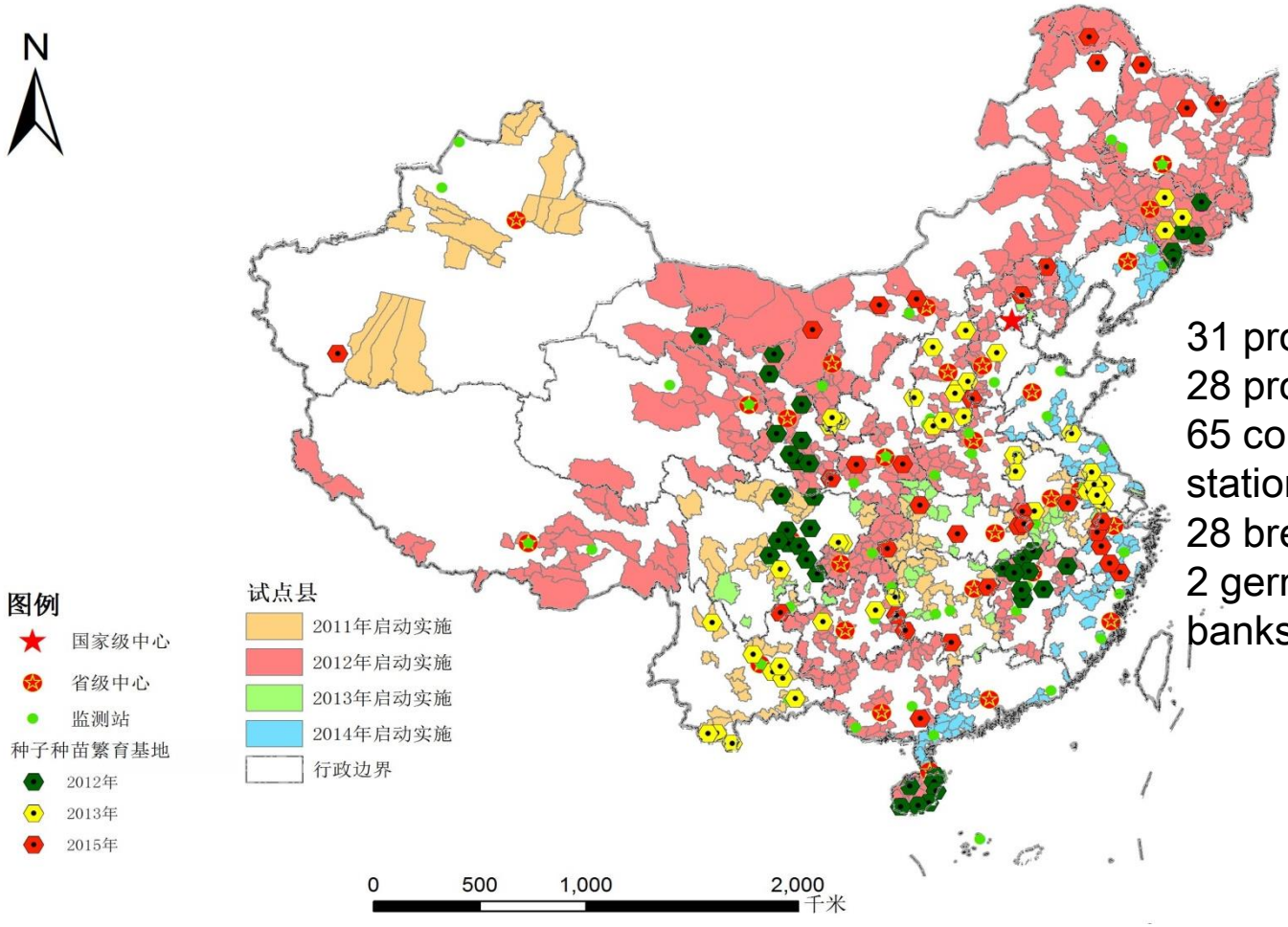
The research of sustainable utilization on Traditional Chinese medicine resources

China Academy of Chinese Medical Sciences
Huang Luqi

1.Introduction on the pilot work of the national survey on TCM resources

Conduct TCM resources survey and method study; Grasp the resources background information of TCM resources



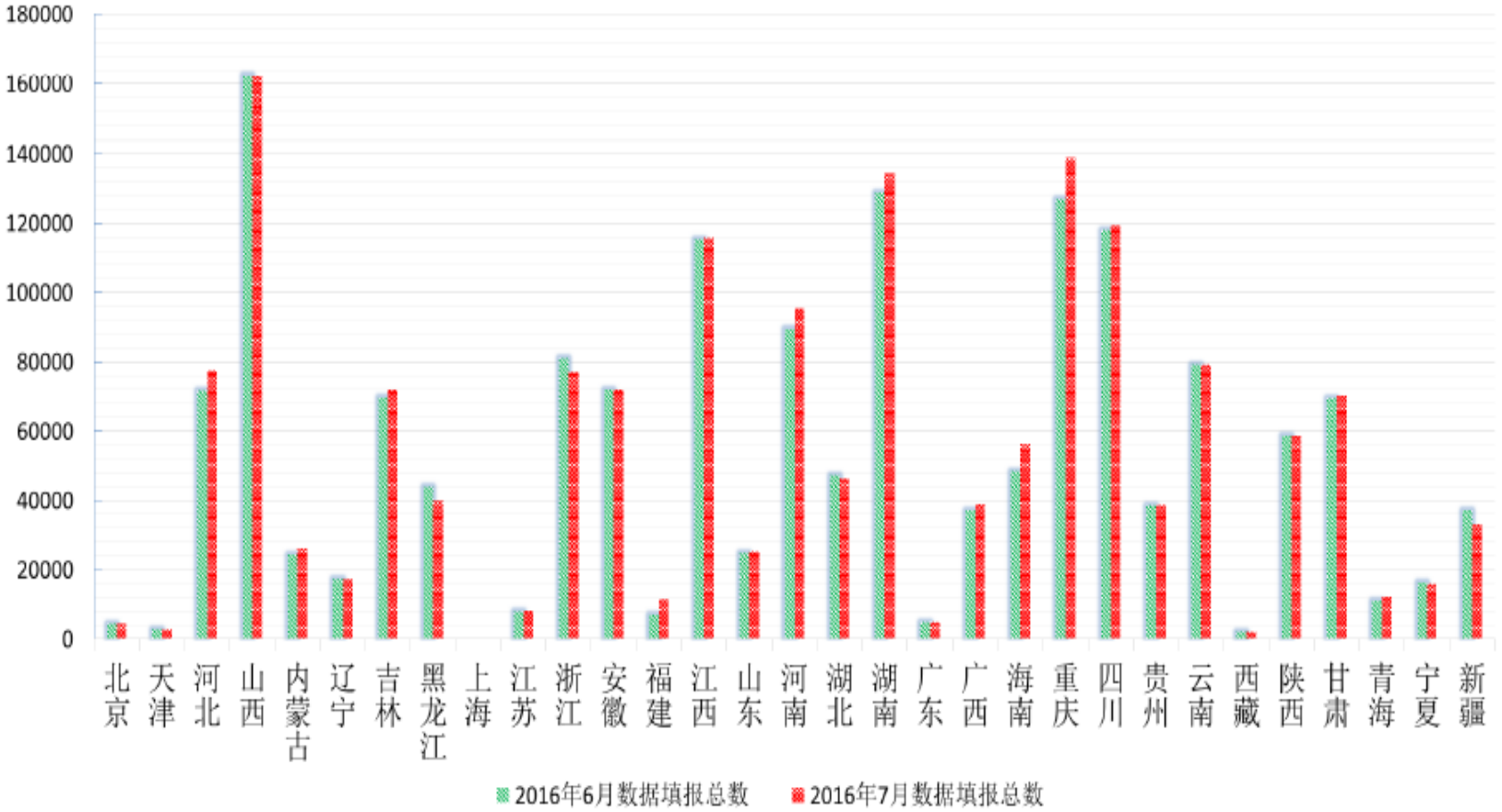
31 provinces 922 counties
28 provincial centers
65 county-level monitoring stations
28 breeding bases
2 germplasm resources banks

TCM resources survey

- A large number of data from TCM resources survey has been stored
- The number of quadrat ：687024（more than 6 hundred thousand ）
- The number of the medicinal resources varieties ： 16048（the 3rd national survey:12807 varieties ）
- The species of those have exploitable reserves ：1995（The country focus the research on 563 varieties ）
- Cultivar ：631 varieties
- Appropriate techniques for planting ：more than 320 techniques
- Traditional knowledge ：9658 items
- The number of photos：4323186（more than 4300 thousands photos）
- Specimens ：1 million specimens have been collected, and 130 thousand have been submitted to Beijing

2016. 8

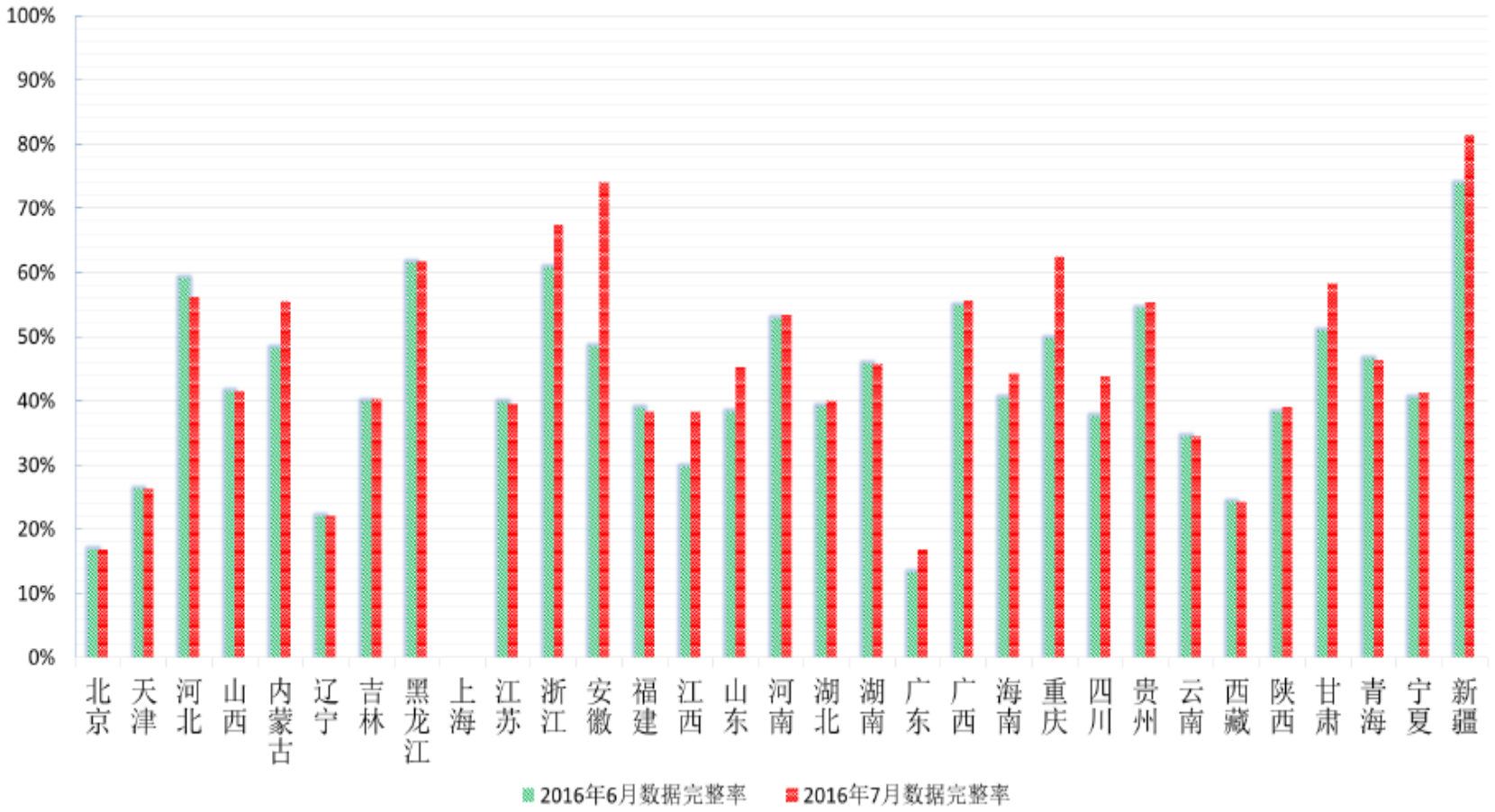
The amount of data for 31 provinces (regions , cities) and pilot counties



Each province submits the survey data successively,

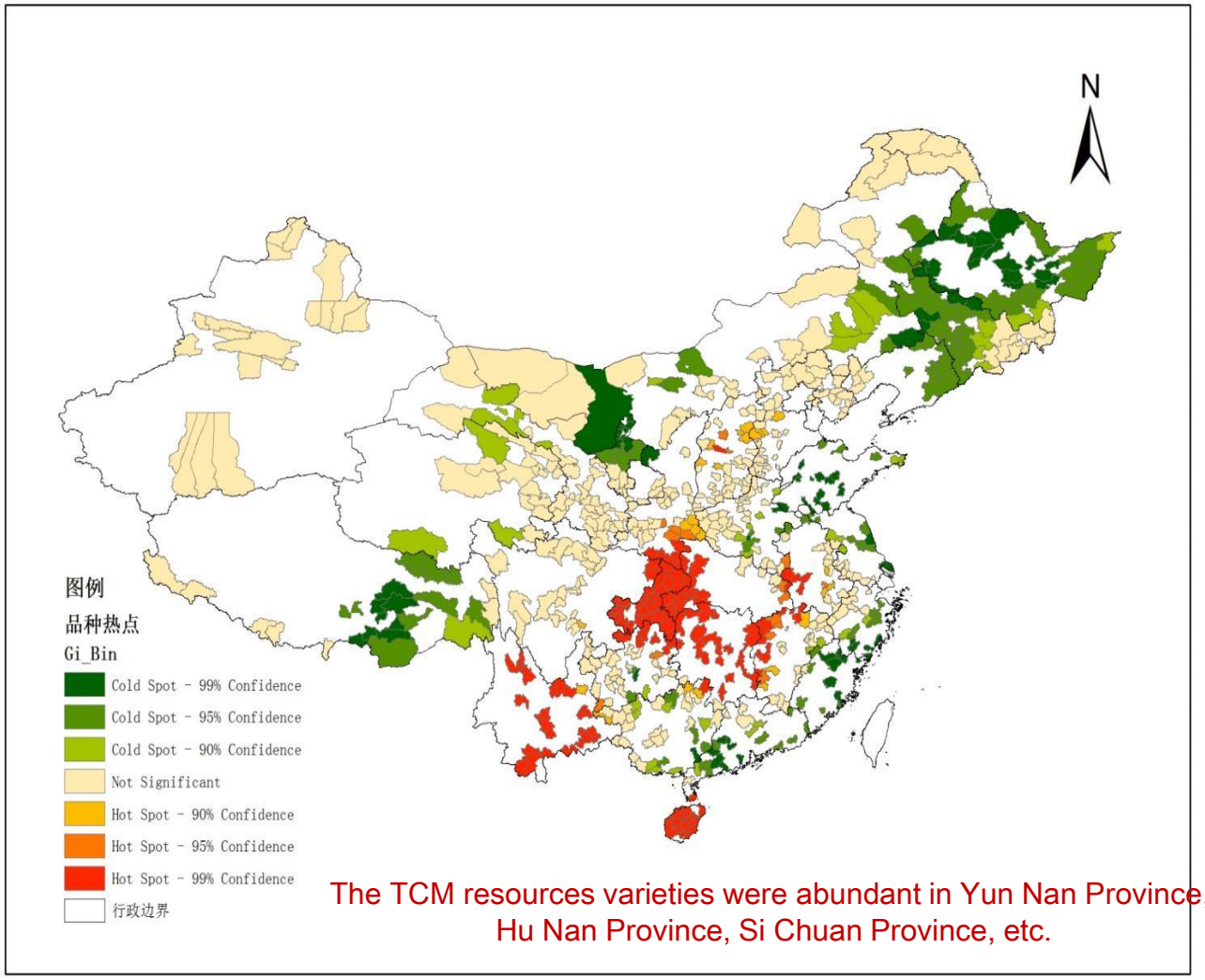
the total number of records has been increasing

The integrity rate of data from 31 provinces (regions , cities) and pilot counties



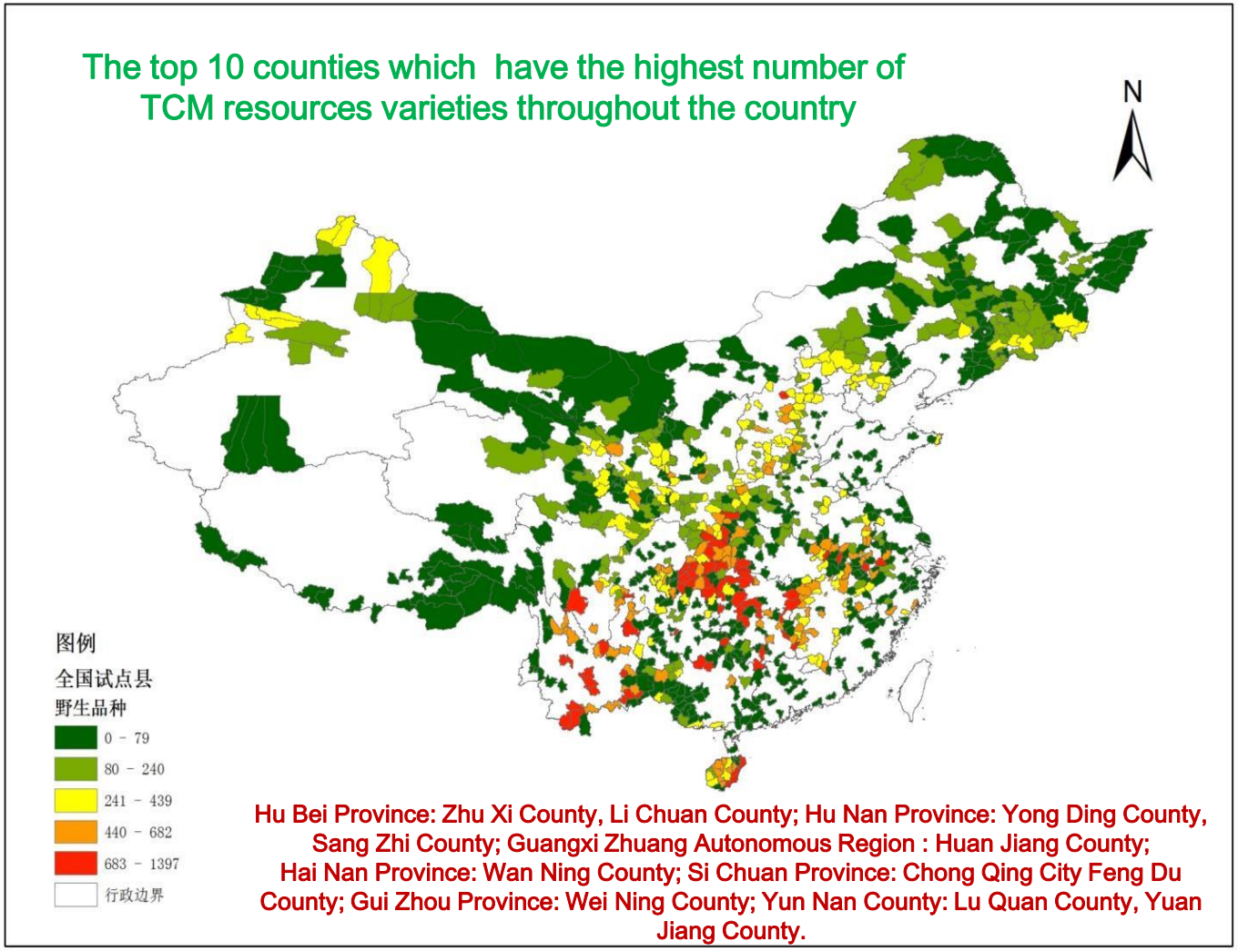
Based on the verification on the data submitted by each county, the proportion of available data from each province is increased and some decreased

Regionalization on species richness of TCM resources



The TCM resources varieties were abundant in Yun Nan Province, Hu Nan Province, Si Chuan Province, etc.

The number of TCM resources varieties in each county



Provide the service to the acceptance check, scientific research and long-term storage for the national survey on TCM resources

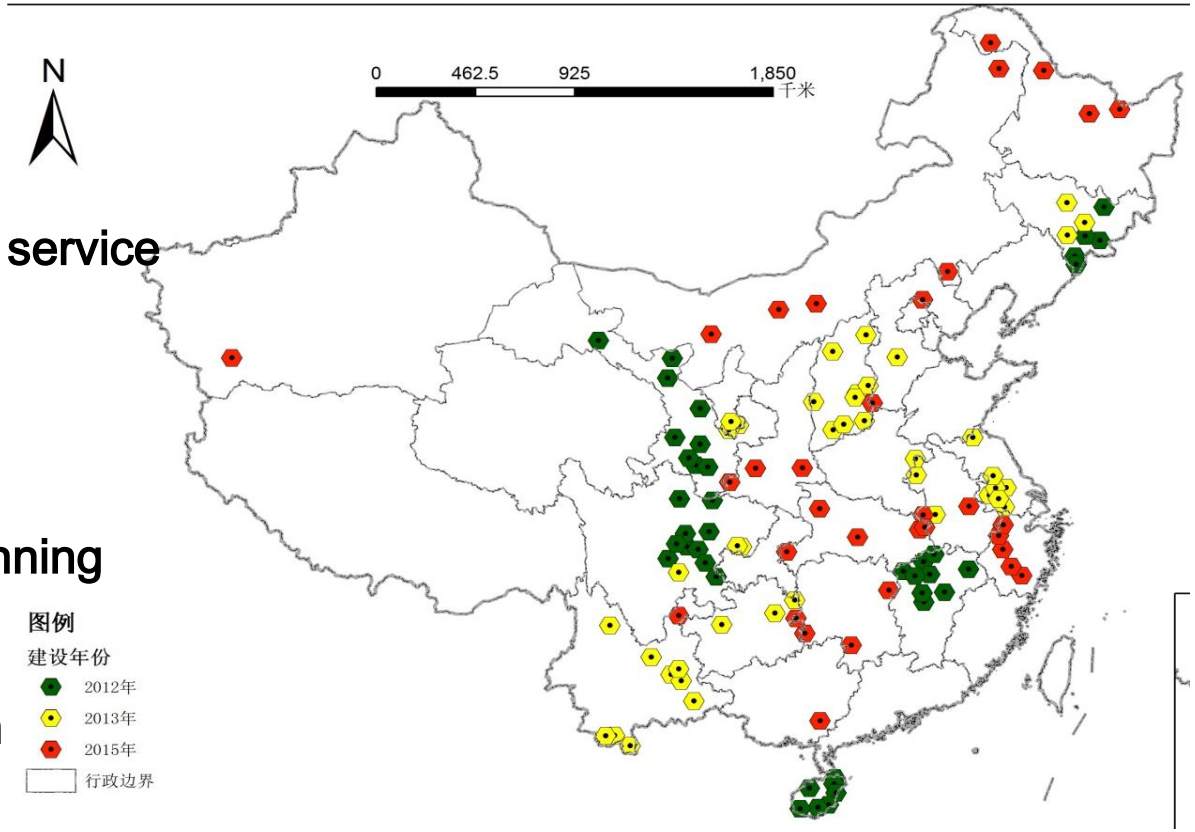
- Submitting
- Verification
- Acceptance check
- Data mining and discovery

Herbarium in Da Xing District

Collating on provincial level

Carry out the basic condition construction and appropriate technology research;
Promote the effective supply for the raw materials of Chinese herbal medicine

- 2012-5
  - 90% completed
  - Got the preliminary service ability
- 2013-11
  - Under construction according to the planning
- 2015-12
  - Construction begin



28 bases, breeding & production focusing on seeds & seedlings of 160 varieties of Chinese medicinal materials

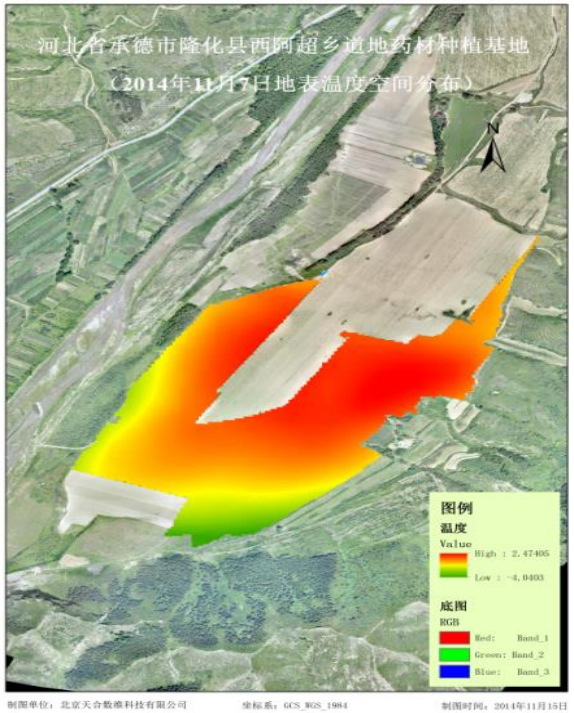
Appropriate technology for Chinese medicinal materials production

Techniques and information of seeds & seedlings are needed

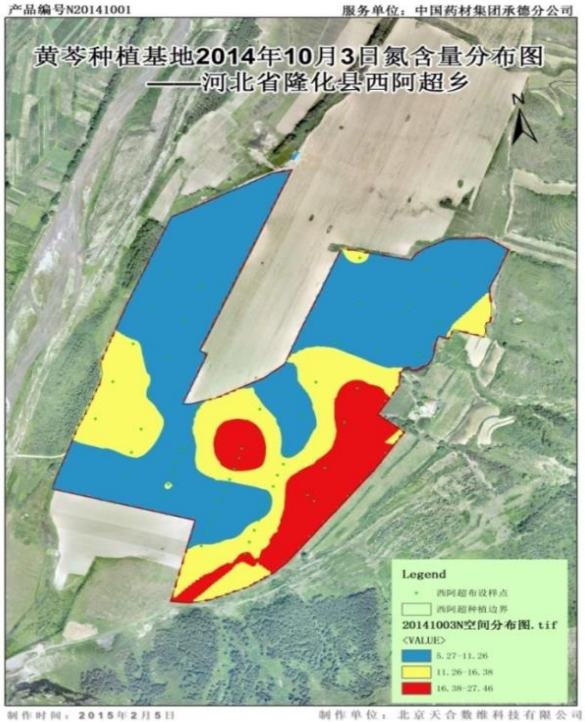
Study on dynamic monitoring system construction and the technical methods research;
Grasp the variation trend of the quantity, quality and the price of Chinese medicinal materials

- Monitoring system starts operating
- Information platform operates stably
- Information released regularly
- Start to provide testing and technical guidance services gradually

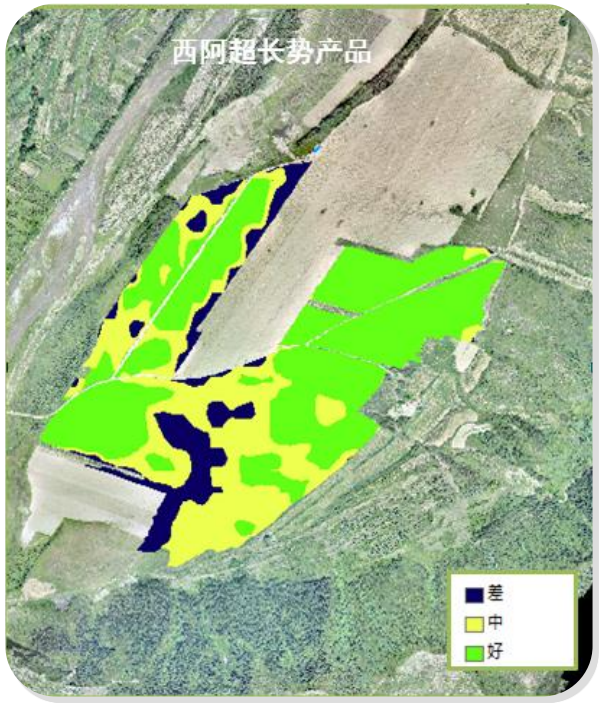
Joint application of monitoring devices and unmanned aerial vehicle (uav), in order to monitor *Scutellariae radix*



Temperature distribution



N elements distribution



Growth status distribution

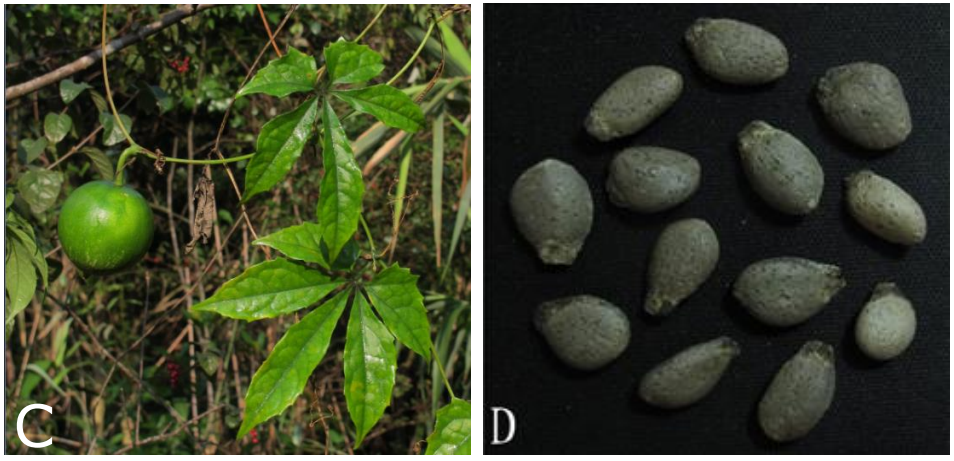
Technical specifications for the national survey on TCM resources

7 industry standard drafts have been formed

Fill the gap of that no technical specifications and industry standards in the field of TCM resources

A new species of *Trichosanthes Linn.* has been discovered. The seed morphology proved that the classification (*Subsect. Hemsleyanae Ku* belongs to *Sect. Involucraria (Ser.) Wight et Arn.*) is correct

那坡栝楼*Trichosanthes napoensis* D. X. Nong & L. Q. Huang



趾叶栝楼*Trichosanthes pedata* Merr. et Chun

Trichosanthes Linn. subordinate classification

Subgenus 1. *Subgen. Trichosanthes*

Group1. *Sect. Trichosanthes*

Group 2. *Sect. Carpesium*

Subgroup 1.叶苞亚组

Subgroup 2.柔毛亚组

Group 3. *Sect. Involucraria (Ser.) Wight et Arn.*

Subgroup 3. *Subsect. Bracteatae Hook. f. et Thoms.*

Subgroup 4. *Subsect. Hemsleyanae Ku*

Subgenus 2. *Subgen. Cucumeroides (Gaertn.) C. Y. Cheng et*

Group 4.王瓜组

Group 5.方子组

Main characteristics of *Sect. Involucraria (Ser.) Wight et Arn.* : simple leaf or compound leaf with digitated structure ; big male flower bracteole ; dark green fruit ; seeds compressed or expanded, no obvious ridge edges.

New understanding

Lycii fructus：【 flavour 】 bitter ， cold .

Revision on “Compendium of Materia Medica”

第 39 卷第 24 期
2014 年 12 月

中国中药杂志
China Journal of Chinese Materia Medica

Vol. 39, Issue 24
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·本草考证·

有关《本草纲目》中北艾产地修订

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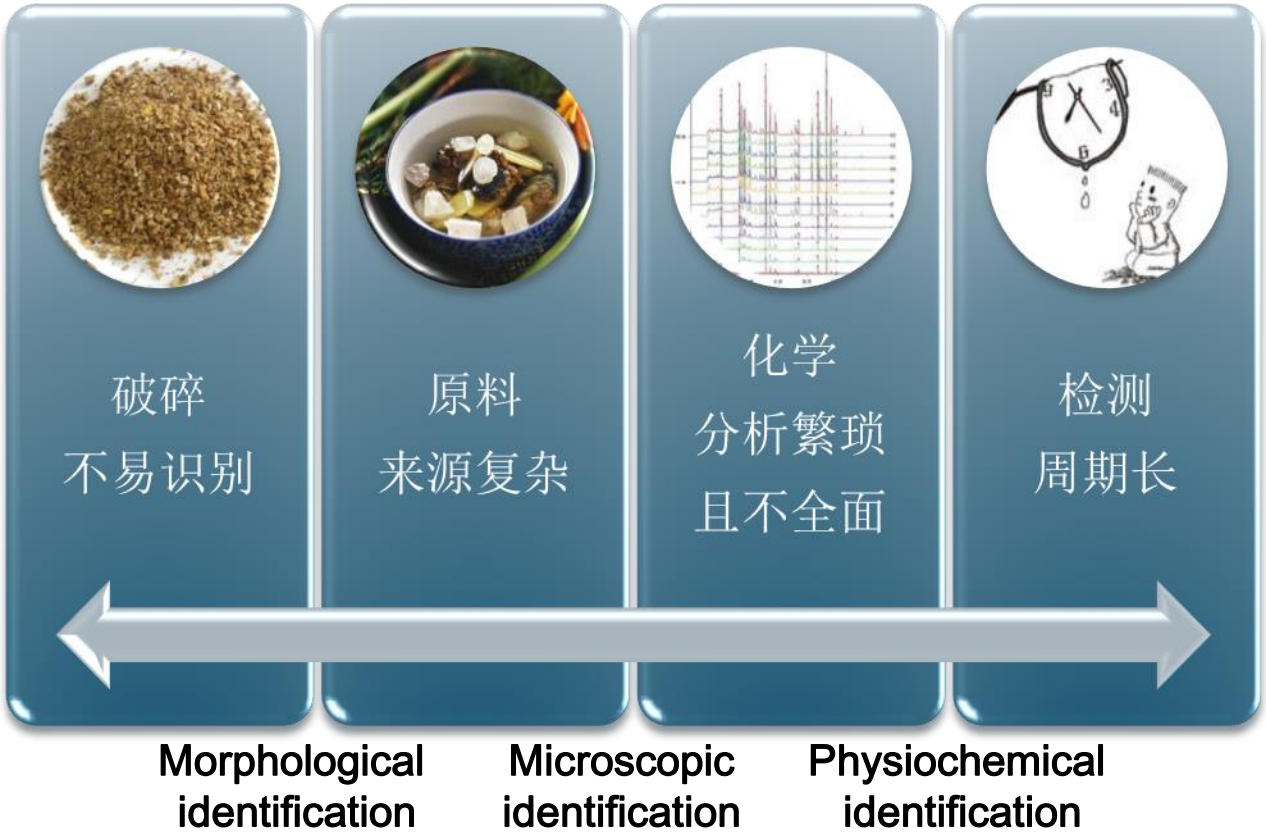
2. 中国中医科学院 中国医史文献研究所，北京 100700）

［摘要］《本草纲目》载北艾产地为汤阴“复道”。查汤阴仅有“伏道”一地，是否“复道”即为“伏道”？作者通过梳理艾叶道地沿革，考证“伏道”地名，同时结合全国中药资源普查实地所得资料，得出“复道”一词首次出现于宋代苏颂《本草图经》，历代沿用，但所指均不甚清楚，至《本草纲目》始出现“汤阴”与“复道”并提。伏道为扁鹊墓地之一，商周时即存在，沿袭至今，未曾变化，汤阴艾因伏道扁鹊墓而得名，可推测李时珍认为“复道”即是“伏道”，北艾产地应为“汤阴伏道”。

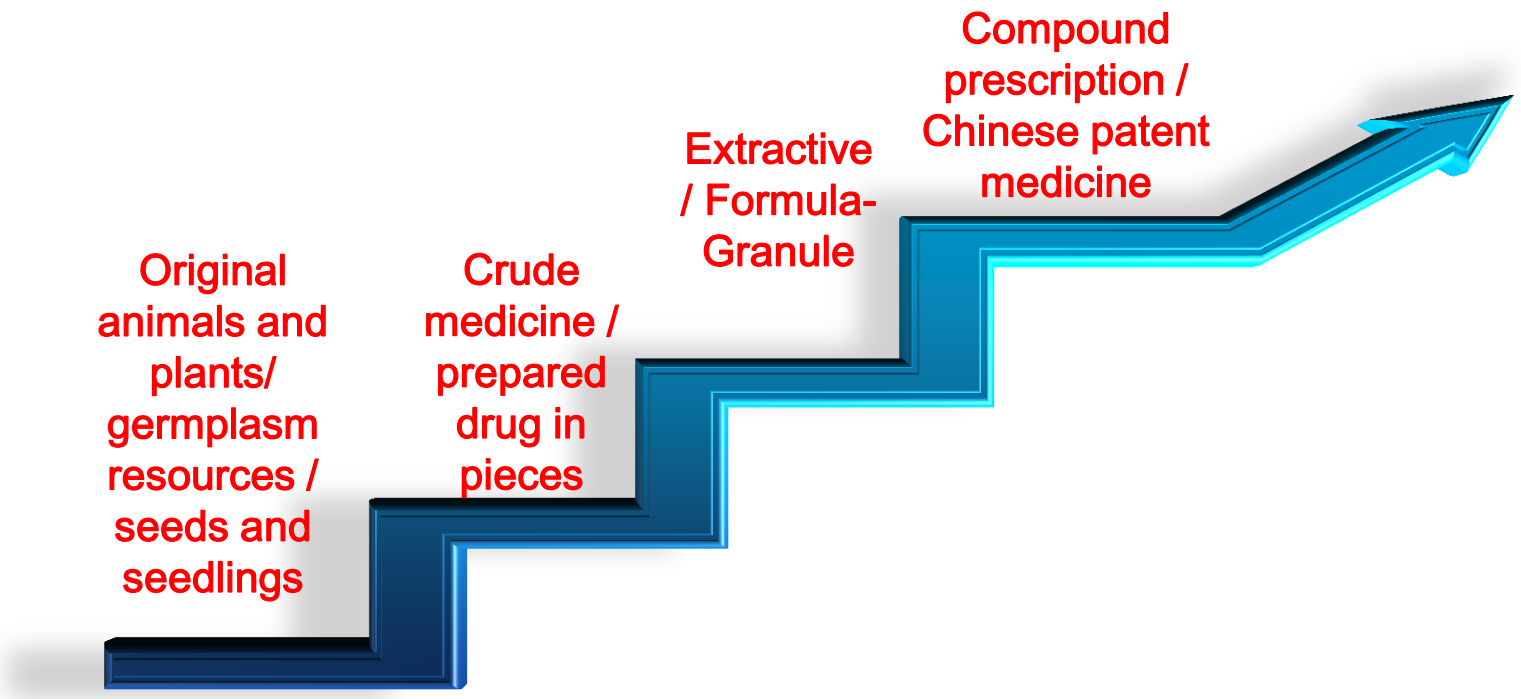
［关键词］ 本草纲目；北艾；汤阴；复道；伏道

2. TCM resources identification

The advantages of molecular identification

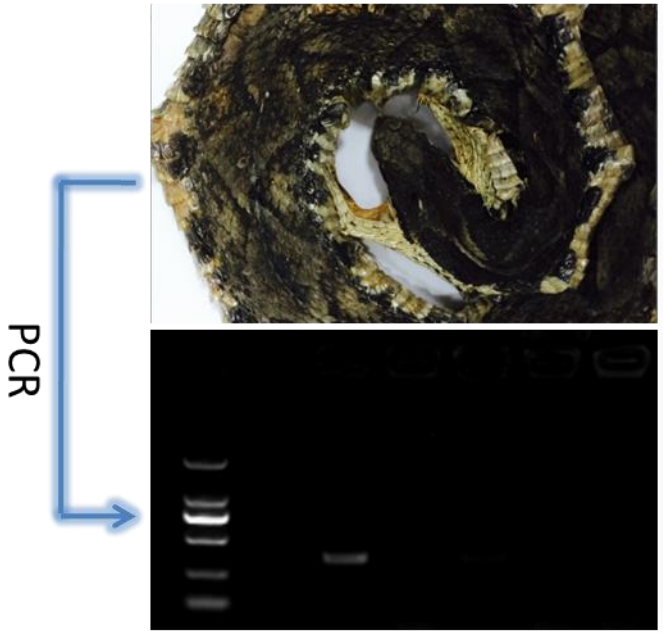


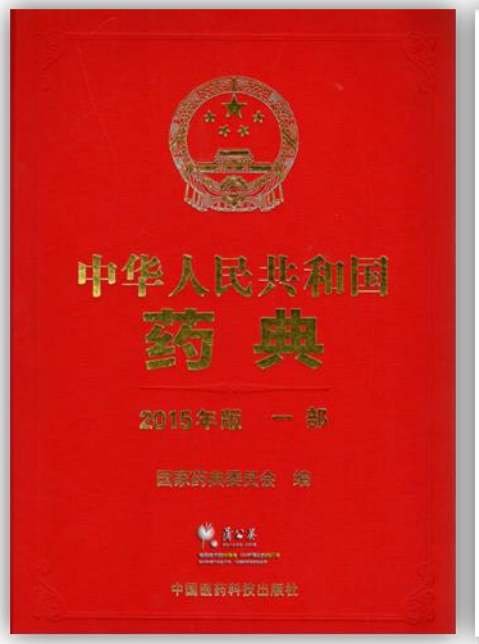
Applied range of molecular identification



Crude medicine / prepared drug in pieces

- Molecular identification on prepared drug in pieces of snakes





PCR identification for the specificity of *AGKISTRODON* and *Zaocys* is the first molecular identification method recorded in "Chinese pharmacopoeia".

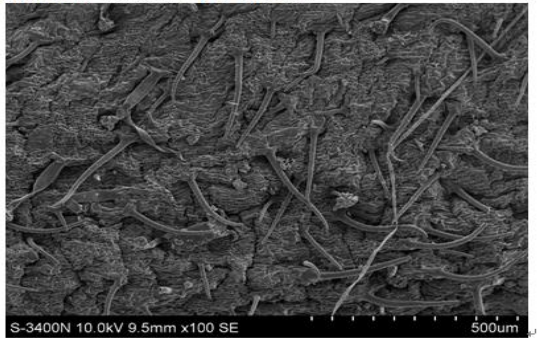
Germplasm resources / seeds and seedlings

- *Lonicerae japonicae flos* germplasm identification

1. 河北红银花

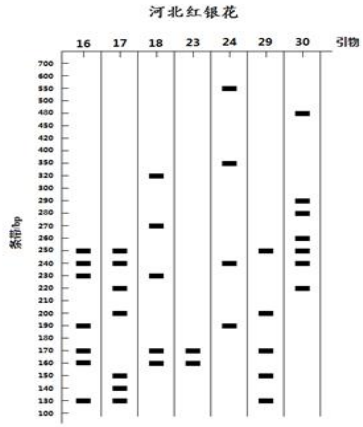
采集日期：2013.5.31
采集地：河北省邢台市巨鹿县堤村乡
N 37° 09′ 33.8″
E 115° 05′ 37.3″
H 30 m
生长环境：平原，土壤为黄壤土，较干旱，采用地下水灌溉。
性状特征：株型树形，平均株高为 87.5 cm，茎干较细，为黄色或黄白色；分枝少，上部缠绕，新枝红紫色，最细，平均直径为 0.168 cm，节间短，平均节间长为 2.813 cm；叶片为椭圆形，较厚，平均厚度为 0.039 cm，绿色，幼叶紫红色，平均叶长为 5.272 cm，平均叶宽为 2.889 cm，叶背和叶面皆有稀疏披毛，叶脉和叶柄均为红紫色，叶柄较短，平均长 0.397 cm；花蕾较少，散生，平均单枝花蕾数为 26 个，花梗较长，平均长度为 0.633 cm，花萼为披针形，临花对生成一定角度，花蕾较长，平均长 2.541 cm，平均直径为 0.325 cm，红色，顶端膨大，不弯曲，花丝黄白色，花药较大，平均花药长 0.349 cm，平均花药宽 0.08 cm，柱头最大，平均直径为 0.097 cm；开花早，花期短，盛花期为 5 月中下旬。

非腺毛特征：花蕾表面具薄壁非腺毛和厚壁非腺毛，厚壁非腺毛短而稀疏，平均长度为 3.039 mm，平均密度为 47.5 个/cm²。



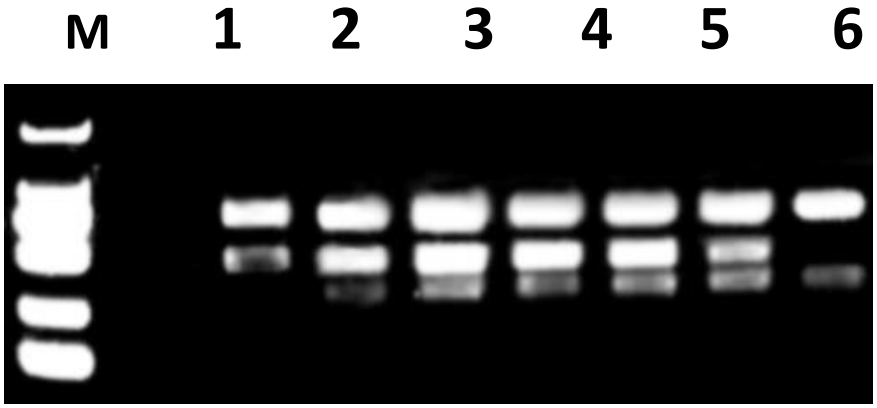
SSR 分子标记指纹图谱：

引物编号	1	2	3	4	5	6	7
指纹图谱	01110111	**00110110110**	0111001	000001	**10100001010**	000010111	00110111



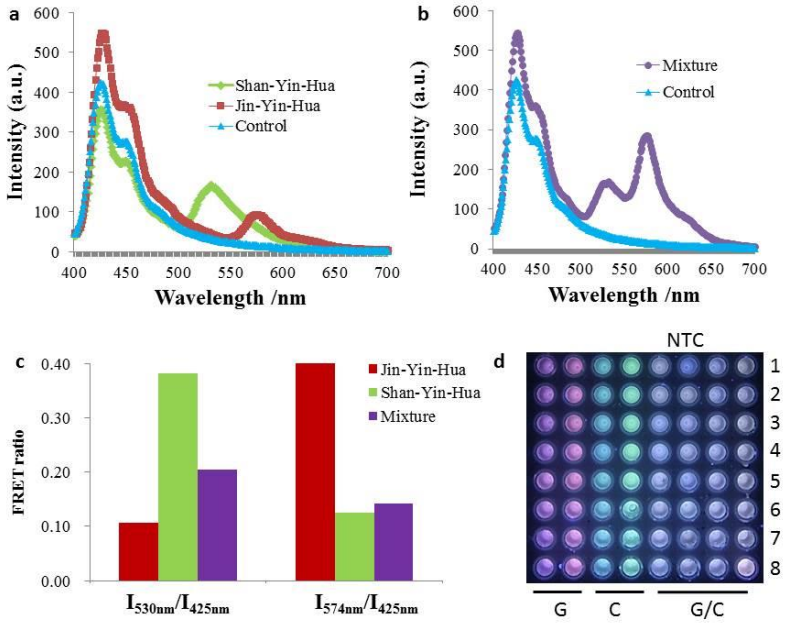
Mixed product identification technology

Specificity PCR/ng
(Apply to the identification on the crude medicine / prepared drug in pieces)



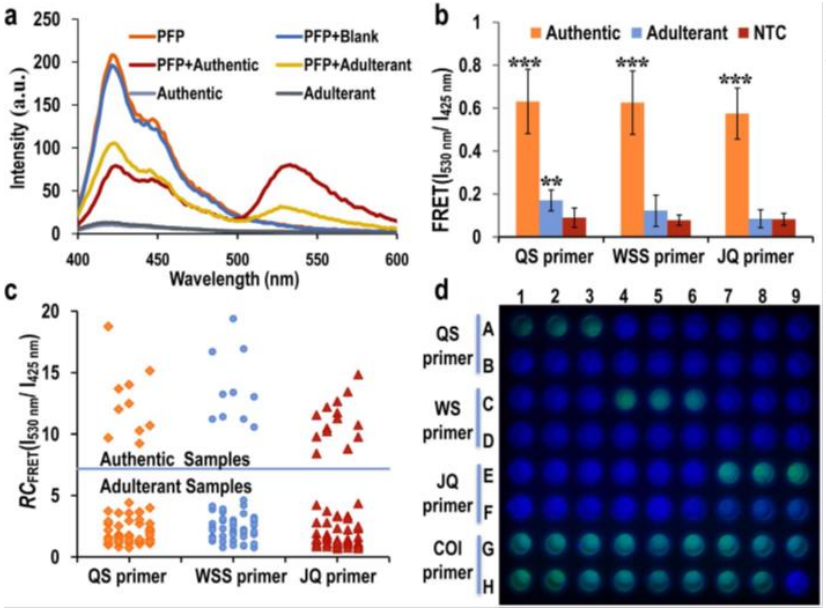
M：DL2000 marker；1： Negative control ；2-7：Different proportion of *Lonicera confusa (Sweet) DC.* blended in *Lonicera japonica Thunb.*，2，1%；3，5%；4，10%；5，20%；6，50%；7，90%；8：*Lonicera confusa (Sweet) DC.* 。

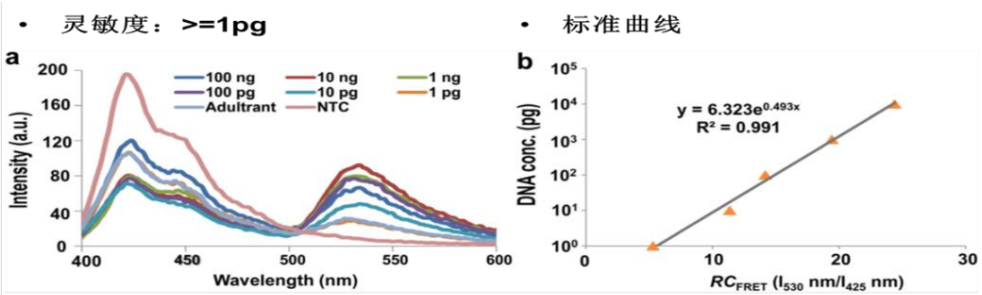
CCP-FRET/pg
(Apply to the identification on the Extractive / Formula-Granule)



Compound prescription / Chinese patent medicine

- Molecular identification on snakes raw materials in Chinese patent medicine





• 中成药原料鉴定

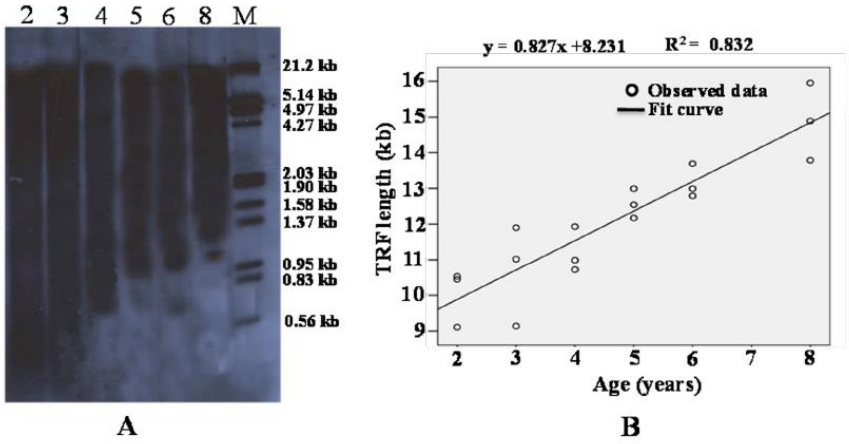
中成药名	RCFRET (I530 nm/I425 nm)			BLAST 结果 (Best Hit)			
	QS primer	WSS primer	JQ primer	Species	E value	Idnetity(%)	Accession
再造丸	12.67	4.20	3.34	*D. acutus*	1e-141	99	AF038883.1
人参再造丸	10.23	3.01	2.34	*D. acutus*	7e-149	99	AF038883.1
消栓再造丸	9.79	1.68	2.79	*D. acutus*	7e-148	98	EU913476.1
清眩治瘫丸	7.85	8.48	4.23	*D. acutus*[a]	3e-147[a]	98[a]	AY223560.1[a]
				Z. dhumnades[b]	1e-116[b]	96[b]	AF236676.2[b]
大活络丸	4.20	12.02	2.48	*Z. dhumnades*	1e-110	95	AF236676.2
乌蛇再造丸	3.01	3.66	9.68	*B. multicinctus*	0	99	AJ749344.1

DNA identification fluorescence spectrum of snakes medicinal materials of CCP-FRET: (a) Identification on *Bungarus parvus* and its adulterants(b) FRET rate of CCP-FRET identification on *AGKISTRODON*, *Zaocys* and *Bungarus parvus*; (c) Identification threshold value of *AGKISTRODON*, Zaocys, *Bungarus parvus* and their adulterants; (d) Test results by visual inspections under the 365 nm uv lamp .

Jiang C, Yuan Y*, Luqi Huang*, Scientific report, 2015

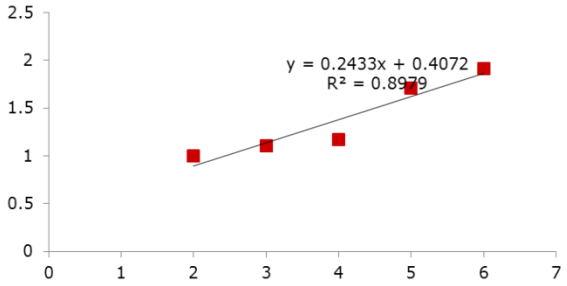
Rapid assessment on growth year of the Chinese medicinal materials

Introducing the telomeres theory

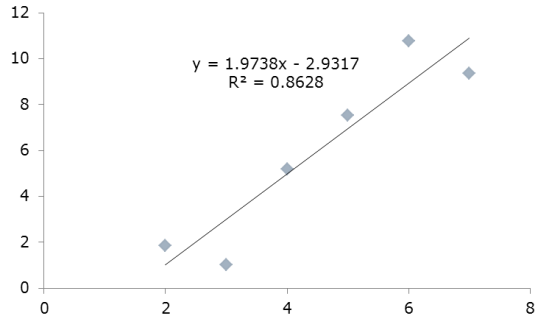


Fitted curve of ginseng telomeres and growth year has been established, based on the find of that the length of ginseng telomere will grow along with the age growth. Took a 5 years raw ginseng randomly, and measured the telomeres length is 12.56kb. Put it into the fitted formula, and got the result of that the growth year of ginseng is 5.23, which is consistent with the actual value.

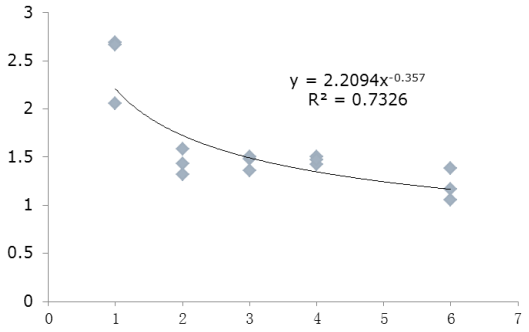
qPCR rapid identification



Ginseng



Panacis Majoris Rhizoma



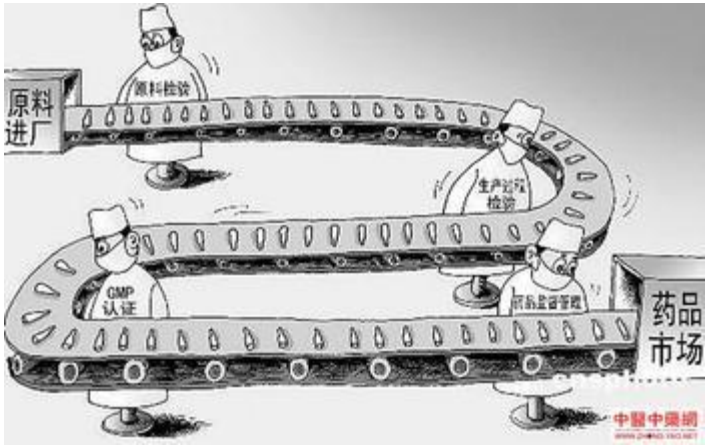
Paeoniae Radix Rubra

Could measure the relative length of ginseng telomeres in 2 hours
50mg samples are enough to be tested

Liang J，Jiang C，Luqi Huang*，
Scientific report，2015

On-site and fast demand

Accurate, fast, high throughput, low cost



On-site operation of molecular identification

- DNA rapid extraction technique– DNA alkali pyrolysis
  - Simple reagent：

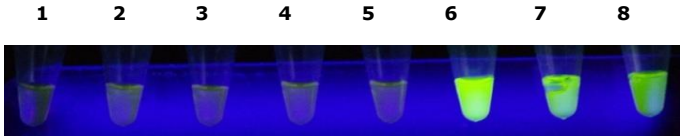
1. Extracting buffer（A）： NaOH（KOH）and additive agent KCl、NP-40、β-mercaptoethanol、PEG、glycocoll、Tween 20、PVP； 2. neutral buffer（B）： HCl、NaAc、Tris

  - The operation is simple, and no instrument needed：

Put medicinal powder into solution A , vibrate for 1 minutes, and then put into solution B , ,vibrate for 1 minutes, get supernatant for future determination.

- Rapid PCR technique

Bungarus parvus
29 min



1：Natrix annularis Hallowell；2：Bungarus parvus；3：Zaocys；4：AGKISTRODON；5：Elaphe carinata；6：Bungarus parvus；7：Bungarus parvus；8：Bungarus parvus

- PCR amplification time can be reduced to 30 minutes;
- Without using electrophoresis equipment, visual inspection is ok for getting the result.

Jiang Chao，Huang Luqi，Yuan Yuan*， Chinese Journal of Pharmaceutical Analysis ，2013；Zheng qi，Huang Luqi，Yuan Yuan*，China Journal of Chinese Materia Medica ，2014；Jiang Chao，Huang Luqi，Yuan Yuan*， China Journal of Chinese Materia Medica ，2014；Chen Kang，Jiang Chao，Huang Luqi，Yuan Yuan， China Journal of Chinese Materia Medica ，2014

On-site operation of molecular identification

DNA extraction — DNA alkaline lysis method 5-10 min

Amplified reaction — Rapid PCR technique 30min

Test and authenticity — Fluorescent dye technique 1-2min

便携式细胞破碎仪

恒温金属浴

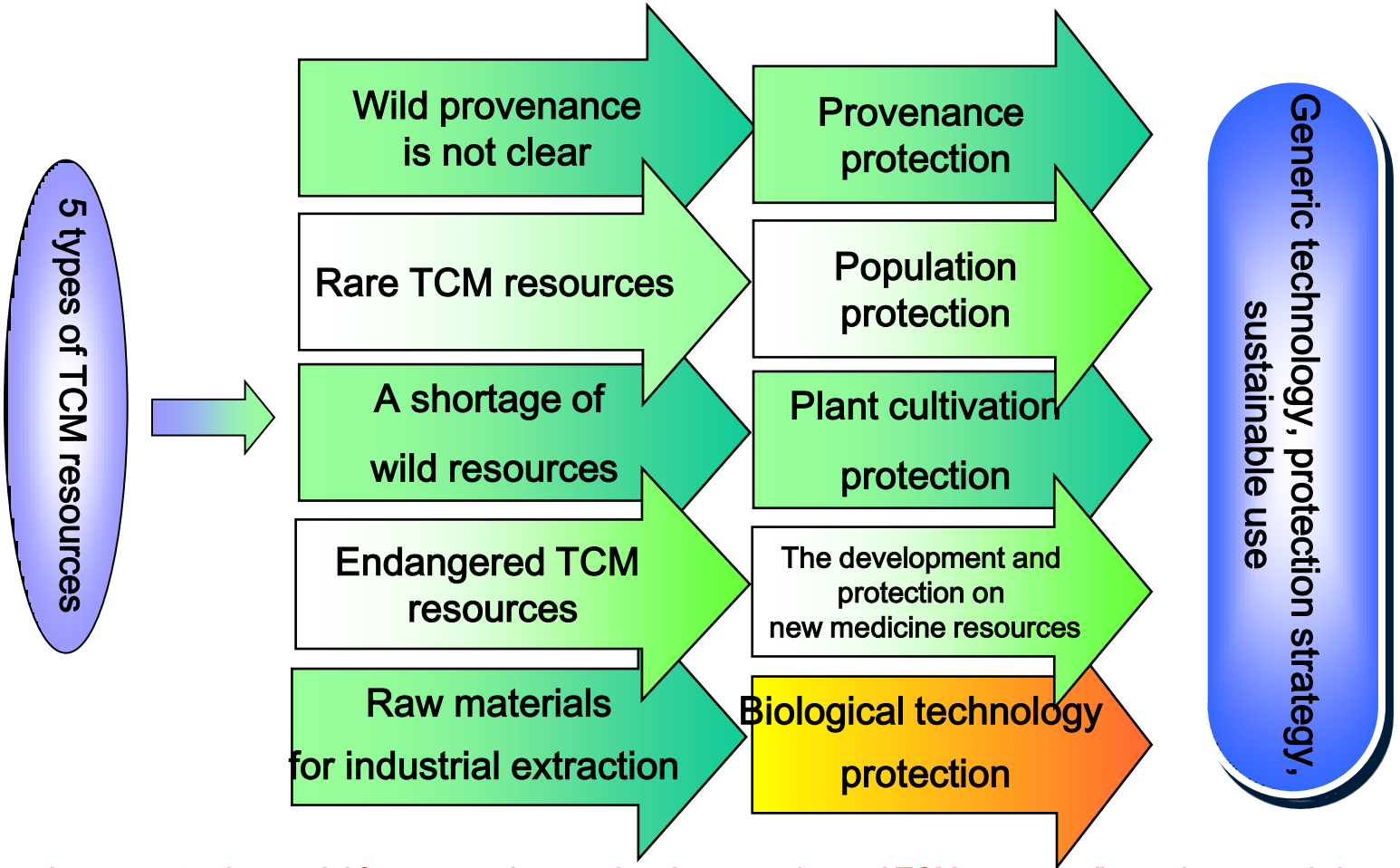
手持式紫外灯

掌上PCR

Yuan Yuan,Huang Luqi*, China Journal of Chinese Materia Medica, 2013.

3. Research on TCM resource protection

The research on 5 protection model for rare, endangered and commonly used TCM resources



"The research on 5 protection model for rare, endangered and commonly used TCM resources" won the second class prize of national award for progress in science and technology (the 1st author , 2008)

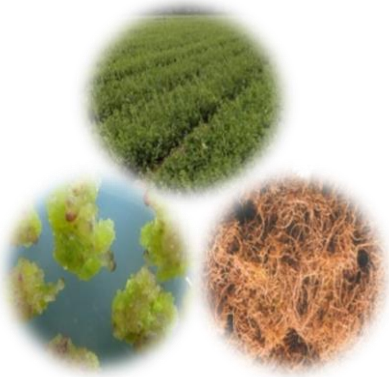
Sustainable Utilization of the Traditional Chinese Medicine Resources

香山科學會議

Synthetic Biology and Sustainable Utilization of the Traditional Chinese Medicine Resources

Active ingredients of medicinal plant

Secondary metabolites



Artemisinin

Biosynthetic pathway

Improve by metabolic engineering

Produce by synthetic biology

High-throughput Sequencing

NCBI

Abundant Data Base

High Resolution Mass Spectrum

Genomics
- Phylogeny and evolution
- GWAS
- Gene cluster

Transcriptomics
- Deep transcriptome
- Co-expression network

Metabolomics
- Metabolite annotation
- Change in development, stress and genotype

Data processing, databasing, integration

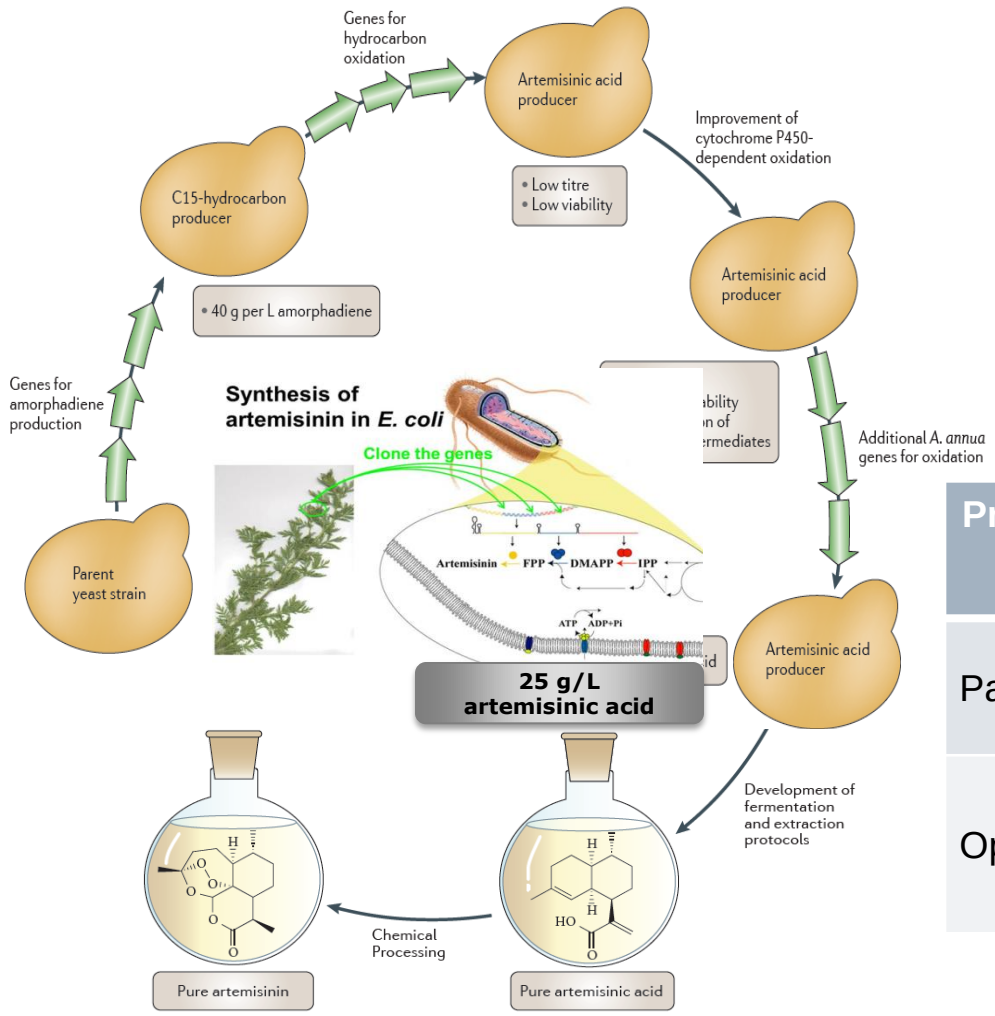
Hypothesis generation by systems analysis

Reverse genetics/biochemistry/chemistry, mathematical modeling

Functional genomics (hypothesis validation) and biotech application (synthetic biology)

Saito K. Current opinion in plant biology, 2013(16):373-380

Heterologous production of high value natural products



Canada

PhytoMetaSyn

Synthetic Biosystems for the Production of High-Value Plant Metabolites

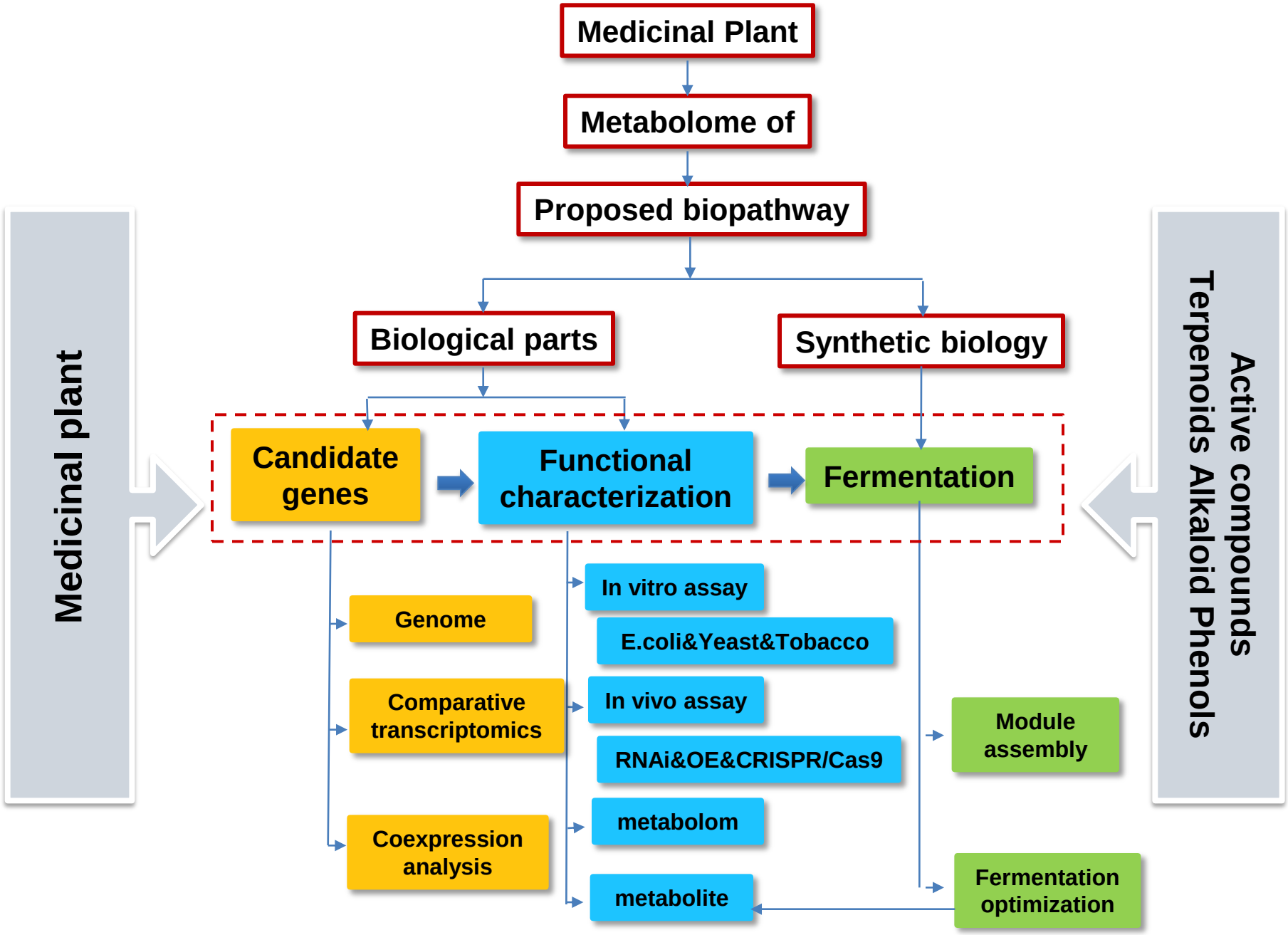
The total project budget is $13,602,100 over 4 years.

75 plants that produce high - value natural products.

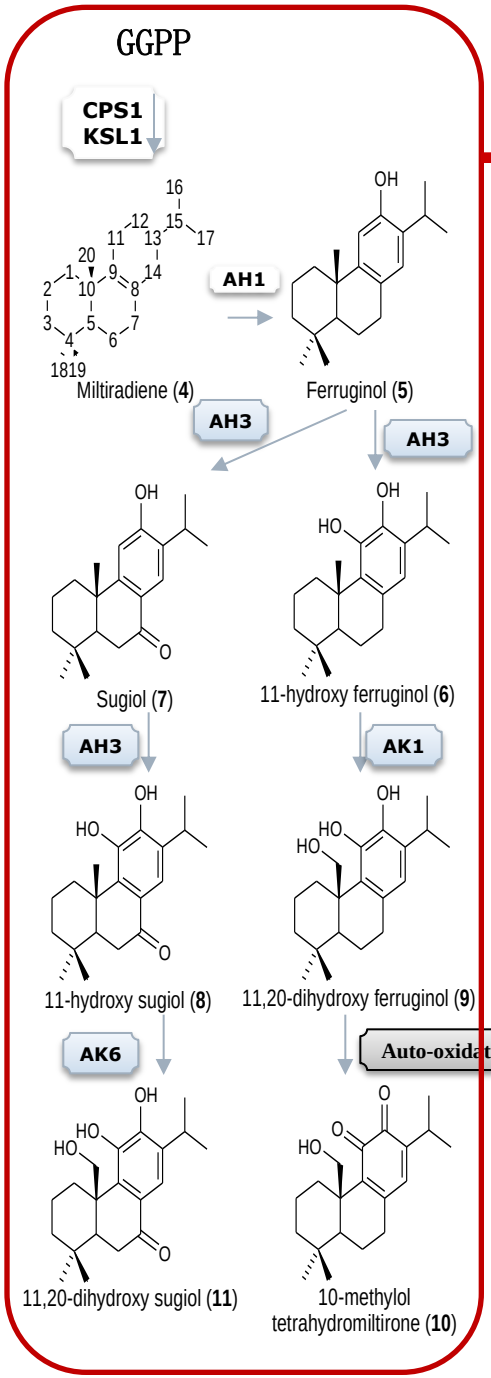
Products	Microorganisms	Progress	References
Paclitaxel	*Escherichia coli*	Taxadiene 1g/L	Ajikumar et al. ***Science***,2010
Opioids	*Saccharomyces cerevisiae*	Hydrocodone 0.3μg/L	Galanie et al. ***Science,*** 2015

Ginsenosides	Curcumin	Resveratrol

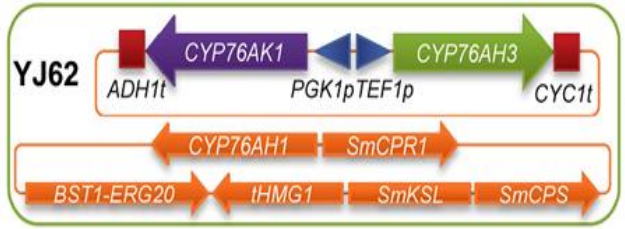
Paddon et al. **2013, *Nature***

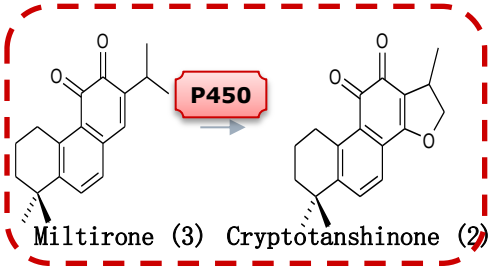

Medicinal Plant
Metabolome of
Proposed biopathway
Biological parts
Synthetic biology
Medicinal plant
Candidate genes
Functional characterization
Fermentation
Active compounds
Terpenoids Alkaloid Phenols
Genome
Comparative transcriptomics
Coexpression analysis
In vitro assay
E.coli&Yeast&Tobacco
In vivo assay
RNAi&OE&CRISPR/Cas9
metabolom
metabolite
Module assembly
Fermentation optimization

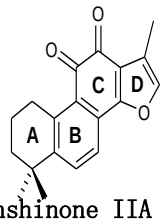
Biosynthesis and heterologous production of tanshinones



Three genes involved in the upstream terpenoid bio-pathway
HMGS, FPPS, GGPPS
Six genes involved in tanshinone biosynthesis
SmCPS1, SmKSL1, CYP76AH1, CYP76AH3, CYP76AK1, SmCPR1
Four engineered yeast strains for tanshinone production.

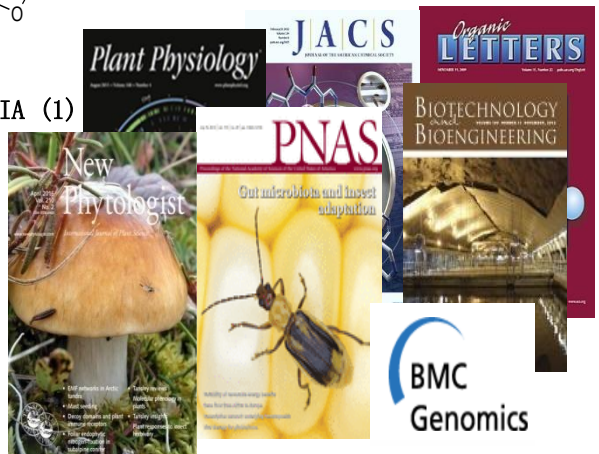




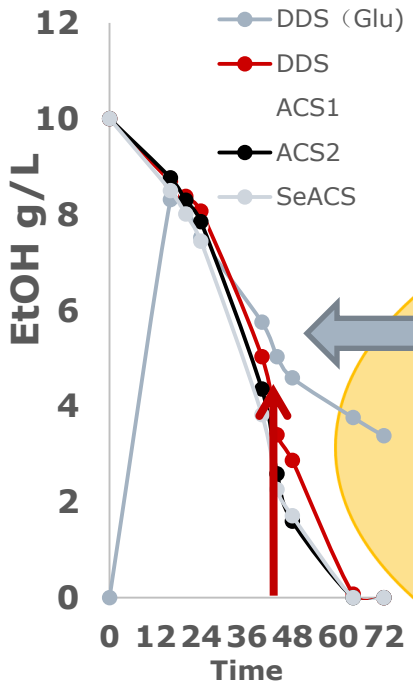


The First Prize in Science and Techonology Awards of the Chinese Pharmaceutical Association

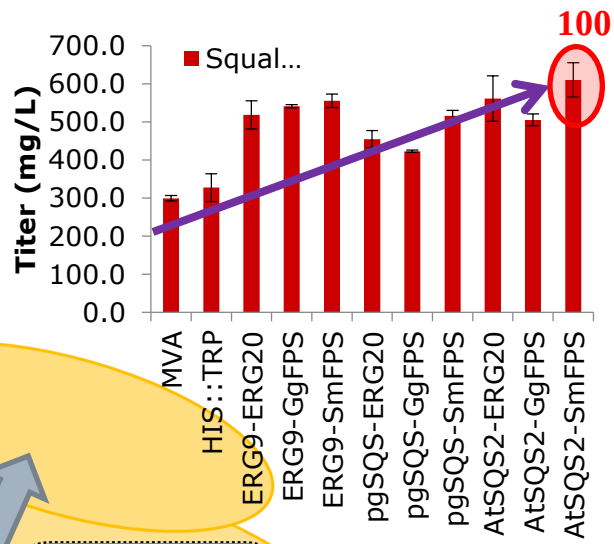
Guo, Ma, Zhao, Huang *et al*.. 2016, *New Phytologist*
Cui G, Huang L, Peters, R. J., Qi, X. et al. 2015, *Plant Physiol*
Guo, Zhou, Peters, Zhao, Huang *et al*.. 2013, *PNAS*
Zhou, Gao, Huang, Zhao *et al*.. 2012, *JACS*
Dai, Huang, Zhang *et al*.. 2012, *Biotech and Bioeng*
Gao, Peters, Huang et al.. 2009, *Organic Letters*

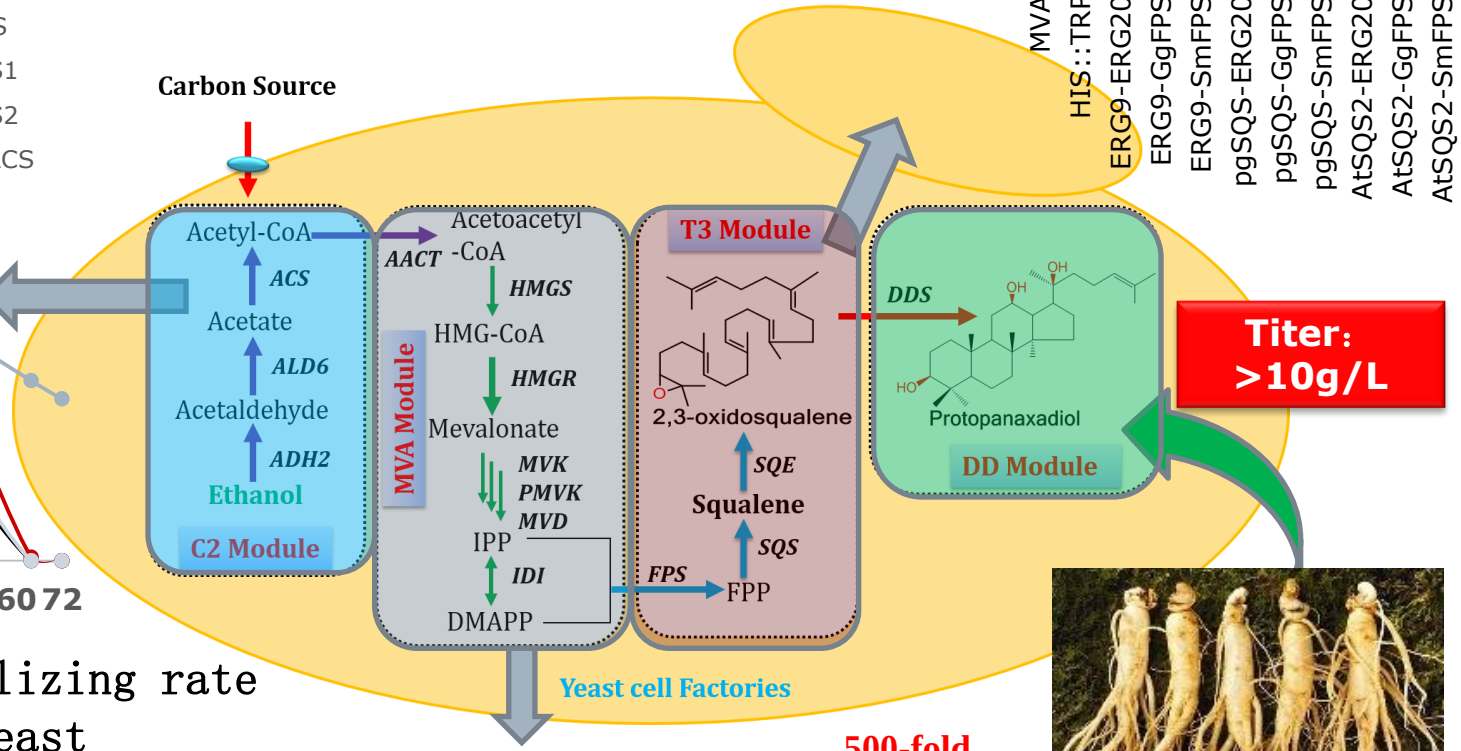


Ginsenosides yeast



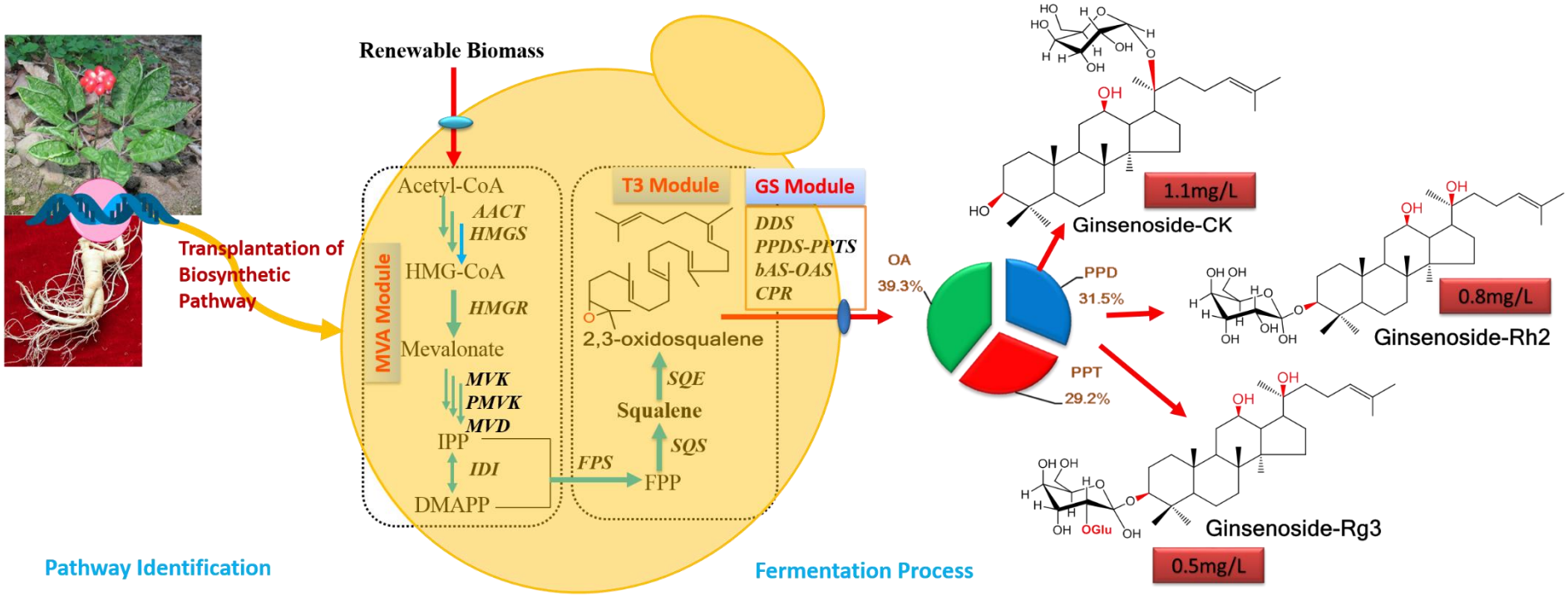
Ethanol utilizing rate of yeast





Improve the utilization rate of ethanol
Improve precursor production
Codon optimization
Ginsenosides production:
1.3 g/L PPD and 10 g/L dammarenediol-II(DD)

Ginsenosides yeast



Finally, the biosynthetic pathways of Rh2 and Rg3 were constructed by introducing the UDP-glycosyltransferases genes from P.ginseng, with fermentation titres of 0.5mg/L Rg3 and 0.8mg/L Rh2. which realized the microorganism production of ginsenosides.

Thank You!