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Effect of different growth temperatures on alkaloid content and nicotine metabolism in roots, stems and leaves of tobacco (*Nicotiana tobacum* L.) plants

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P. R. **CHINA**

OVERVIEW

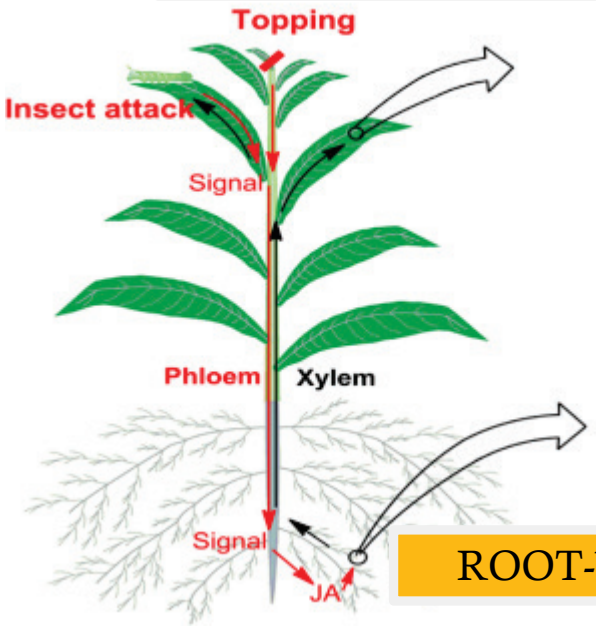
- ✧ Background information
- ✧ Objectives of this experiment
- ✧ Experimental procedure
- ✧ Main Results
- ✧ Brief Conclusion
- ✧ Acknowledgements

BACKGROUNDS—Alkaloids & Nicotine

- The **commercial tobacco** typically produced **alkaloids** at levels between **2%-4%** of total dry weight, with important biological functions including **antiherbivore defense and smoking addiction**;
- As the predominant pyridine alkaloid in tobacco, nicotine usually accounted for **~90% of the total alkaloid content**.

Composition of pyridine alkaloids

LEAF-accumulation



ROOT-biosynthesis

- Besides nicotine, three other important pyridine alkaloids in tobacco:**nornicotine**, **anatabine, and anabasine**, constitute nearly all of the remaining 10% of the alkaloid pool;
- Nicotine is synthesized in the tobacco roots, then is translocated into the xylem, and finally accumulated in leaf tissues.

Biosynthesis of pyridine alkaloids

THREE main steps

Pyrrolidine ring formation

ODC, ADC, PMT, MPO

Pyridine ring formation

AO, QS, QPT

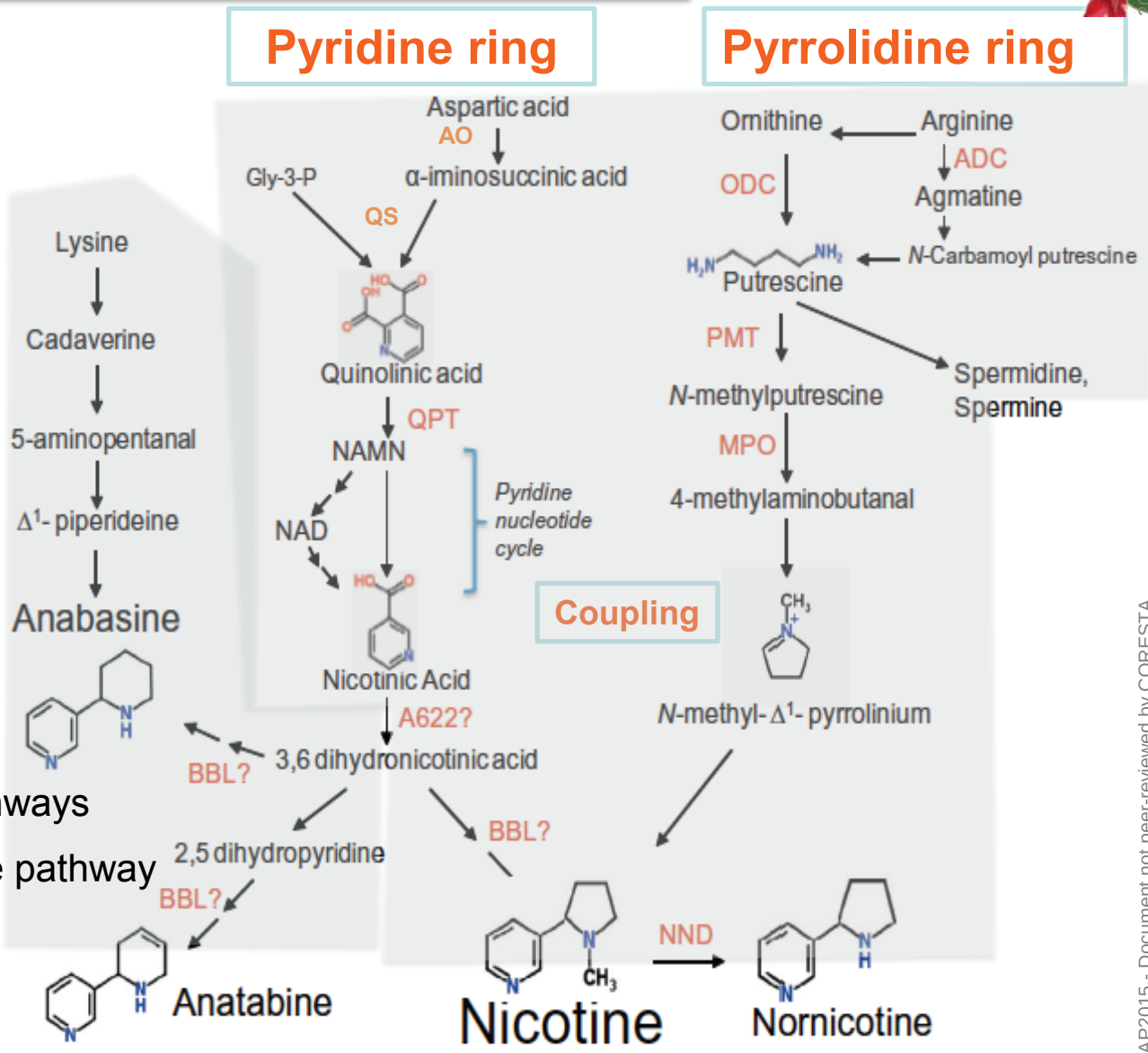
Coupling of two rings

A622, BBL ?

Nicotine/Nornicotine—both pathways

Anatabine/Anabasine—Pyridine pathway

(**Dewey & Xie, 2013**)

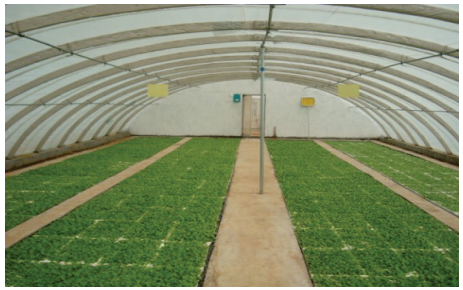


OBJECTIVES OF THE PRESENT WORK

- ✧ **Nicotine** (together with other pyridine alkaloids) functions as important part of **aroma quality** of tobacco in **tobacco products**, ;
- ✧ China—one of the biggest tobacco producing countries: many tobacco-planting areas with **different climate conditions**;
- ✧ **The Question** is:

same variety of tobacco, grown at different areas➔ very different aroma type ➔ What is the influence of growth temperature on nicotine metabolism?

Experimental Procedure

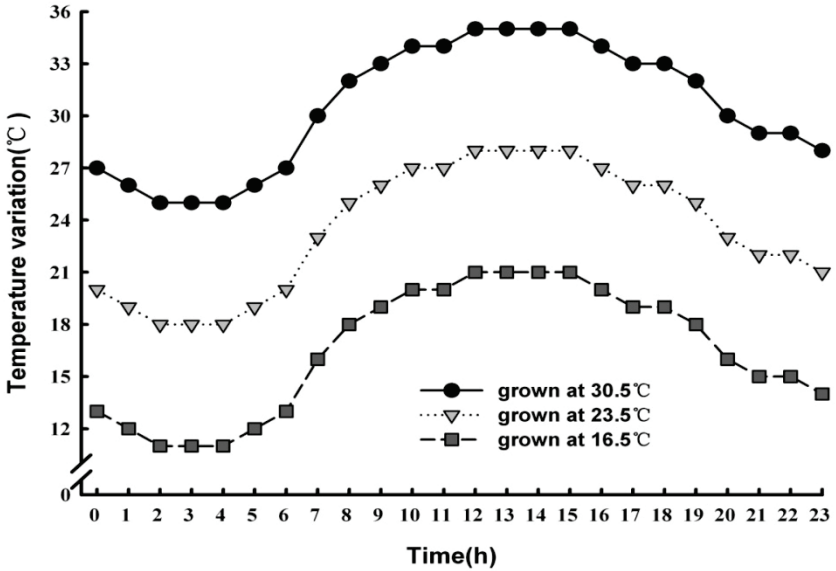


Yunyan 87

30.5℃

23.5℃

16.5℃



Daily temperature fluctuation in the growth chambers set to 30.5 ℃ (circles), 23.5 ℃ (triangles), and 16.5 ℃ (squares)

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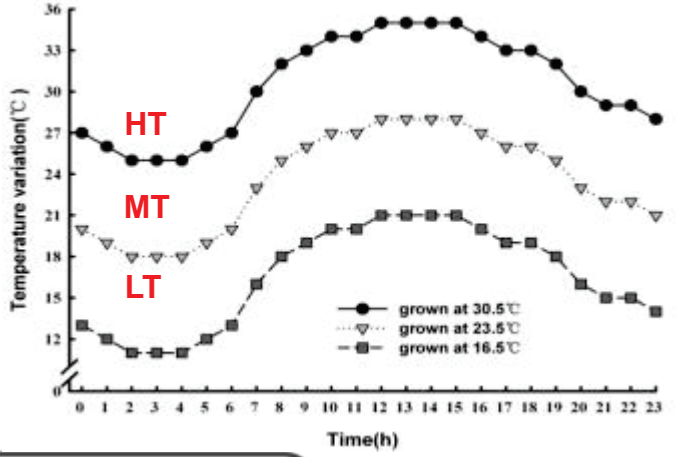
Experimental Procedure

N. tabacum (var. Yunyan87)



HT/MT/LT treatment
(daily average 30.5/23.5/16.5 ℃)

Root/Leaf samples for GC/MS analysis

NICOTINE composition

Root/Leaf samples for RNA preparation

Mixed RNA samples for pair-end normalized library

Illumina sequencing (WHOLE transcriptome)

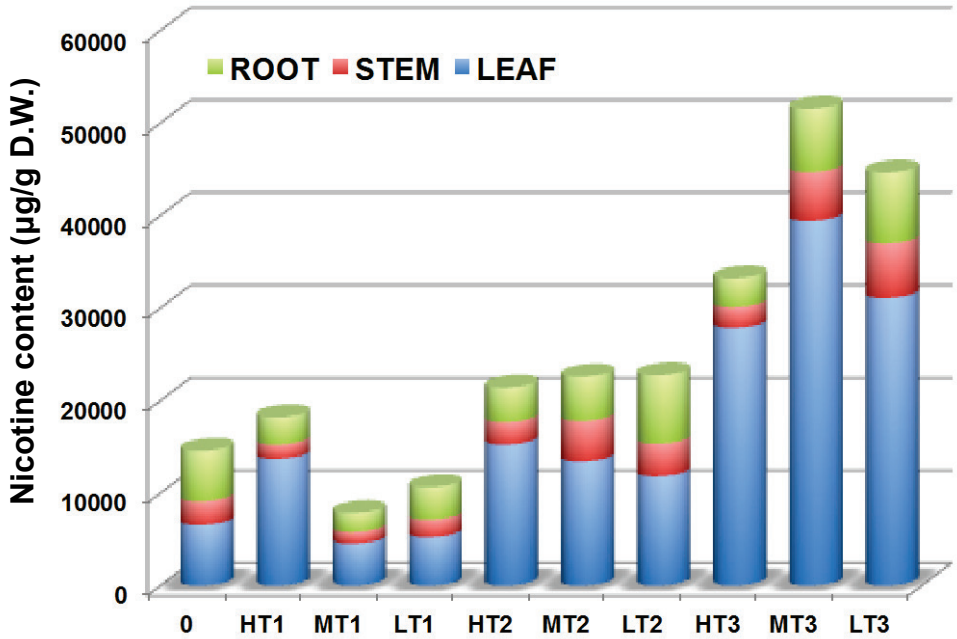
Functional annotation (as a comprehensive reference)

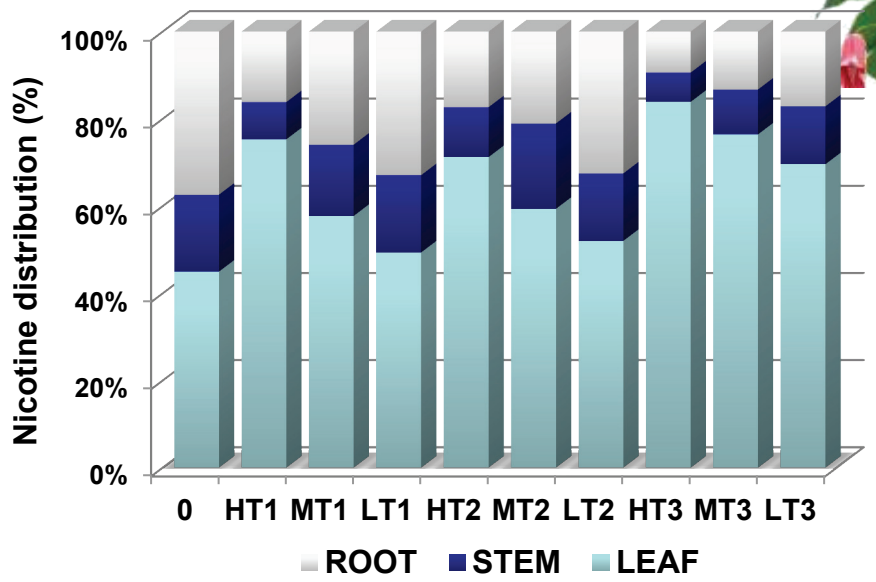
Each RNA sample for single-end library

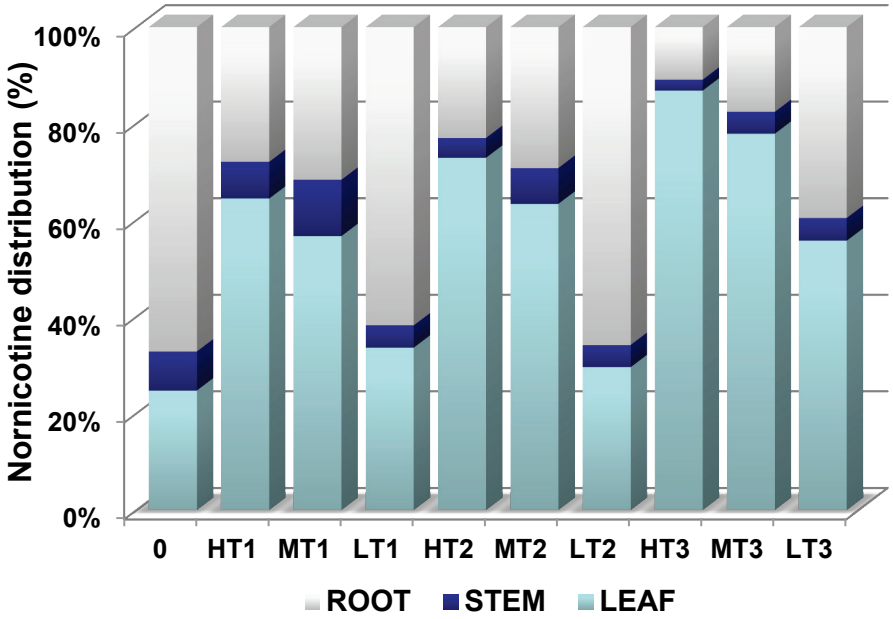
Illumina sequencing (QUANTITATIVE RNA-seq)

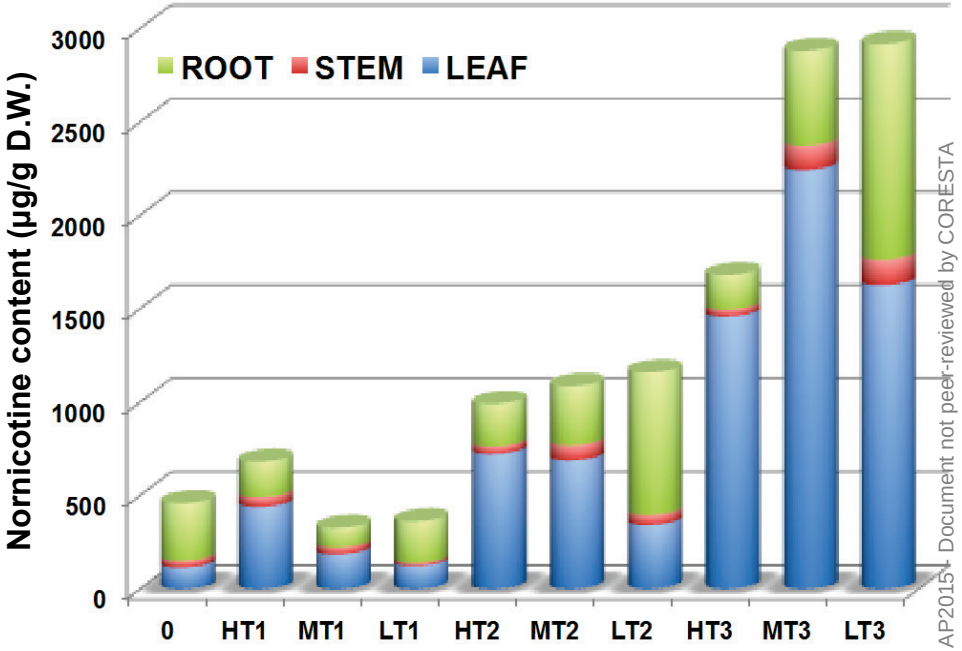
Gene expression profiles (among organs and treatments)

❖ Main results I. Pyrodine alkloids composition—Nicotine/Nornicotine

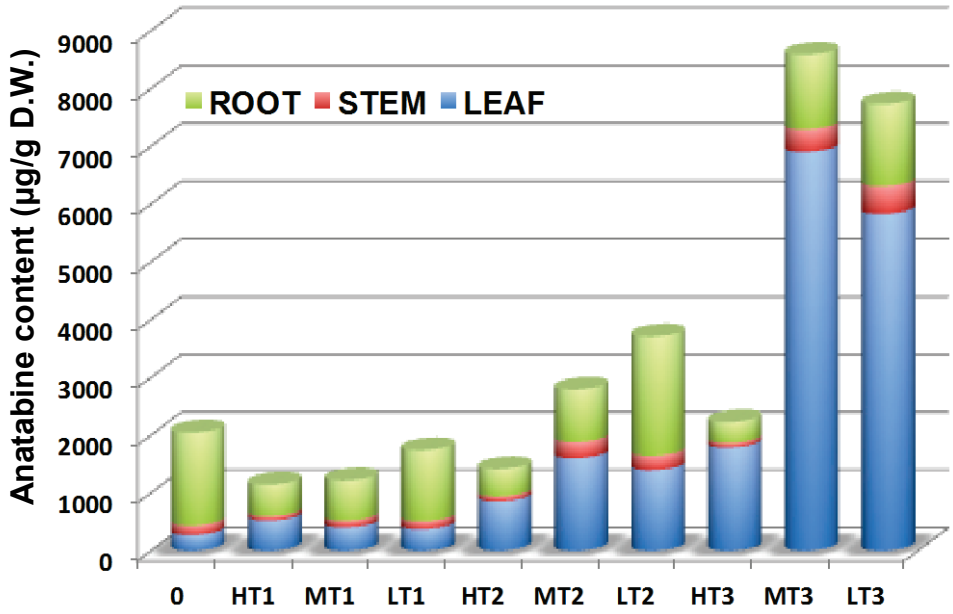


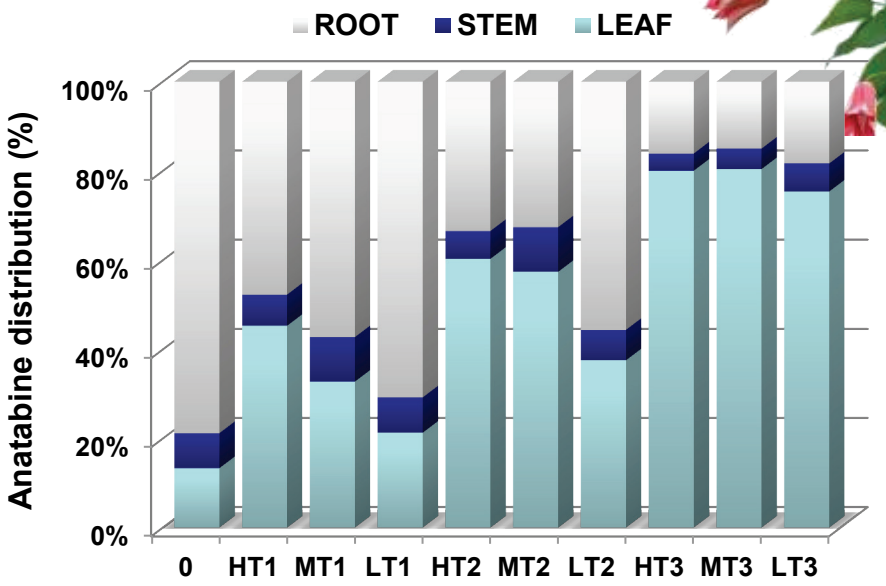


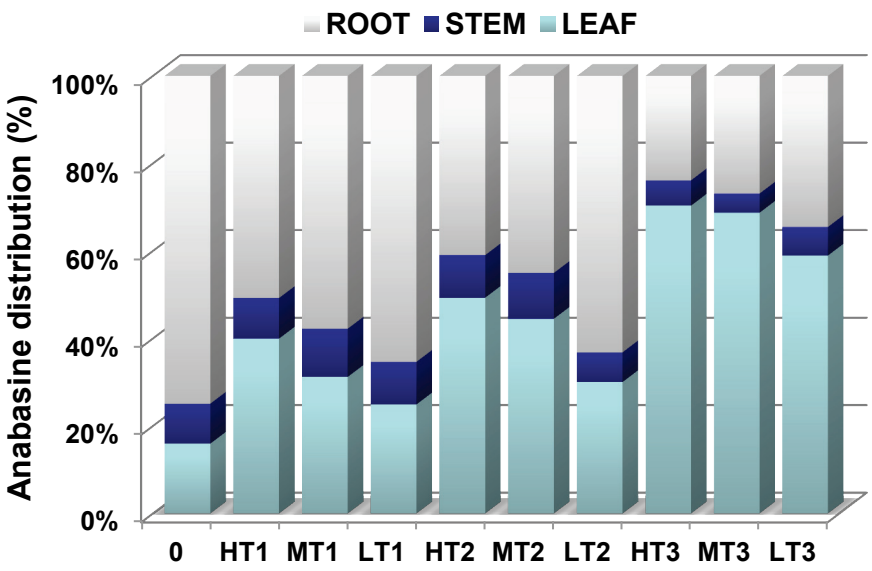


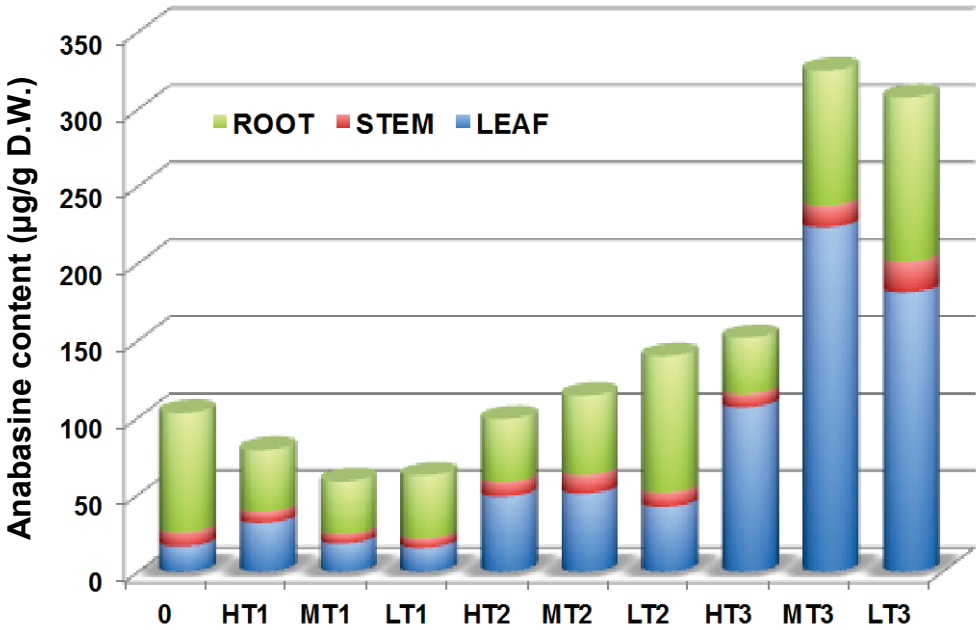


❖ Main results I. Pyrodine alkloids composition—Anatabine/Anabasine









❖ Summary I. Pyrodine alkaloid composition

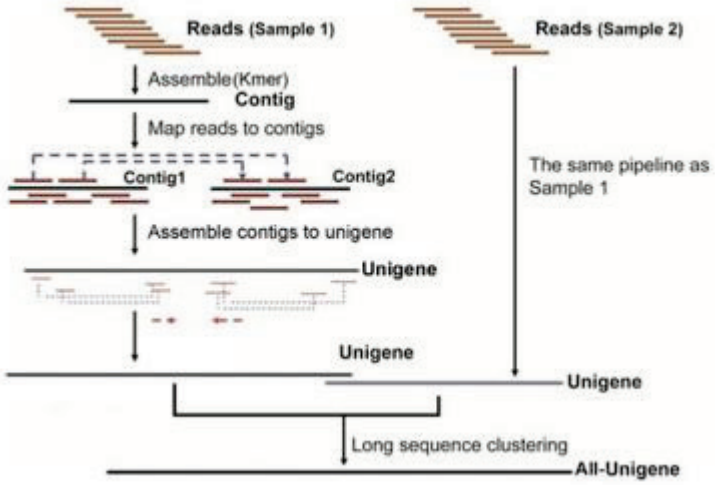
- ✧ **Development: positive effect on pyridine alkaloids formation;**
- ✧ **High temperature: quickly stimulated (within 20 d) the formation of nicotine and nornicotine;**
- ✧ **Medium/Lower temperatures: contribute to long-lasting accumulation (60 d) of all four pyridine alkaloids, especially MT;**
- ✧ **LT: helpful to accumulation of nicotine in stems and roots;**
- ✧ **Higher temperatures: stimulates the translocation of pyridine alkaloids from roots to aerial parts (leaves and stems);**

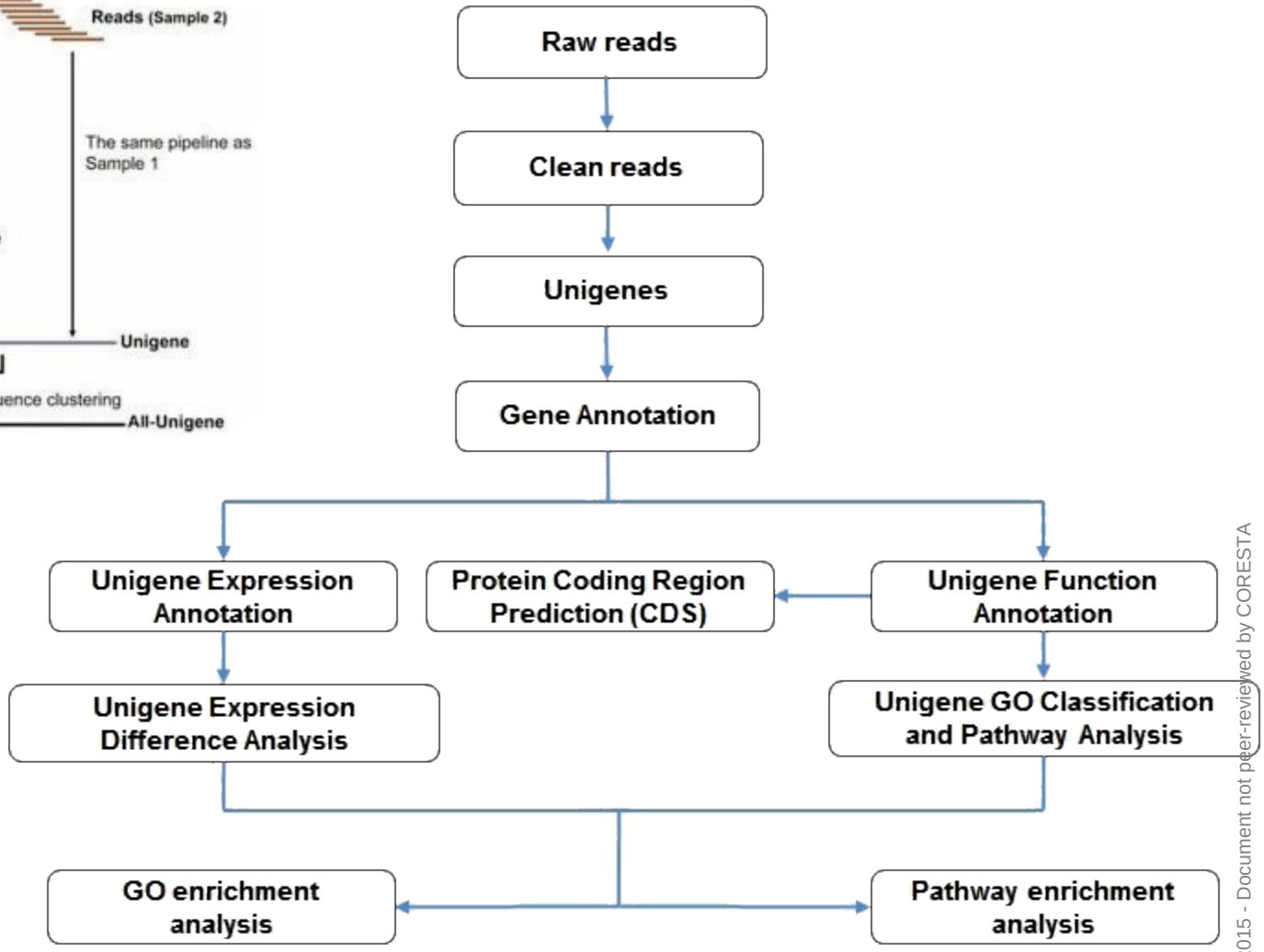
⇩ **Hiseq2000**

What is the molecular mechanism?

Main results-II. Transcriptome analysis

- **Pipeline of Bioinformatics analysis**





❖ II. Transcriptome analysis

By using the Illumina Hiseq™ 2000 for whole transcriptome sequencing, approx. 54 million clean reads were obtained, and assembled into about 178,000 contigs, of which 83,061 were identified as unigenes.

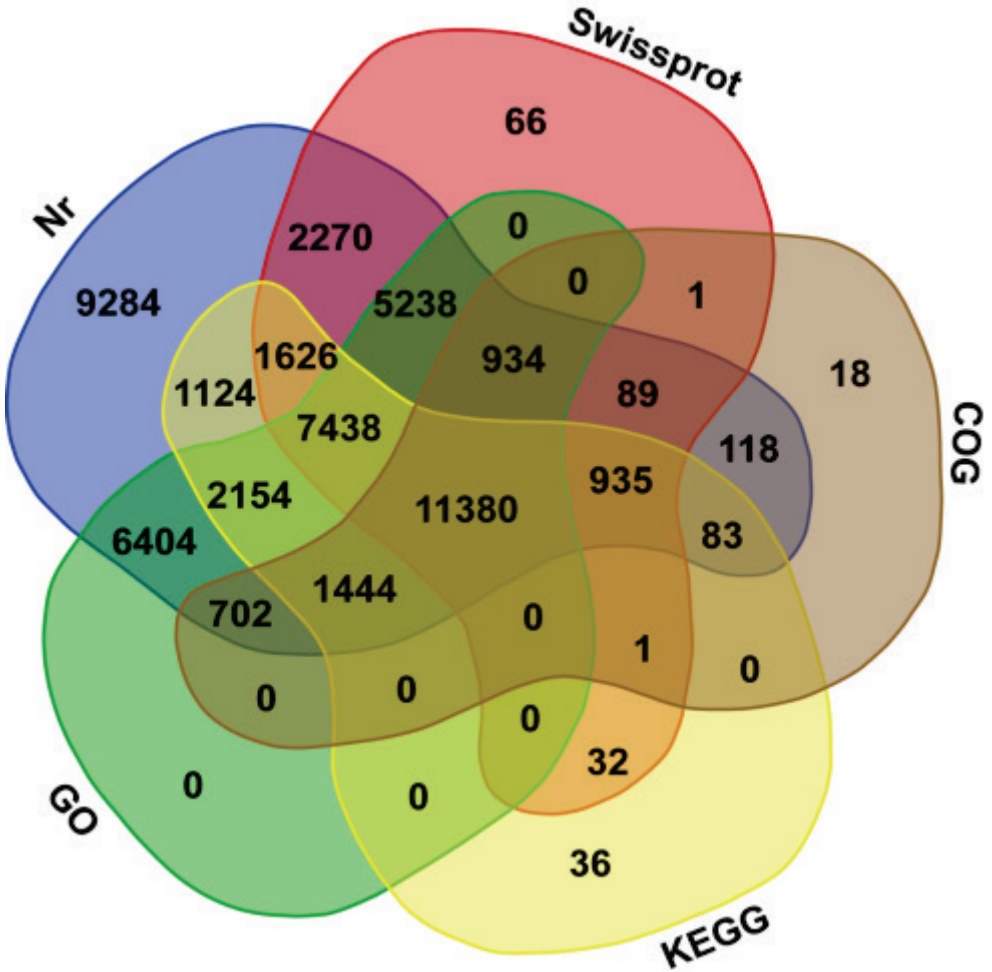
Table1. Output statistics of sequencing.

	N.Tabacum (Yun87)
Total Raw Reads	58,445,490
Total Clean Reads	54,019,256
Total Clean Nucleotides (nt)	4,861,733,040
Q20 percentage	97.91%
N percentage	0.00%
GC percentage	43.25%

Table2. Statistics of assembly quality.

	Contig	**Unigene**
Total Number	178,704	83,061
Total Length(nt)	51,540,645	52,072,023
Mean Length(nt)	288	627
N50	447	1088
Total Consensus Sequences	-	83,061
Distinct Clusters	-	32,115
Distinct Singletons	-	50,946

Venn diagram of shared unigenes among five references databases



NR	NT	Swiss-Prot	KEGG	COG	GO	ALL
51,218	54,822	30,010	26,253	15,705	35,694	58,964

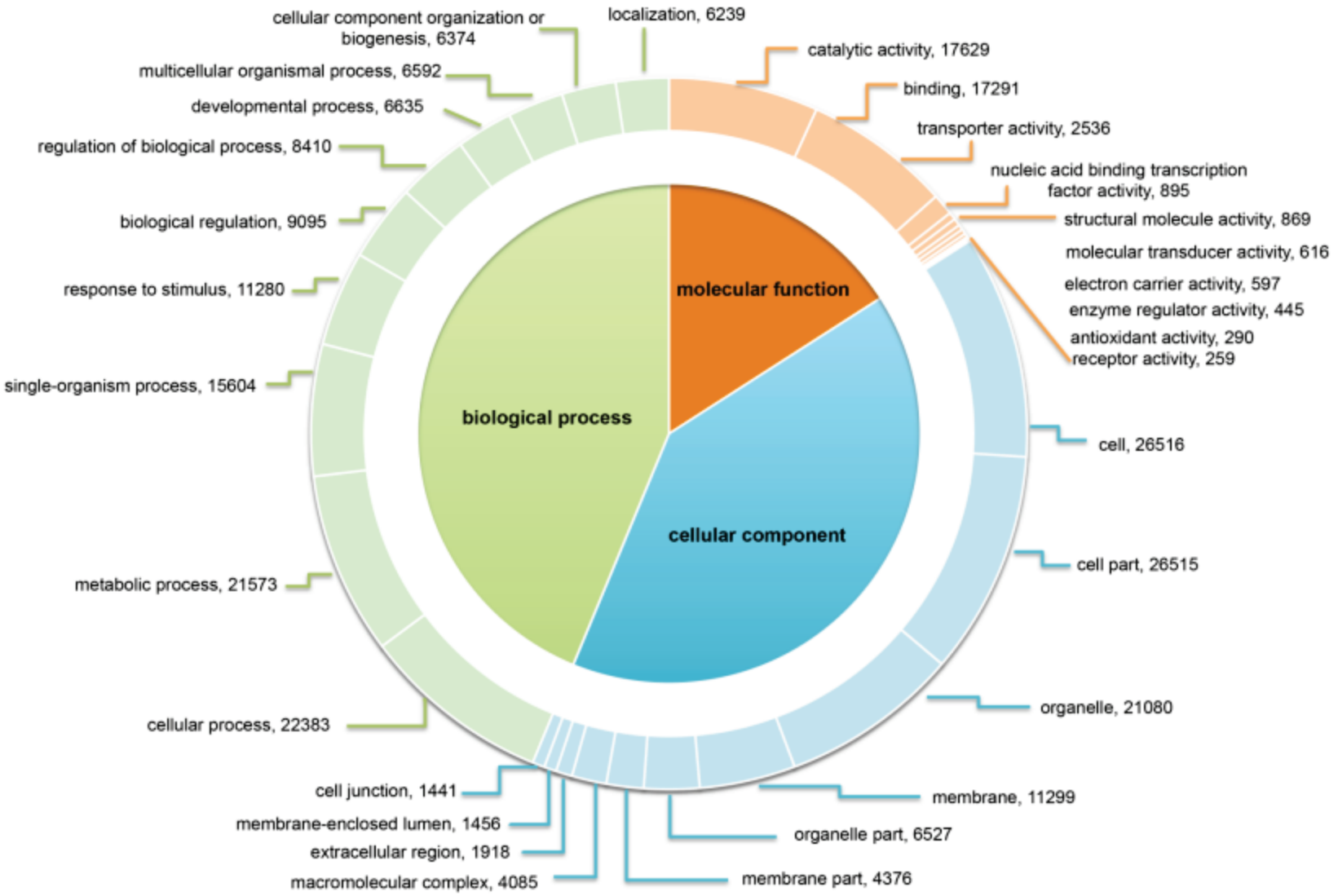
❖ II. Transcriptome analysis

Functional classification of Unigenes

GO (Gene Ontology）

- With the aid of protein Nr annotation, GO functional annotation of these Unigenes was performed.
- As we know, GO has three ontologies: Molecular function, Cellular component and Biological process.
- Firstly, Blast2GO program was used to get GO annotation of all Unigenes, then WEGO software was exploited for GO functional classification.

The most highly annotated GO terms (top 10) in each of the three GO categories



a number of 47 Unigenes

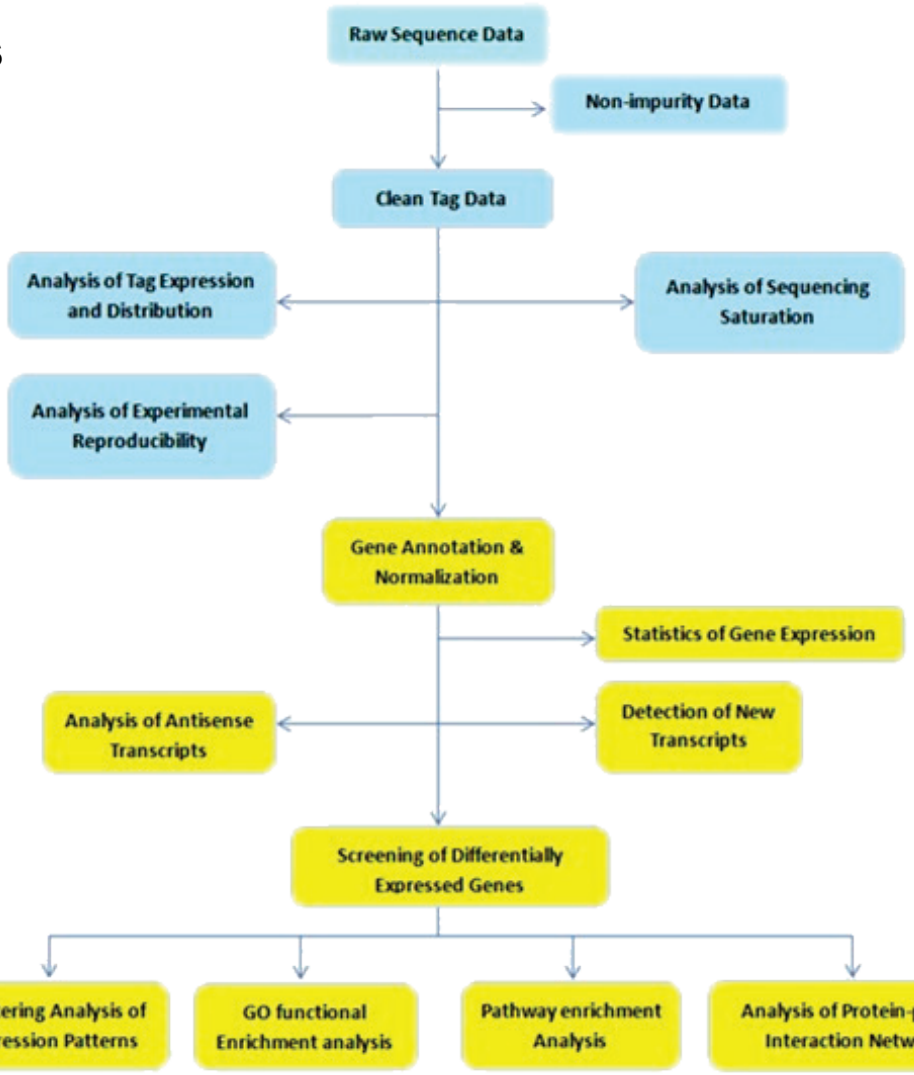
❖ II. Transcriptome analysis

Table3. Potential candidates involved in pyridine alkaloids biosynthesis

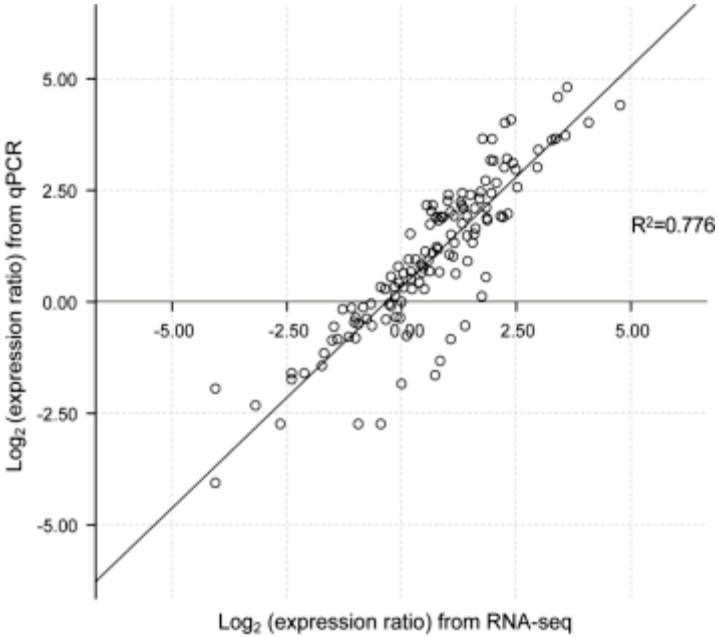
Steps	Gene name (Abbr.)	No. of Unigenes
pyrrolidine ring formation (12)	Arginine decarboxylase (ADC)	7
	ornithine decarboxylase (ODC)	2
	Putrescine N-methyltransferase (PMT)	1
	N-methylputrescine oxidase (MPO)	2
Pyridine ring formation (11)	Aspartate oxidase (AO)	6
	quinolinate synthase (QS)	3
	quinolinic acid phosphoribosyltransferase (QPT)	2
Coupling of two rings (6)	isoflavone reductase-like protein A622	1
	berberine bridge enzyme-like protein (BBL)	5
Nicotine demethylation	nicotine N-demethylase (NND)	9
Nicotine translocation	nicotine uptake permease (NUP)	8
	multidrug and toxic compound extrusion (MATE)	1

❖ III. Digital Gene Expression (DGE) analysis

- **Pipeline of Bioinformatics analysis**



- **Validation of RNA-seq profiles by qPCR (R=0.881)**



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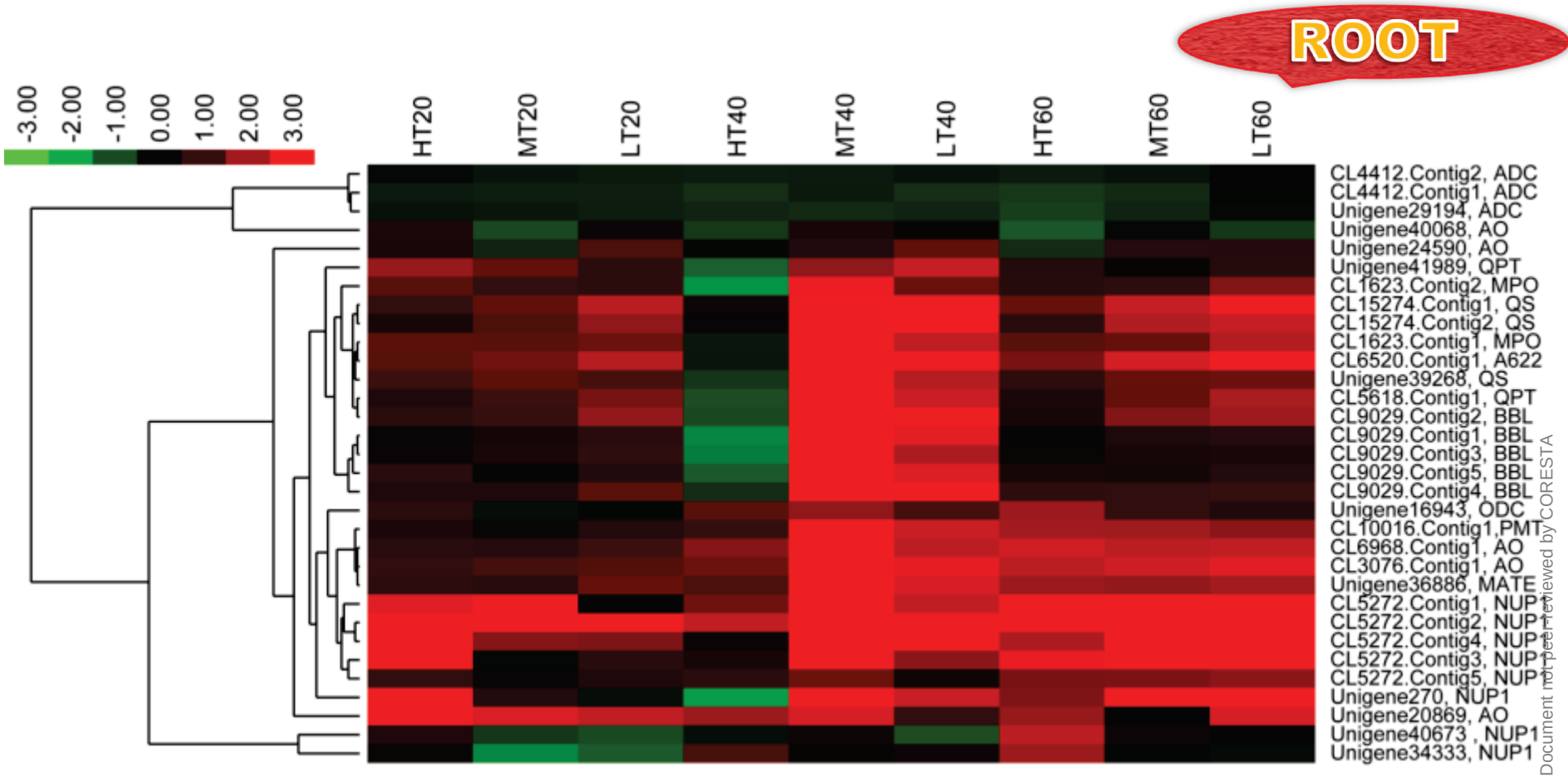

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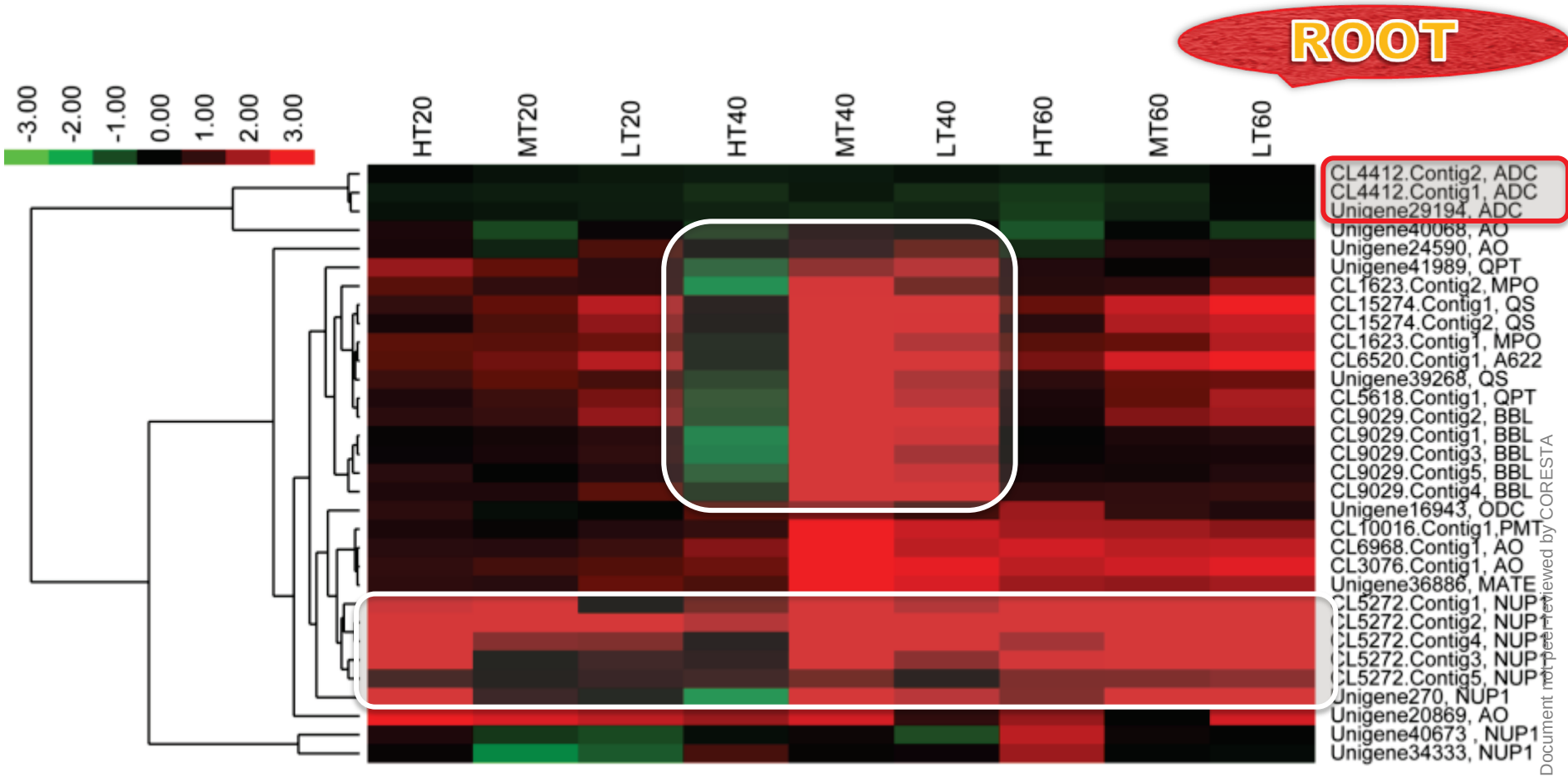


Brief Conclusions

activation repression/unchanged

- ✧ **MT/LT (over 40 d)**

  Pyrrolidine/Pyridine/coupling

  ↓

  Accumulation of nicotine/nornicotine

- ✧ **MT/LT (later stage, 60 d)**

  More metabolic flux into pyridine pathway

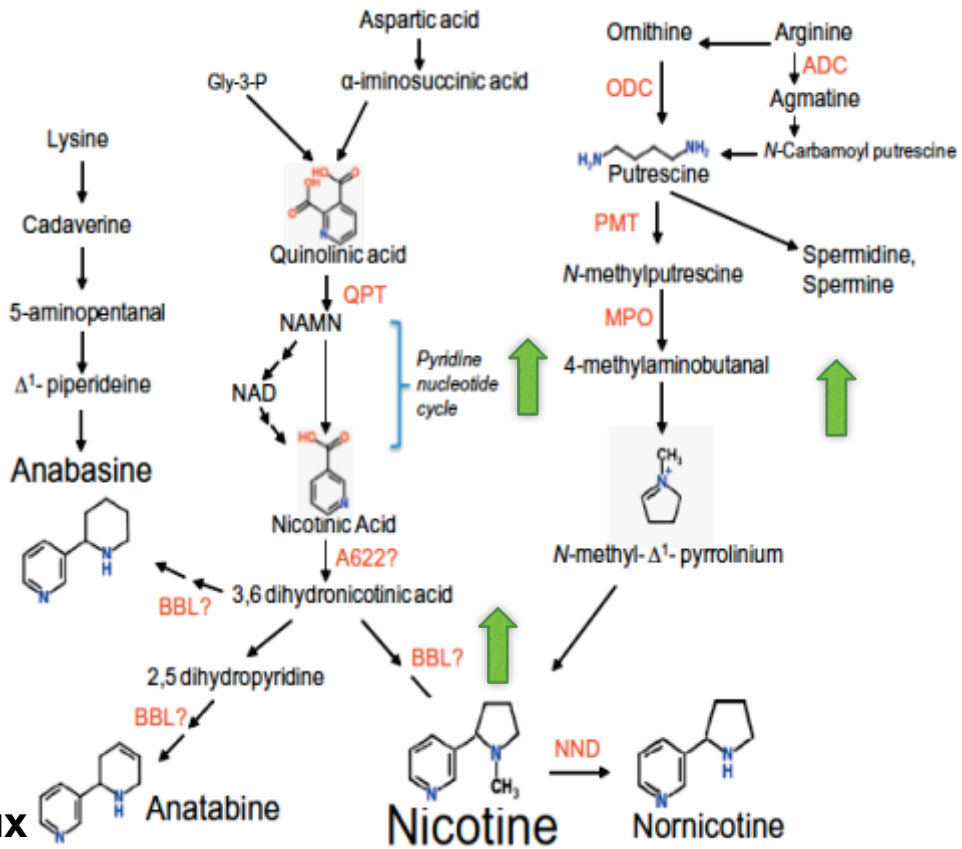
  Repressive/unchanged pyrrolidine pathway

  ↓

  Formation of anatabine/anabasine

- ✧ **Pyrrolidine ring formation**

  ODC, rather than ADC➔metabolic flux



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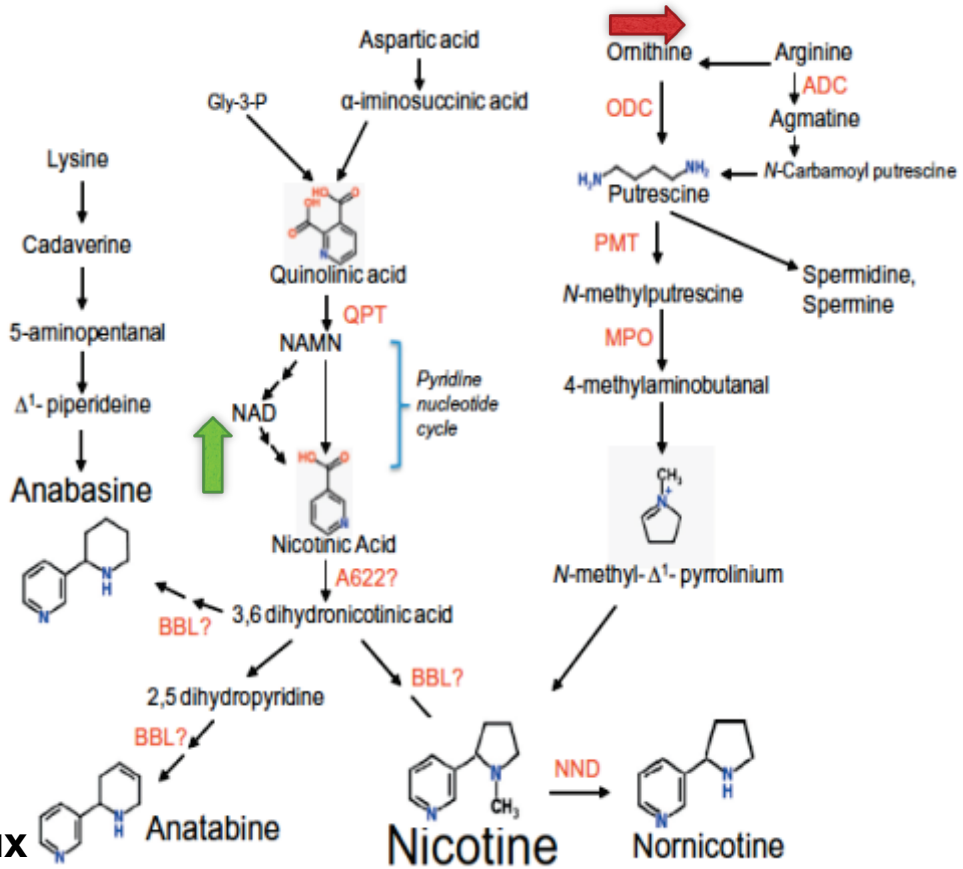

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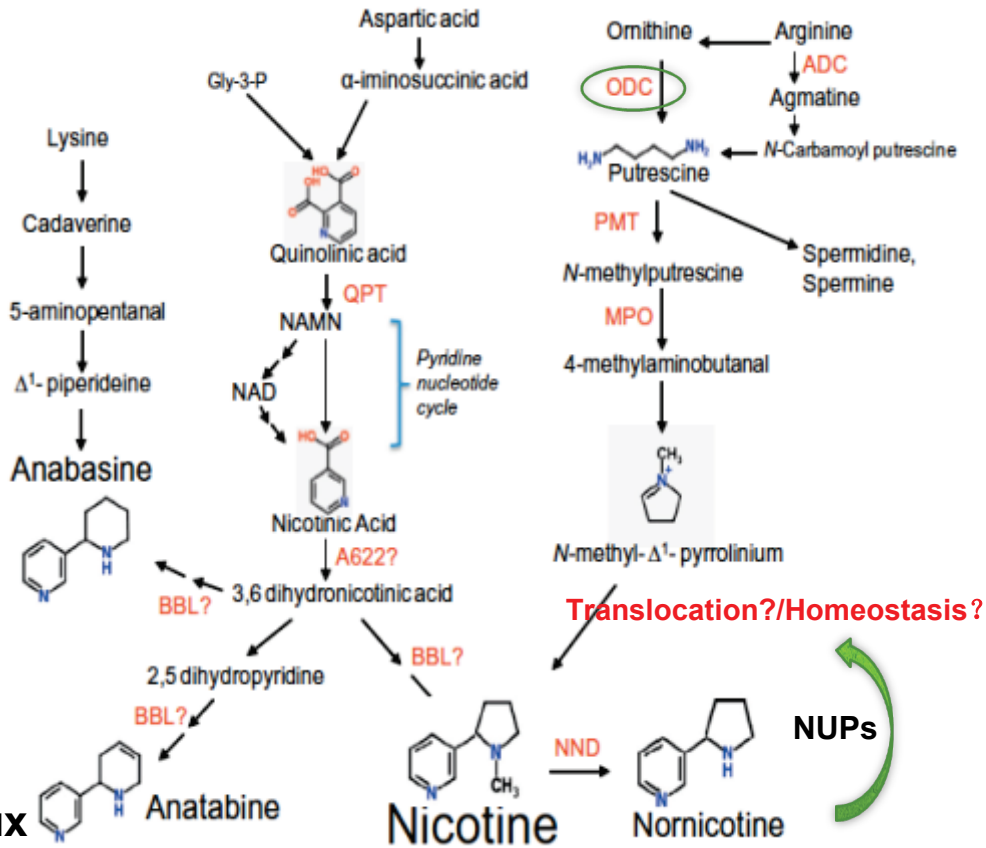

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ACKNOWLEDGEMENTS

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- We are grateful to Dr. Li for providing the experimental materials;
- The technical support for gas chromatography-mass spectrometry from Dr. Pang is greatly appreciated.

Thanks for your attention

谢谢！