中国烟草总公司 郑州烟草研究院
ZHENGZHOU TOBACCO RESEARCH INSTITUTE OF CNTC

Effects of genotype and environment on metabolite profiling of *Nicotiana tabacum*

China Tobacco Gene Research Center
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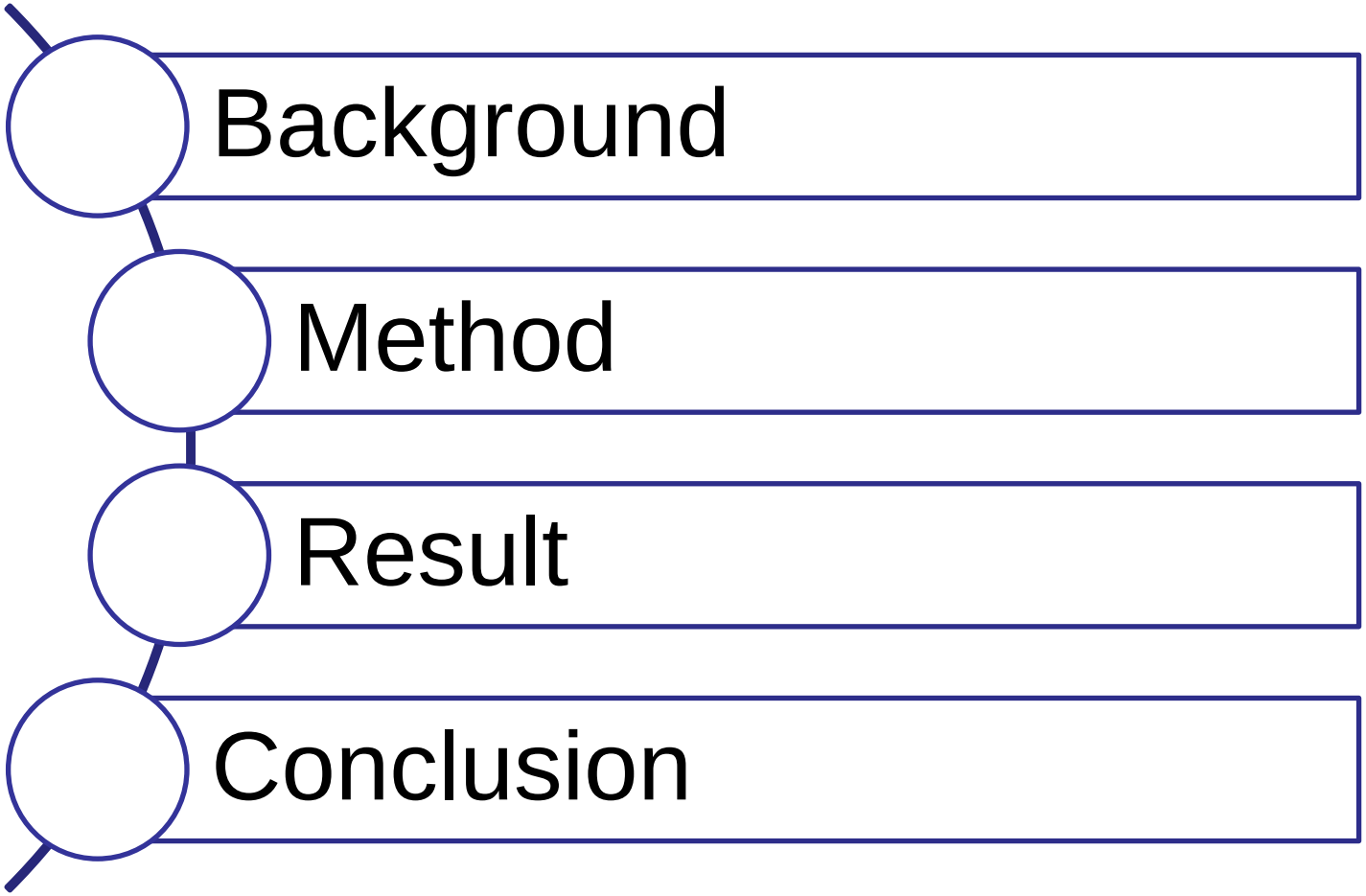
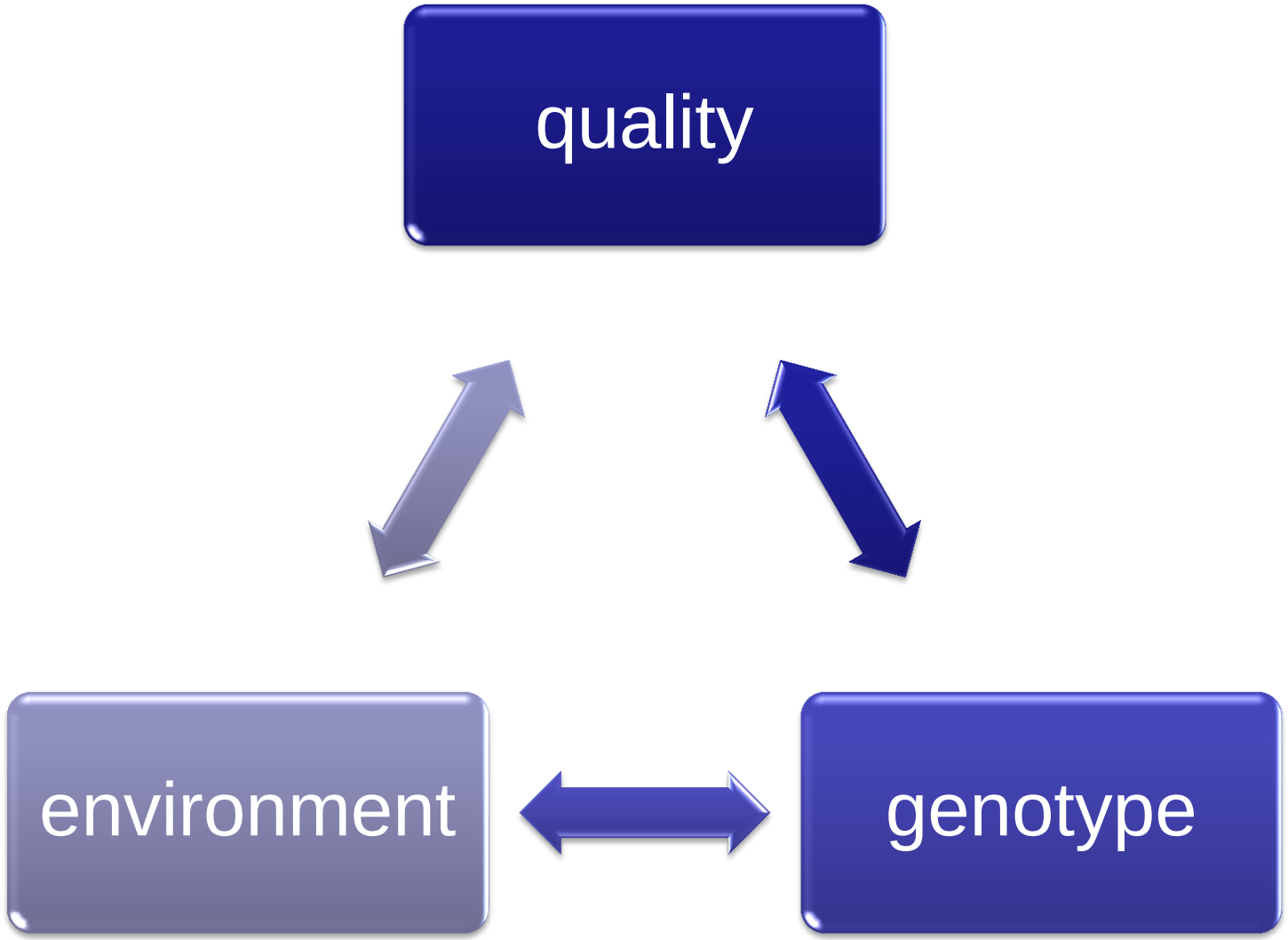
2017.10.23

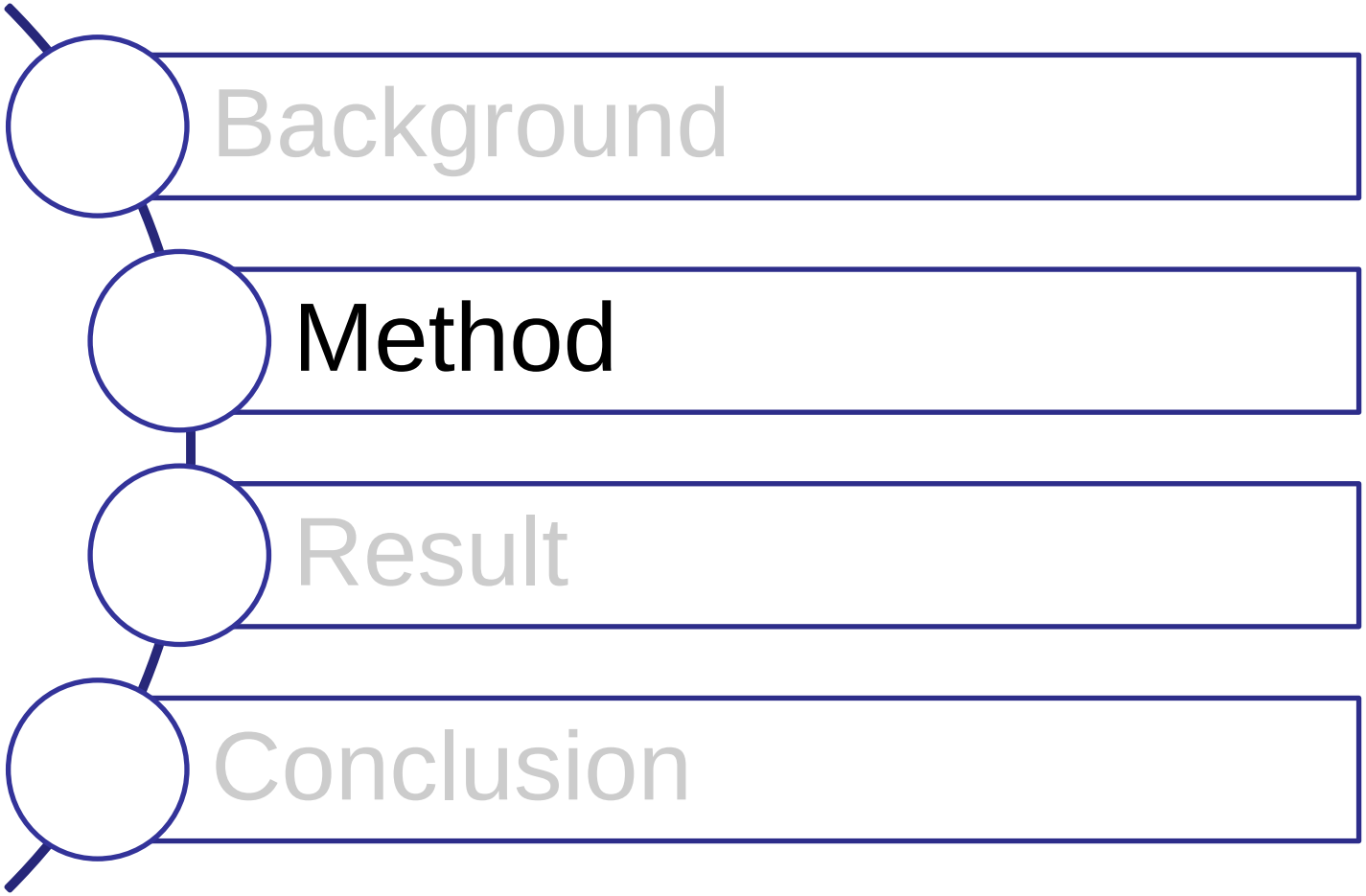
Outline



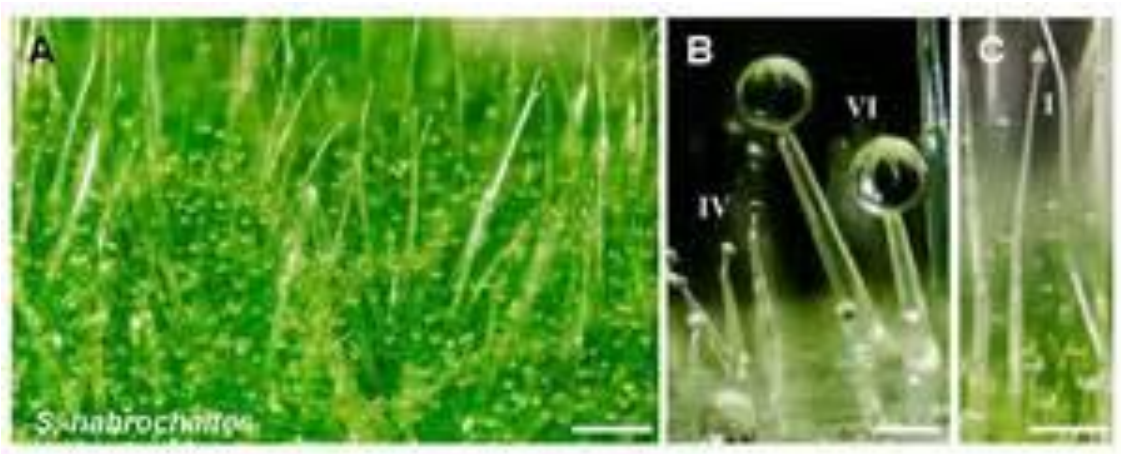
Genotype, Environment <-> Quality

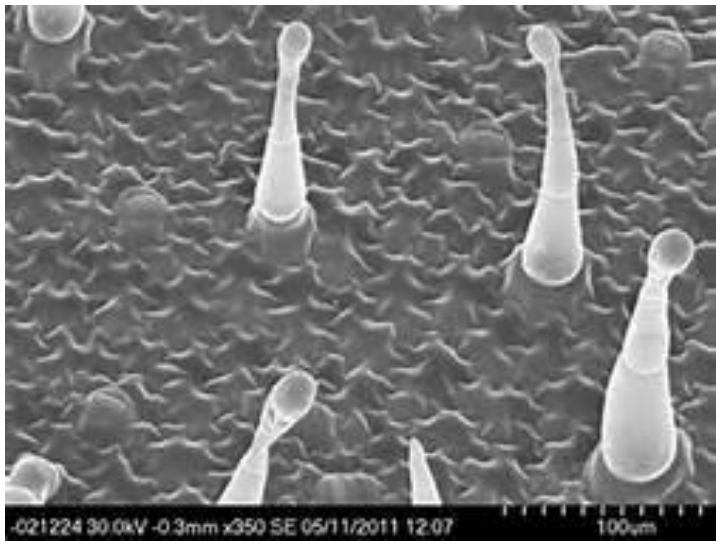


Outline

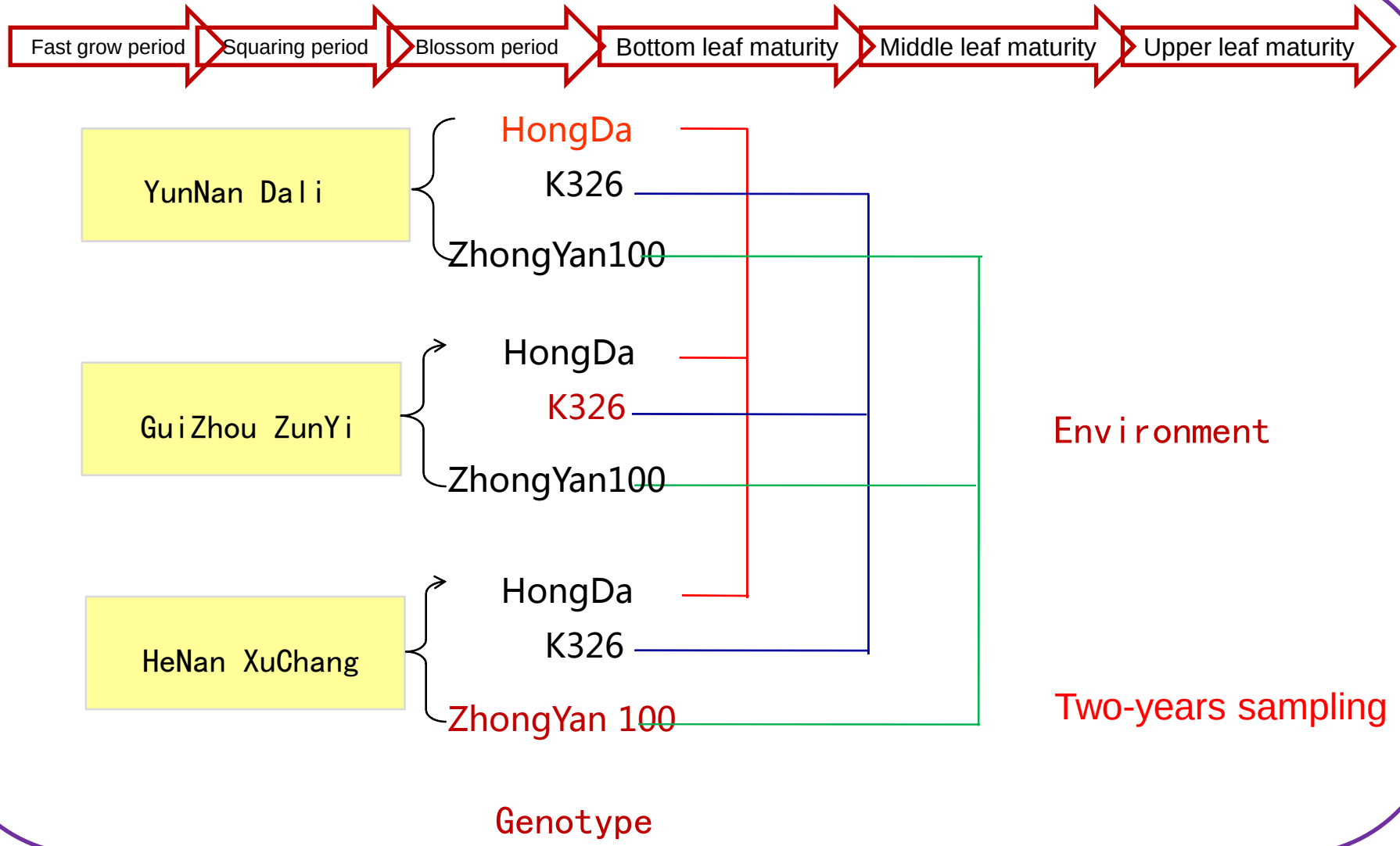


Tobacco---a model plant for metabolism research

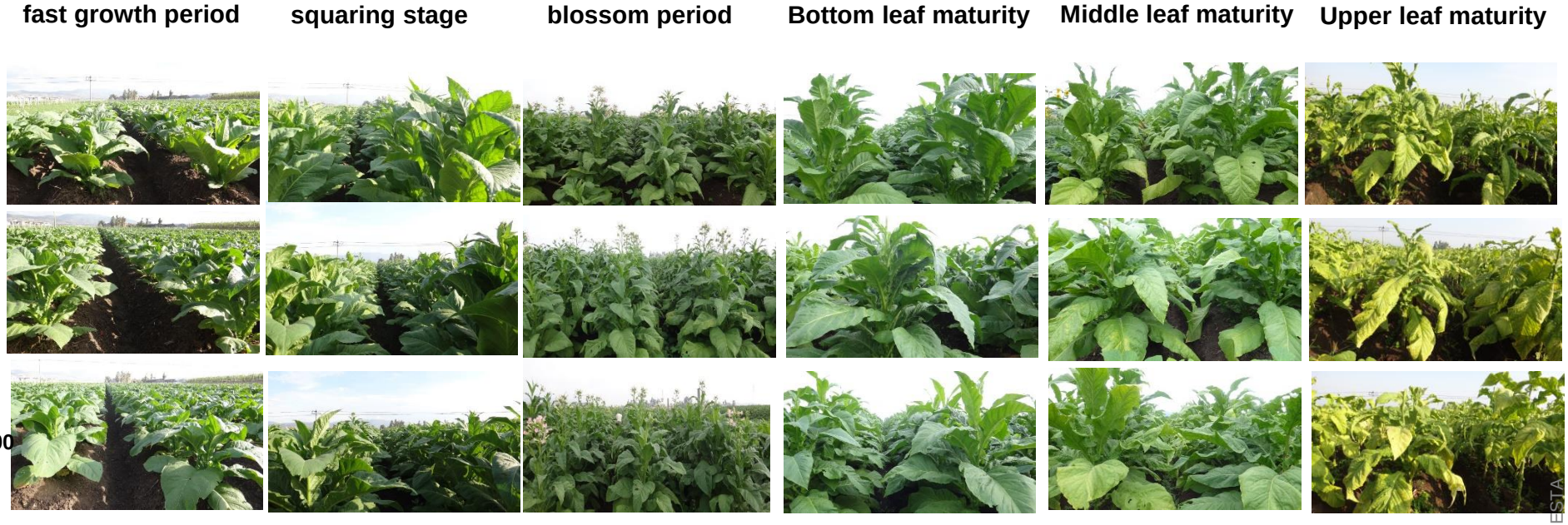




Experiment design



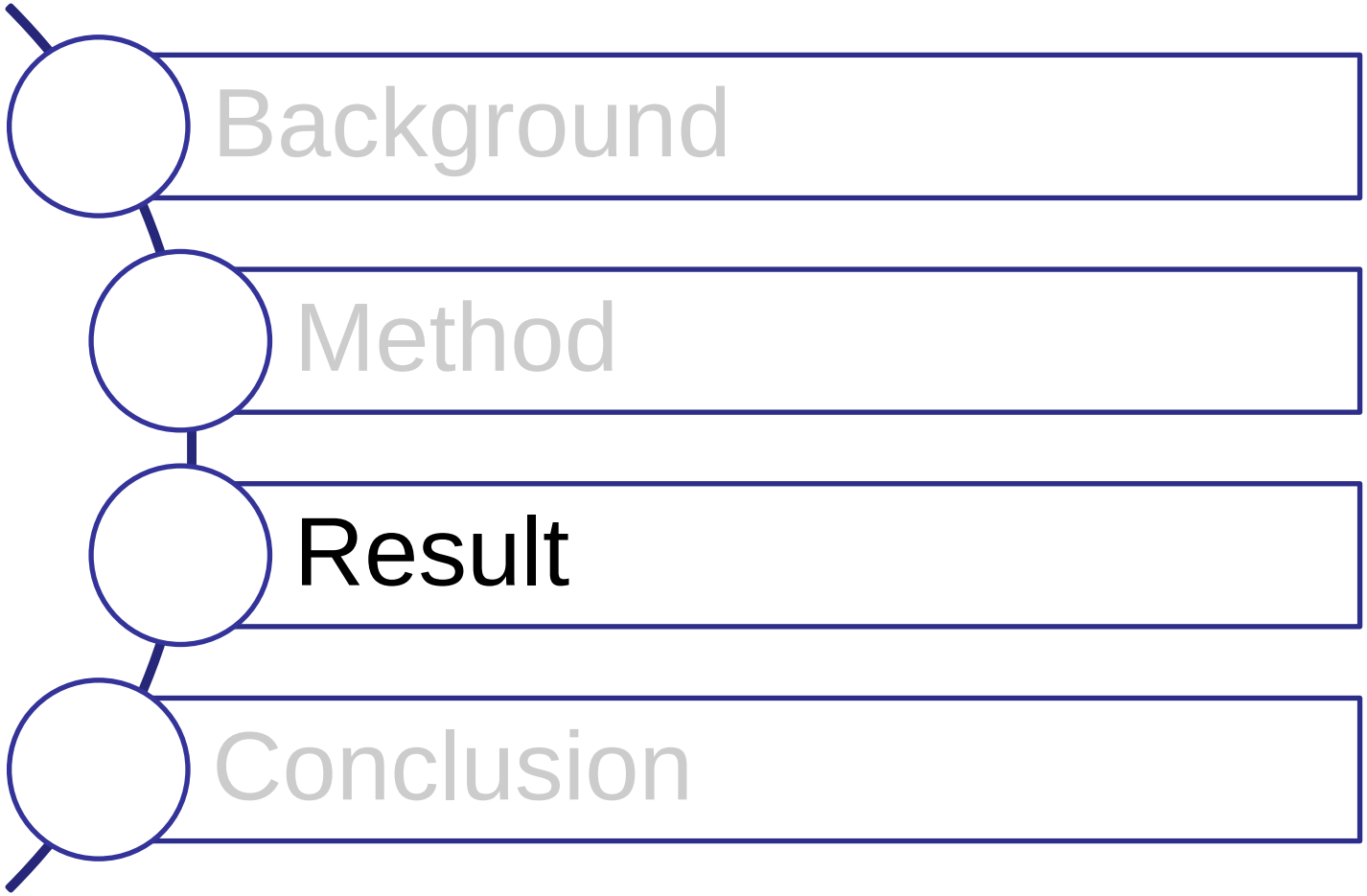
Sample phenotype



12 methods for metabolite identification

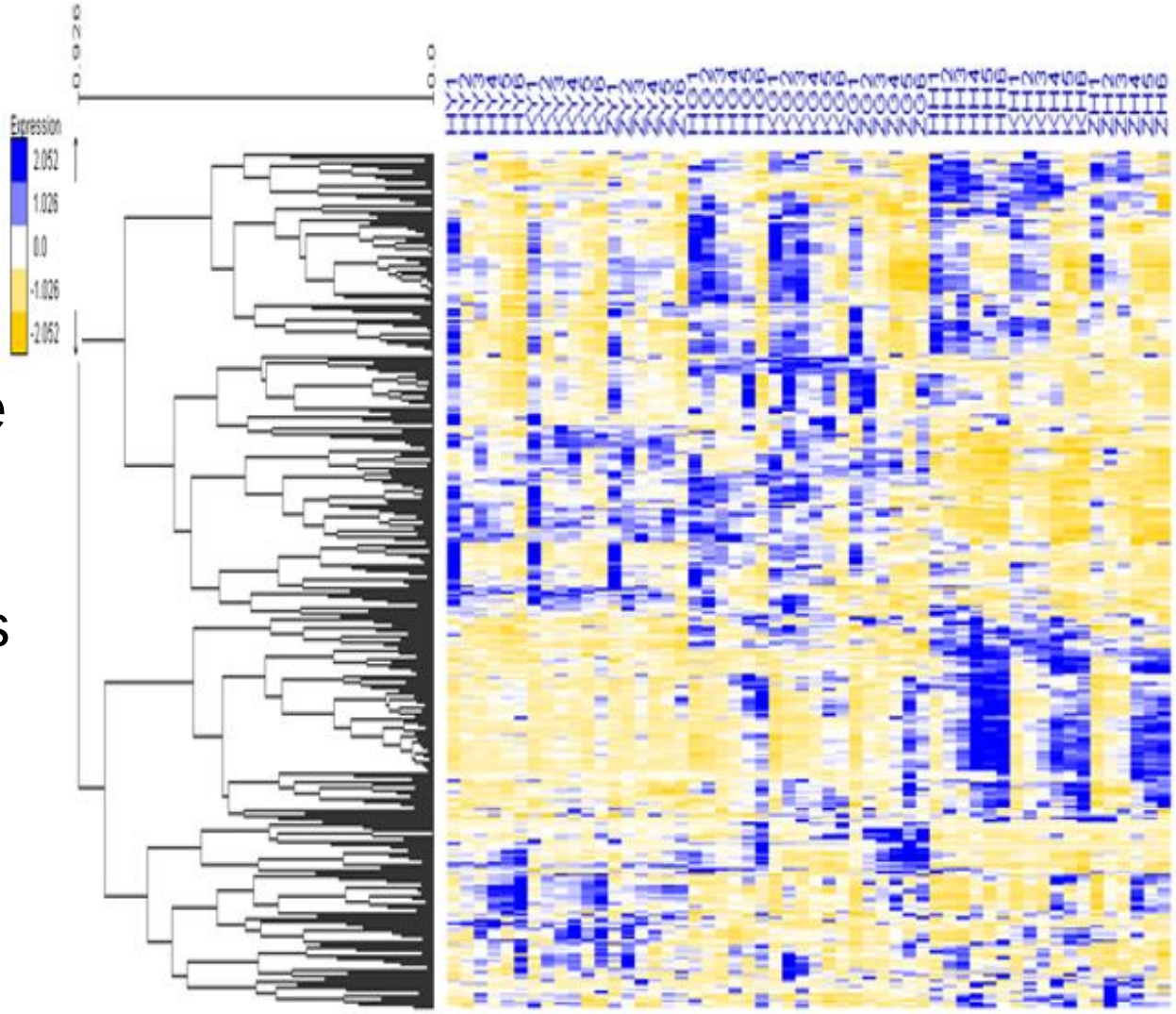
num	target metabolites	method	pathway
1	amino acid	UPLC-SQD	amino acid metabolism
2	organic acid	GC-MS	fatty acid metabolism
3	sterol	GC-QQQ/MS	
4	terpene	GC-QQQ/MS	MEP,MVA and terpene synthase
5	pigment	HPLC-UV	
6	alkaloid	GC-MS	alkaloid biosynthesis pathway
7	polyphenol/flavonoid	HPLC-UV,HPLS-MS	polyphenol/flavonoid biosynthesis
8	lipd	LC-MS	lipid biosynthesis pathway
9	Non-target derivatization GC-MS	GC-MS	TCA,Sugar,Amino acid
10	Non-target direct injection GC-MS	GC-MS	terpene
11	Non-target LC-MS	LC-QTOF/MS	terpene
12	Non-target CE-MS	CE-MS	nucleoside metabolism, pentosephosphate

Outline

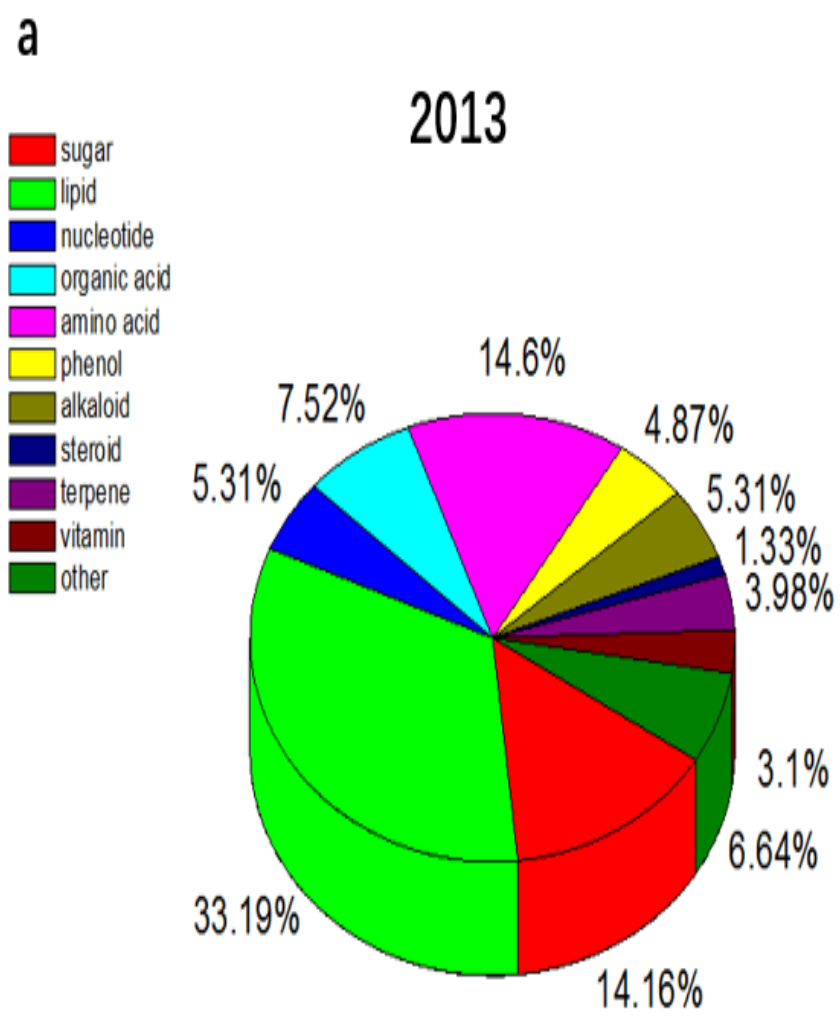


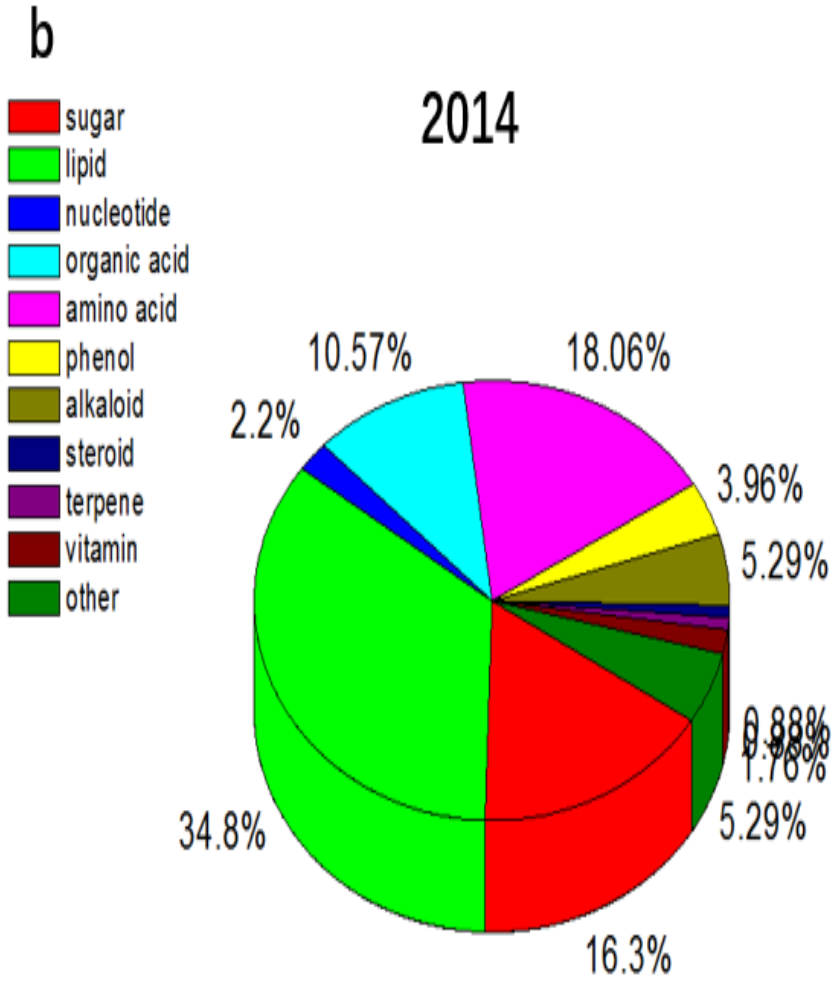
Many differential expressed metabolites were identified

- Around 1000 metabolites
- Around 500 qualitative metabolites
- 200 differential expressed metabolites

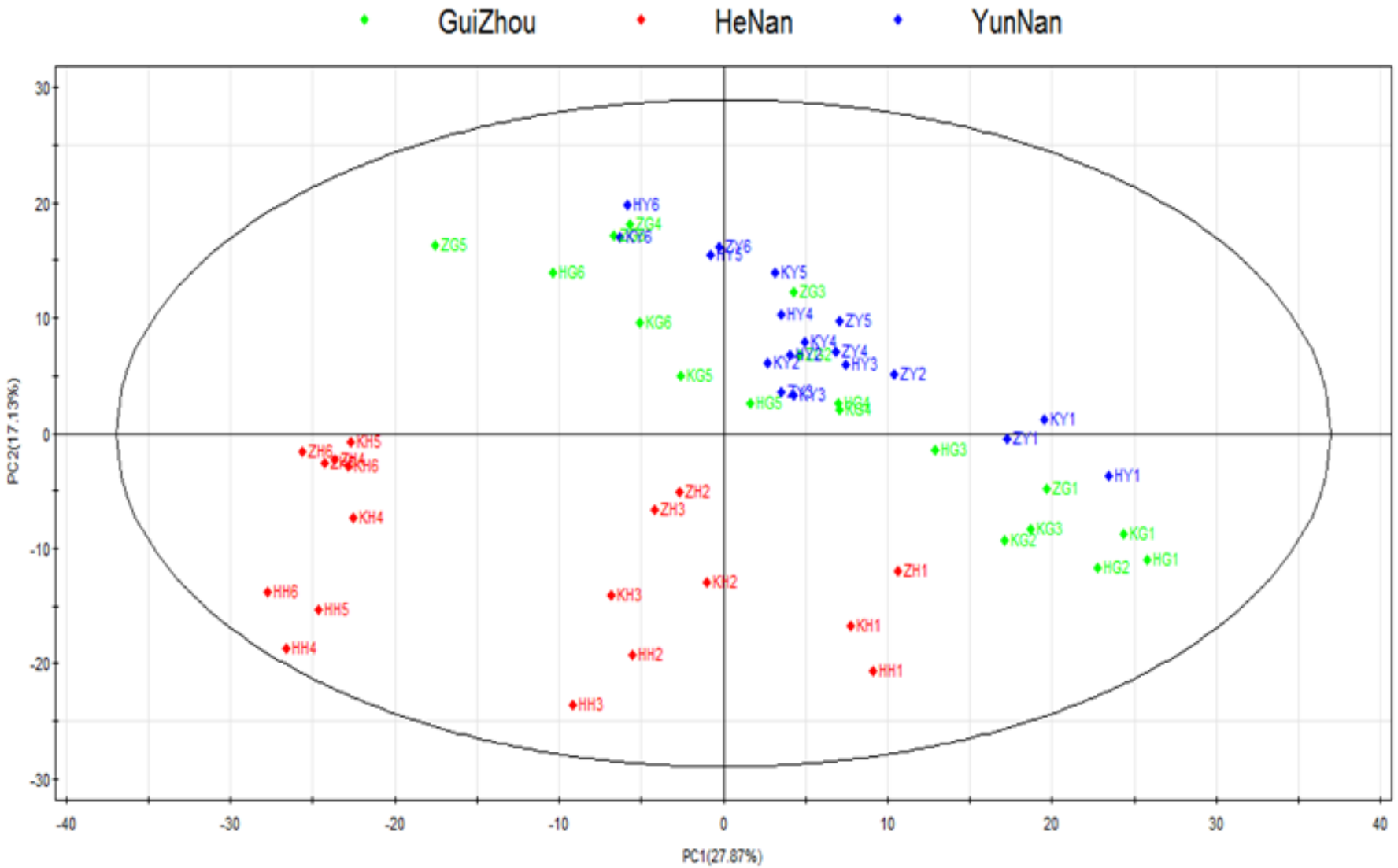


Lipids and sugars are major differential metabolites between three regions

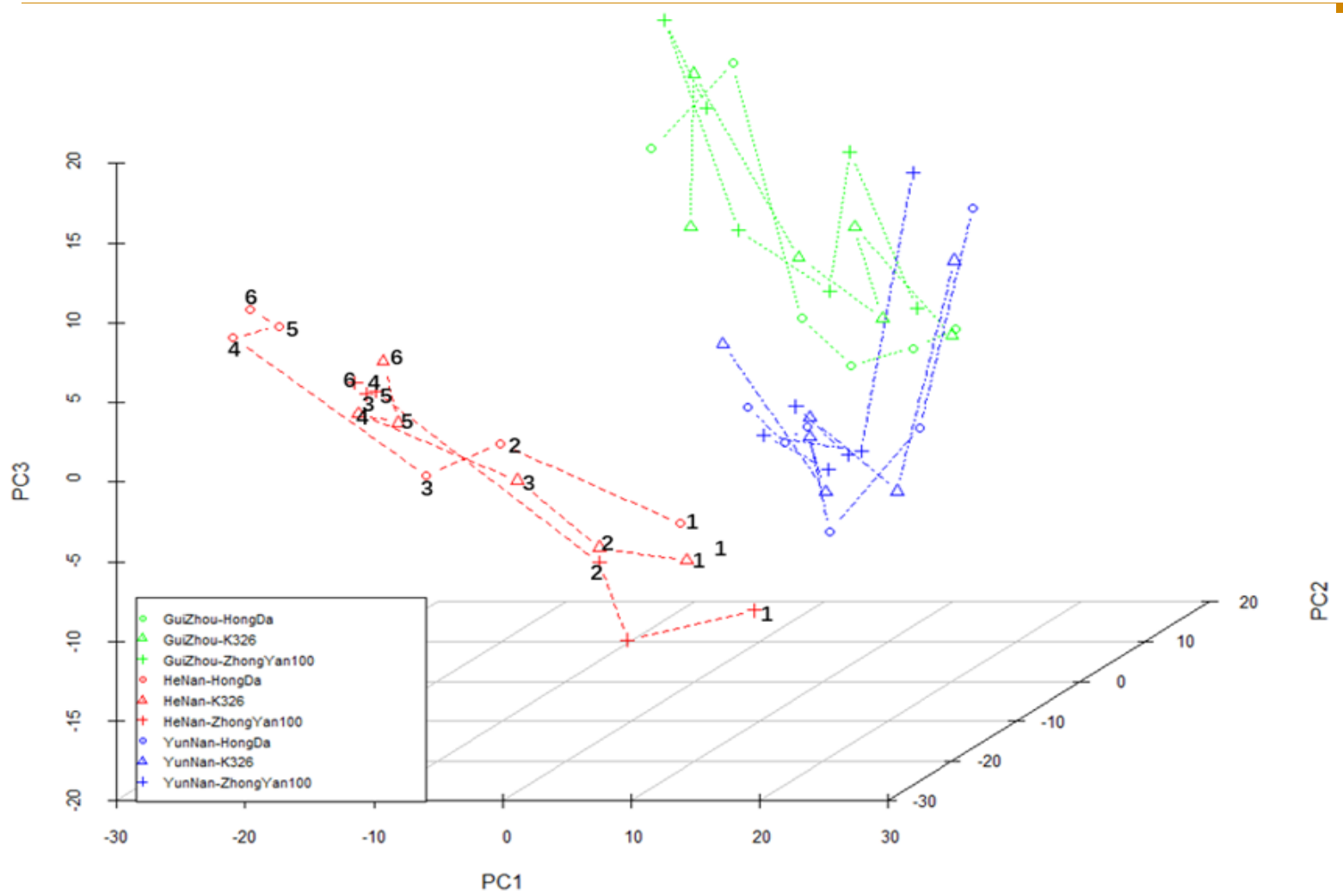




Metabolites of Tobacco have significant region feature



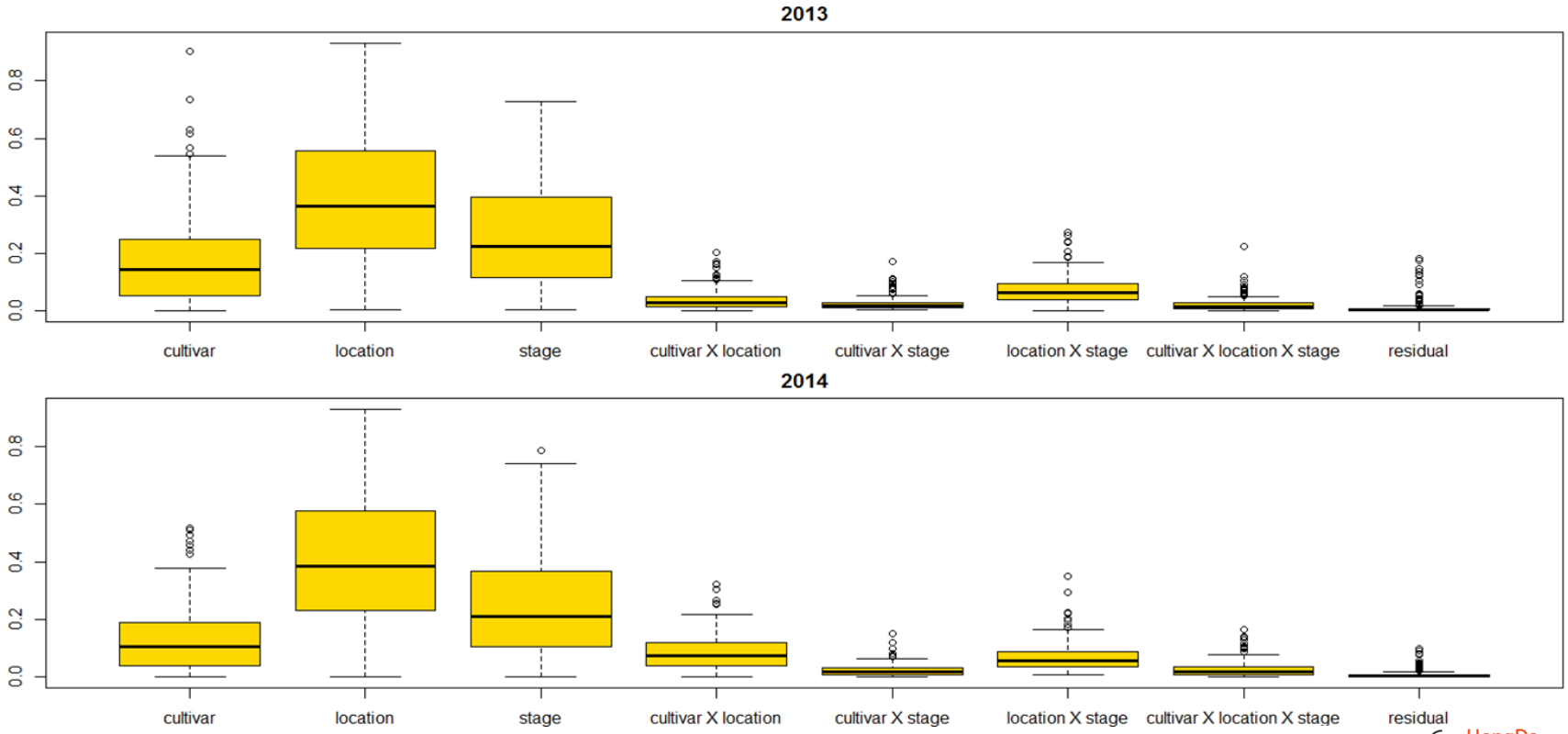
Tobacco metabolites changes with the development stage

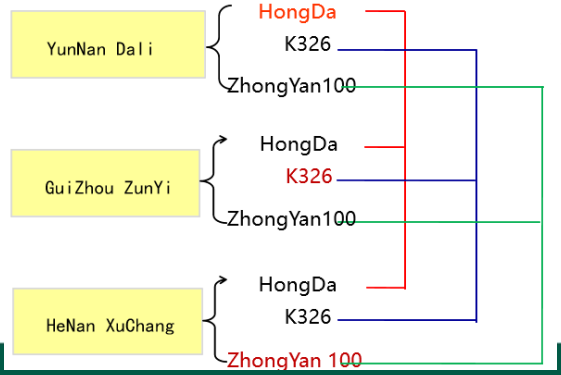


- The metabolites change as the development stage changing
- Region effect > genotype effect

Cultivar and location effect on tobacco metabolites

ANOVA analysis





- Location has much more effect than cultivar

Lipid is mostly affected by location

Metabolite	cultivar	location	stage	C X L	C X S	L X S	C X L X S	residual
D-malic acid	1.45%	92.79%	0.84%	1.14%	0.68%	2.20%	0.61%	0.30%
Ethylene glycol	3.13%	92.65%	0.24%	0.86%	0.59%	1.14%	0.69%	0.70%
DGDG (18:2/18:0)	1.91%	92.04%	2.62%	0.42%	0.34%	2.03%	0.50%	0.14%
Phenol	1.52%	91.59%	1.26%	2.47%	0.57%	0.77%	0.88%	0.94%
glycerol	3.11%	90.44%	1.97%	1.87%	0.52%	1.28%	0.52%	0.29%
glycerol 1-phosphate	1.51%	90.01%	3.66%	1.34%	0.51%	1.81%	0.97%	0.20%
stearic acid	0.99%	88.38%	3.39%	1.42%	1.42%	2.04%	1.41%	0.94%
Quinic acid	1.58%	87.03%	6.28%	1.49%	0.67%	1.90%	0.91%	0.14%
fumaric acid	2.89%	86.45%	5.42%	3.37%	0.34%	1.16%	0.28%	0.08%
D-glucose-6-phosphate	1.78%	86.41%	3.72%	2.18%	0.86%	4.30%	0.55%	0.20%
Hexanoic acid	5.27%	85.71%	4.61%	1.83%	0.15%	1.72%	0.41%	0.31%
Glucuronic acid	5.10%	85.61%	4.22%	1.04%	0.74%	2.16%	1.00%	0.13%
Spermine	3.90%	84.43%	3.49%	5.27%	0.61%	1.54%	0.58%	0.18%
xylitol	1.33%	83.96%	3.46%	7.92%	0.17%	2.76%	0.33%	0.07%
pantothenic acid 2	7.99%	83.77%	1.26%	4.51%	0.27%	1.83%	0.31%	0.07%
succinic acid	3.68%	83.12%	3.43%	5.27%	0.55%	3.30%	0.54%	0.11%
arachidic acid	4.23%	81.80%	1.32%	7.26%	0.85%	3.18%	0.55%	0.82%
DGDG (18:3/18:0)	3.74%	81.79%	11.6%	0.40%	0.14%	1.41%	0.59%	0.30%
L-glutamic acid 2	0.02%	81.60%	11%	1.50%	0.34%	4.99%	0.38%	0.12%
PE (35:3)	6.51%	81.37%	3.98%	3.64%	0.58%	2.84%	0.86%	0.23%
TG (16:0/16:0/18:3)	2.24%	81.11%	8.99%	2.00%	0.97%	4.02%	0.54%	0.13%
quinic acid	3.05%	81.02%	7.92%	2.78%	0.95%	2.81%	0.90%	0.57%

Some secondary metabolites are mostly affected by genotype

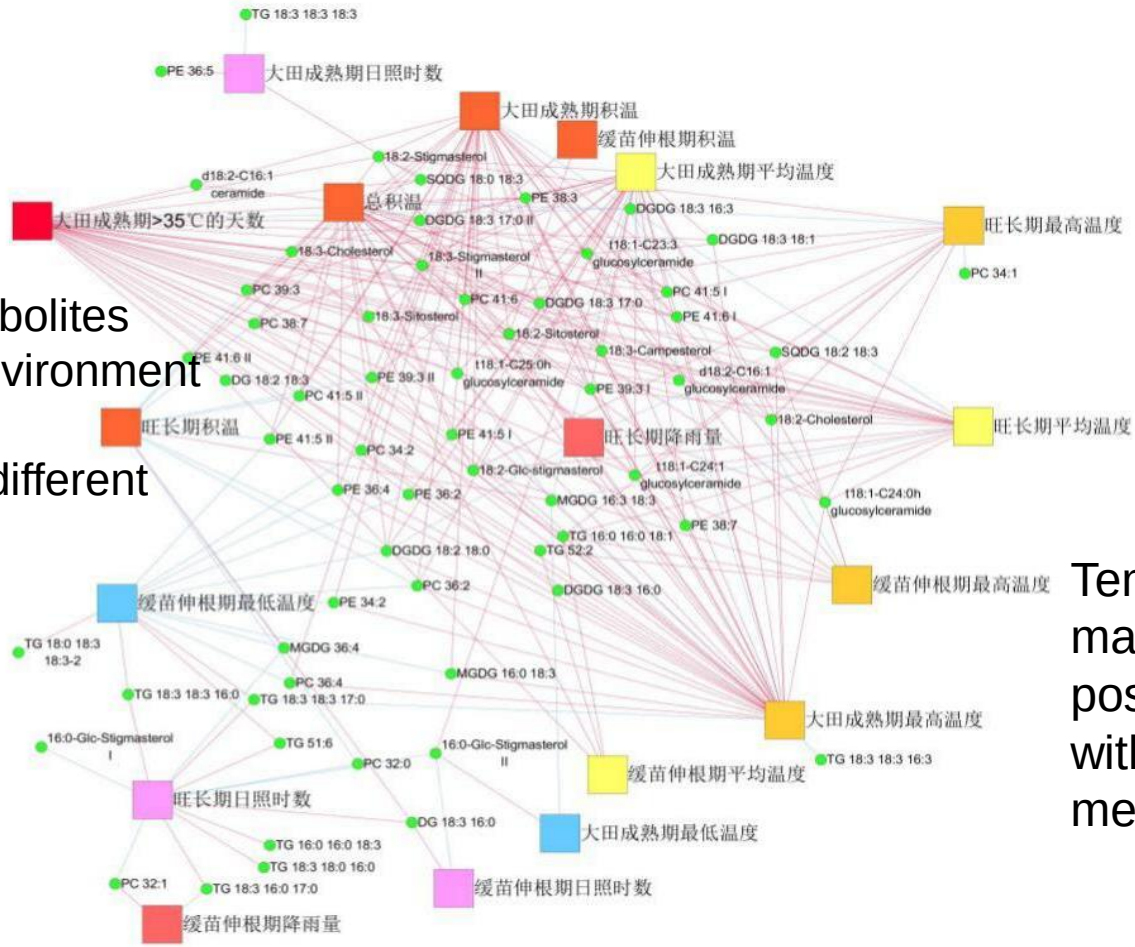
Gene_id	cultivar	location	stage	C X L	C X S	L X S	C X L X S	residual
trehalose	63.12%	4.39%	6.67%	5.91%	6.61%	8.98%	3.82%	0.51%
Nicotine-1-oxo	62.66%	5.78%	12.00%	5.18%	2.76%	7.33%	4.01%	0.27%
tritriacontane	56.47%	26.98%	2.08%	1.82%	4.59%	2.53%	4.07%	1.46%
narcissin	54.53%	11.93%	6.91%	9.69%	1.70%	9.81%	4.95%	0.48%
Glycerol 3-phosphate	51.74%	19.25%	12.57%	6.28%	1.16%	5.68%	2.69%	0.62%
Phosphorylcholine	50.89%	0.19%	25.57%	13.02%	1.51%	6.43%	1.91%	0.47%
chlorogenic acid 2	49.84%	22.40%	2.68%	11.60%	2.95%	5.41%	3.52%	1.59%
Glycerophosphocholine	49.13%	24.44%	7.19%	8.71%	2.77%	4.97%	2.08%	0.71%
dihydrotachysterol	48.27%	5.20%	3.93%	17.36%	9.48%	6.21%	7.51%	2.04%
Imidazole-4-acetic acid	47.29%	24.33%	6.99%	4.18%	2.75%	8.74%	4.84%	0.87%
alpha-cembertriene	47.18%	5.88%	0.53%	21.10%	4.52%	11.96%	6.66%	2.18%
Sinapic acid	46.45%	9.02%	20.17%	14.14%	1.86%	6.90%	1.27%	0.19%
chlorogenic acid 1	46.13%	25.95%	5.17%	11.58%	2.96%	5.41%	2.35%	0.46%
N-Formylmethionine	45.70%	30.13%	0.63%	11.86%	3.56%	5.88%	2.04%	0.21%
hentriacontane	45.67%	36.69%	0.96%	1.40%	5.37%	2.68%	4.80%	2.43%
d18:0-C28:3-Glc-Ceramide	44.44%	14.65%	17.93%	15.13%	2.94%	2.99%	1.66%	0.26%
neochlorogenic acid	44.38%	31.59%	6.69%	8.90%	1.28%	4.07%	2.28%	0.82%
sclareol	44.32%	8.73%	2.20%	17.52%	3.50%	13.44%	6.41%	3.87%
D(+)Xylose	44.12%	14.66%	11.16%	5.04%	2.97%	17.08%	3.96%	1.01%
t18:1-C23:3-Glc-Ceramide	43.33%	45.80%	6.15%	1.00%	0.67%	2.10%	0.80%	0.16%
PE (41:6) I	42.77%	21.11%	10.08%	9.29%	2.05%	10.20%	2.91%	1.60%
t18:1-C24:1-Glc-Ceramide	41.65%	48.71%	2.48%	1.18%	0.63%	4.10%	1.03%	0.22%
heptadecanoic acid	39.82%	10.59%	5.32%	23.91%	2.99%	7.67%	4.33%	5.37%
UDP-N-acetylglucosamine	39.32%	17.86%	21.28%	13.12%	1.50%	3.49%	2.24%	1.19%

Temperature is the most important environment factor

Stage	ID	Name	Num	Stage	ID	Name	Num
fast growth period	Q1-1	temperature	210	Bottom leaf maturity	Q4-1	temperature	200
	Q1-2	temperature difference between day and night	69		Q4-2	temperature difference between day and night	170
	Q1-3	annual rainfall	177		Q4-3	annual rainfall	50
	Q1-4	Sunlight	67		Q4-4	Sunlight	51
	Q1-5	humidity	160		Q4-5	humidity	49
	Q1-6	Lowest temperature	199		Q4-6	Lowest temperature	76
	Q1-7	highest temperature	141		Q4-7	highest temperature	299
	Q1-8	>35°C day	204		Q4-8	>35°C day	353
squaring stage	Q2-1	temperature	161	Middle leaf maturity	Q5-1	temperature	210
	Q2-2	temperature difference between day and night	74		Q5-2	temperature difference between day and night	107
	Q2-3	annual rainfall	176		Q5-3	annual rainfall	112
	Q2-4	Sunlight	73		Q5-4	Sunlight	155
	Q2-5	humidity	89		Q5-5	humidity	83
	Q2-6	Lowest temperature	76		Q5-6	Lowest temperature	183
	Q2-7	highest temperature	179		Q5-7	highest temperature	218
	Q2-8	>35°C day	174		Q5-8	>35°C day	183
blossom period	Q3-1	temperature	157	Upper leaf maturity	Q6-1	temperature	140
	Q3-2	temperature difference between day and night	43		Q6-2	temperature difference between day and night	67
	Q3-3	annual rainfall	39		Q6-3	annual rainfall	61
	Q3-4	Sunlight	157		Q6-4	Sunlight	185
	Q3-5	humidity	63		Q6-5	humidity	275
	Q3-6	Lowest temperature	97		Q6-6	Lowest temperature	33
	Q3-7	highest temperature	243		Q6-7	highest temperature	268
	Q3-8	>35°C day	0		Q6-8	>35°C day	0

Lipid and environment factor interaction network

- Circles represent metabolites
- Squares represents environment factor
- Different color means different development stage

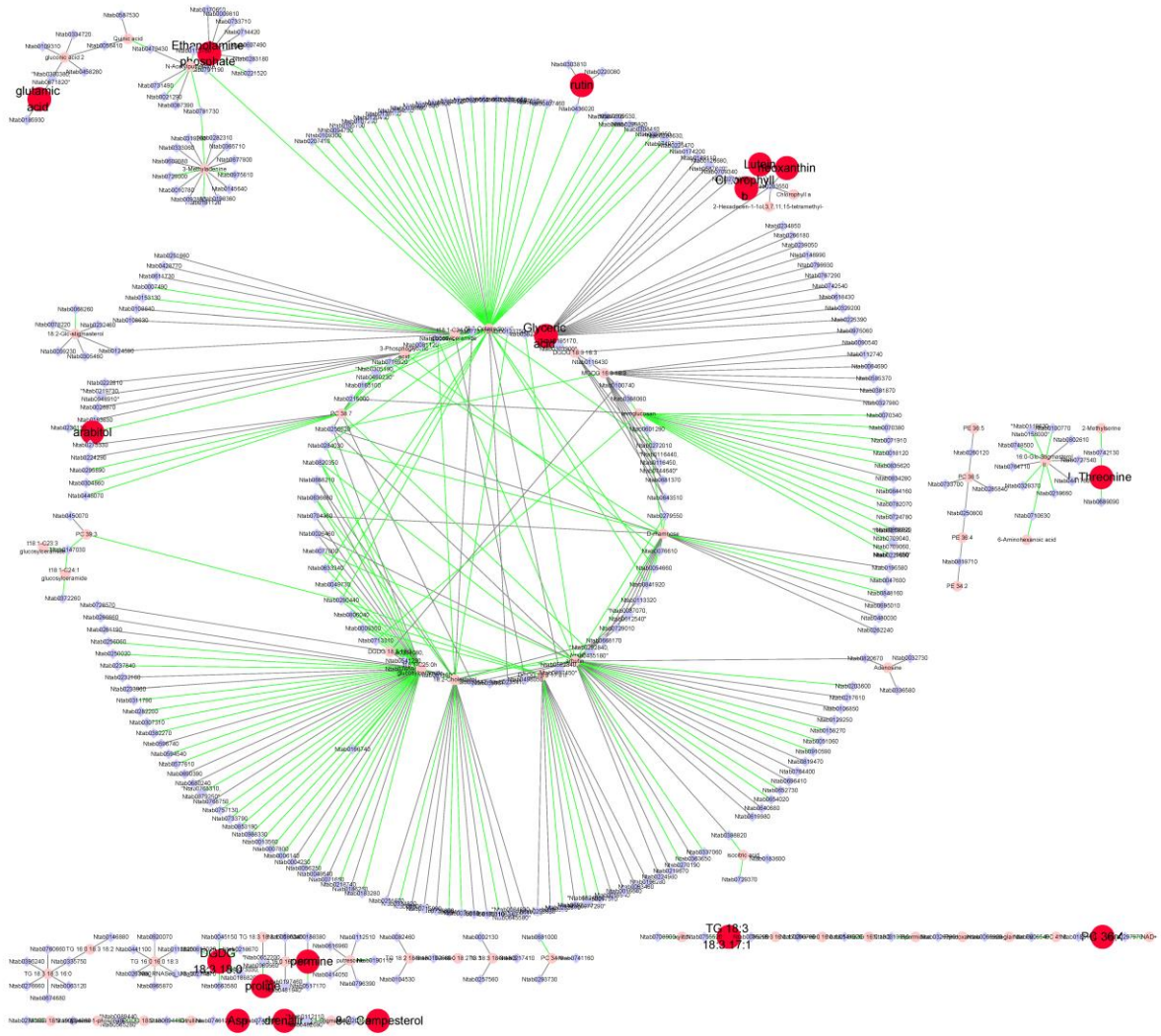


Temperature of maturity stage is positive correlated with most lipid metabolites

- In 2014, lipid is high in HeNan regions
- Temperature is high and annual rainfall is low in HeNan of 2014

Metabolite and gene interaction network

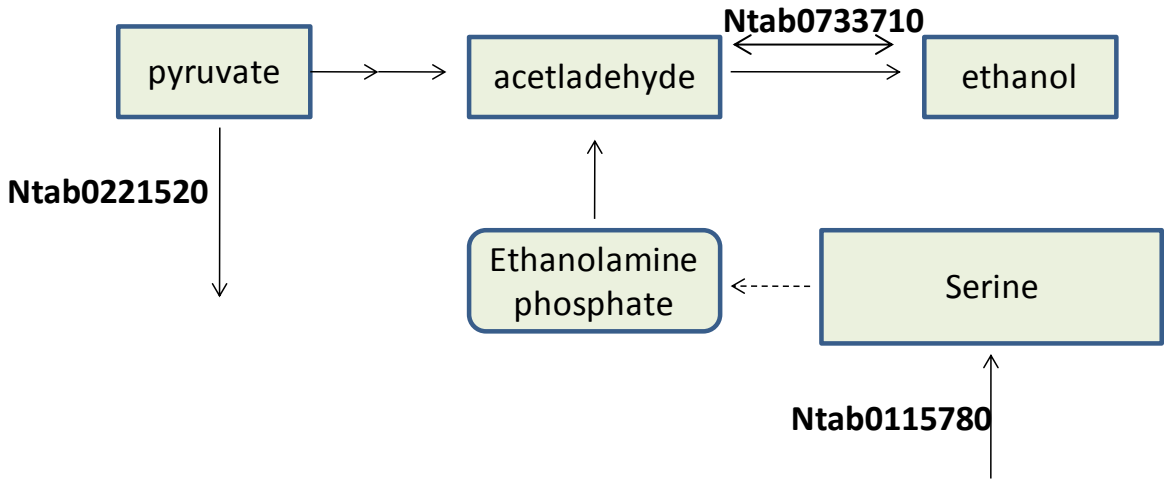
- Circles represent metabolites
- Diamond represents genes
- Red color means metabolites related to quality
- Green color means negative correlated
- Black color means positive correlated



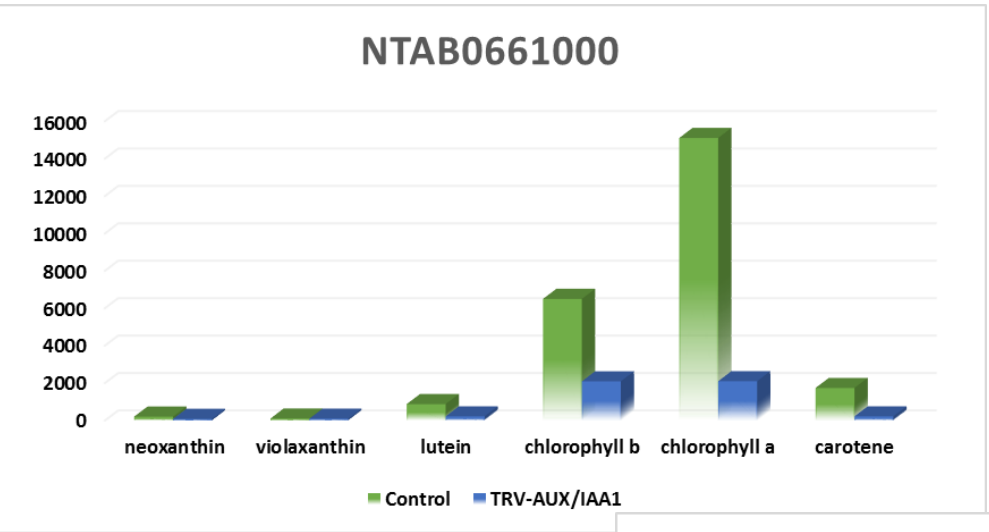
Some new genes are identified

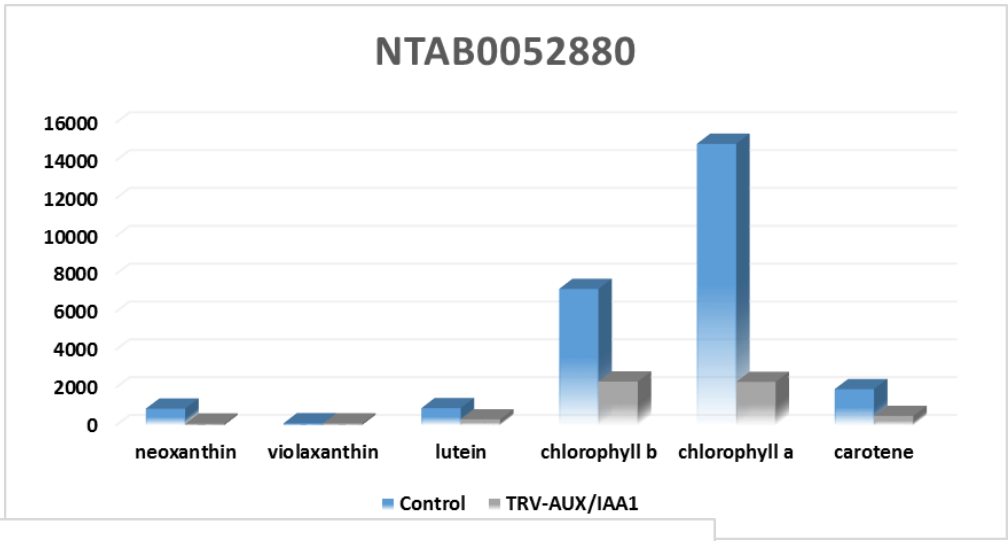
neoxanthin	positive	Ntab0283550	similar to Rac-like GTP-binding protein 5
lutein	positive	Ntab0283550	similar to Rac-like GTP-binding protein 6
chlorophyll b	positive	Ntab0283550	similar to Rac-like GTP-binding protein 7
phytol	positive	Ntab0283550	similar to Rac-like GTP-binding protein 8

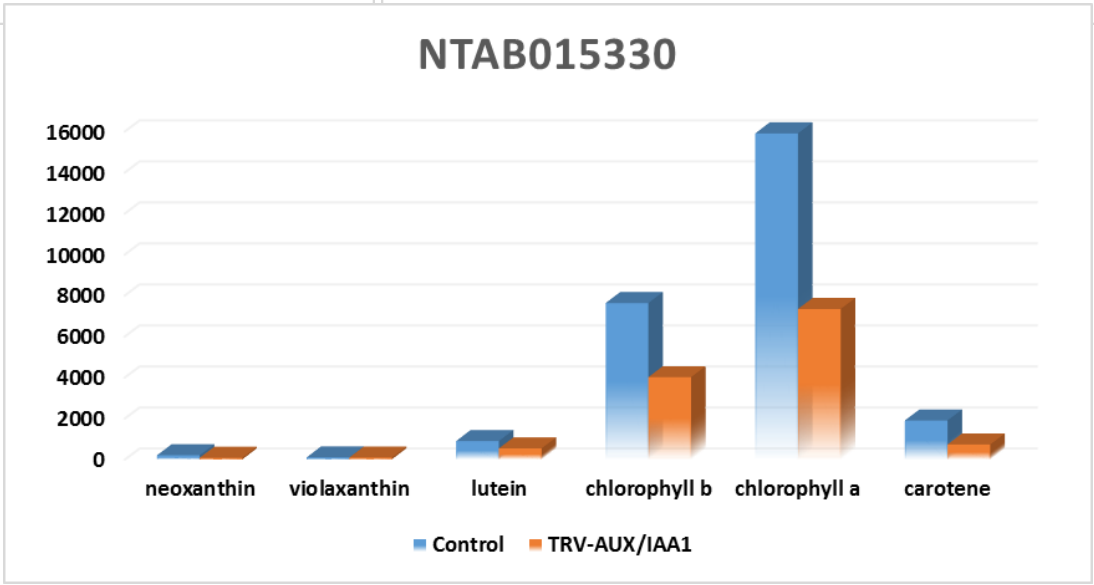
Ethanolamine phosphate	negative	Ntab0221520	similar to Pyruvate, phosphate dikinase
Ethanolamine phosphate	positive	Ntab0115780	similar to Serine acetyltransferase 5
Ethanolamine phosphate	positive	Ntab0733710	similar to Alcohol dehydrogenase class-3



VIGS of new genes related to pigment

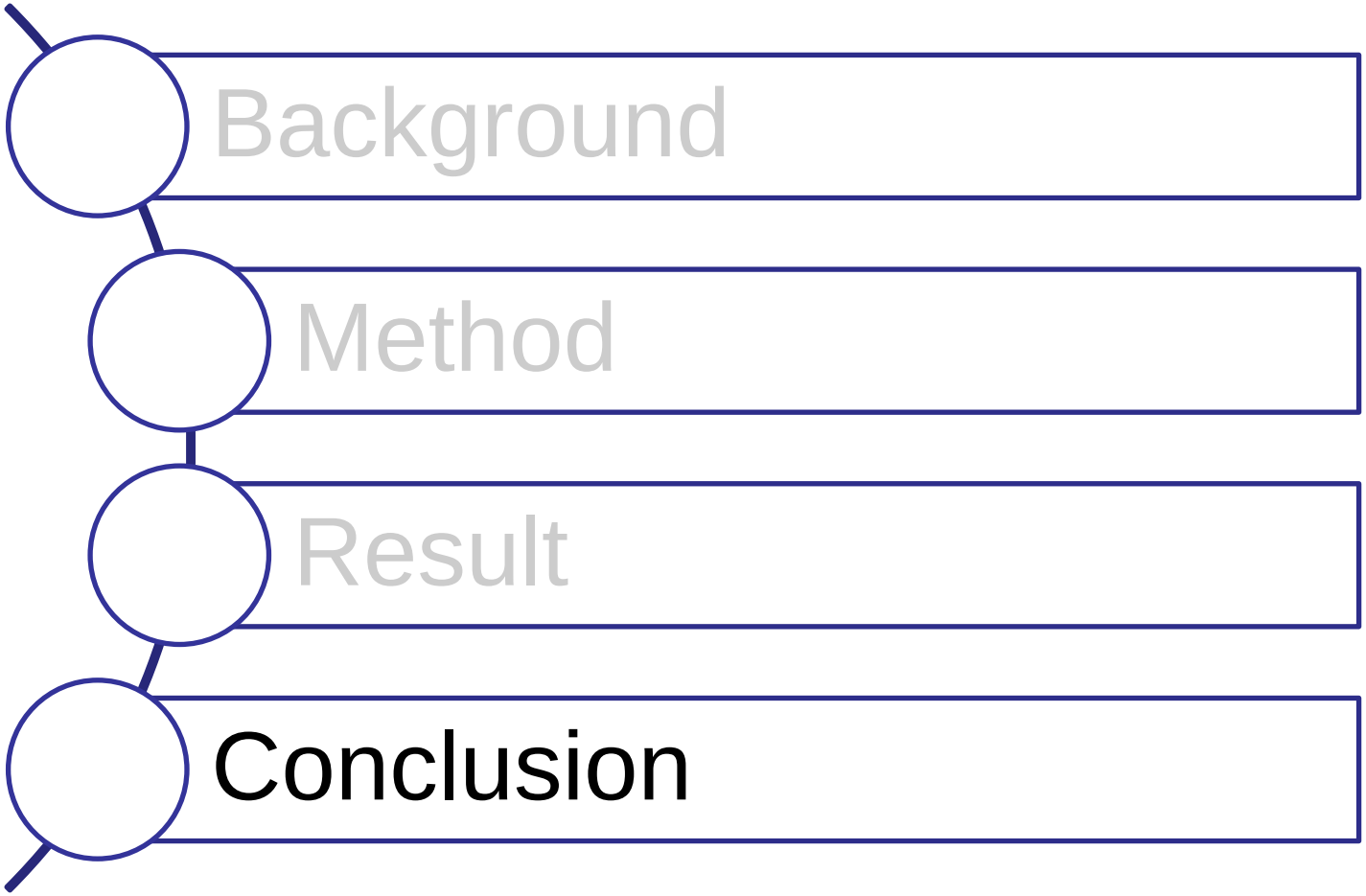






The pigment is reduced after VIGS of Ntab0661000, Ntab0155330, Ntab0052880

Outline



Summary

- Environment affects metabolites of tobacco much more than genotype, around 2-3 times.
- Lipid is mostly affected by environment.
- Some secondary metabolites are mostly affected by genotype.
- Temperature is the most important environment factor.
- Some new genes can be identified by metabolite-gene interaction network.
- For different metabolites, we should use different strategy to regulate them.

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Q&A

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Thank you!