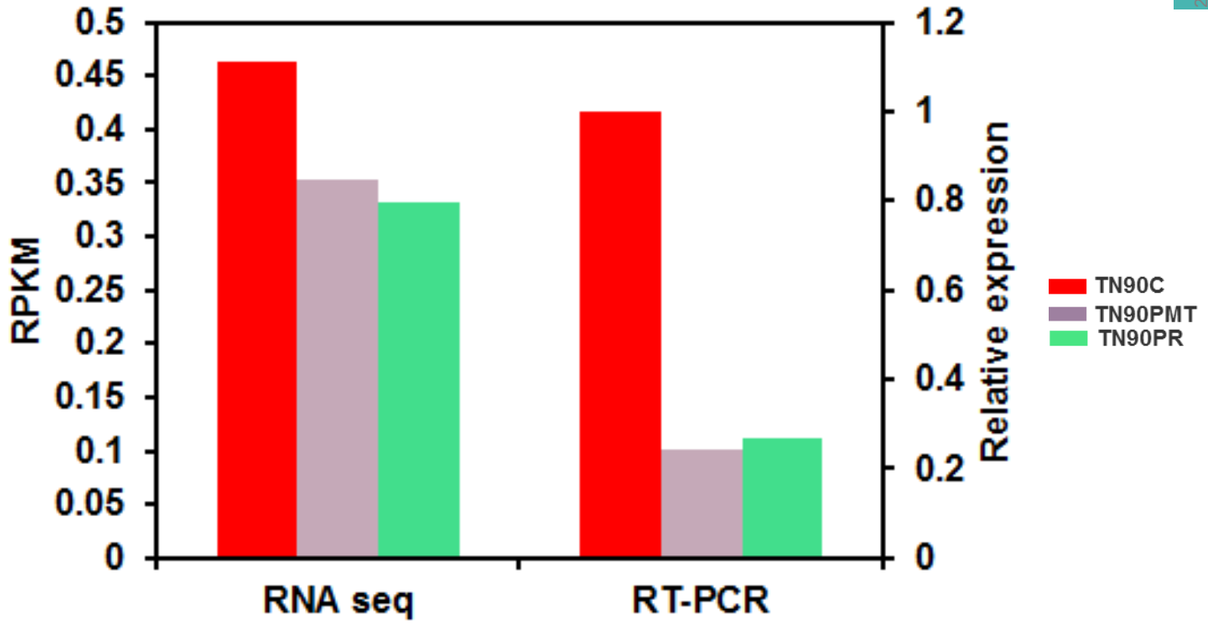
Class 1: Cell wall Related Gene Expression





Class 3: Senescence Related Gene Expression



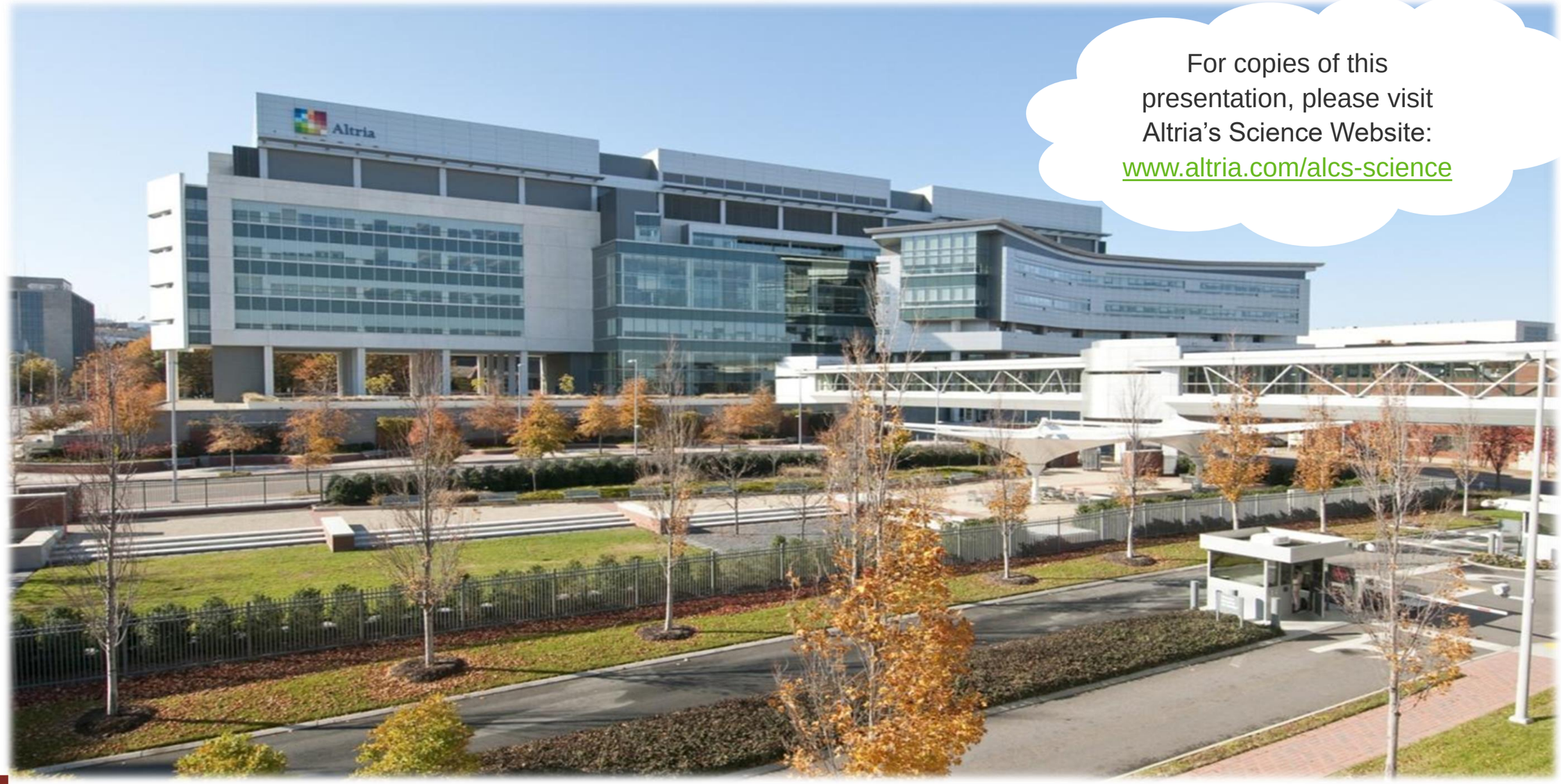


senescence is delayed in VLN leaf

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's Center for Research & Technology (CRT)



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