

ACIAR project "Establishing Sustainable Solutions to Cassava Disease in Mainland Southeast Asia"

# Cassava germplasm evaluation and variety breeding in China

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Alliance



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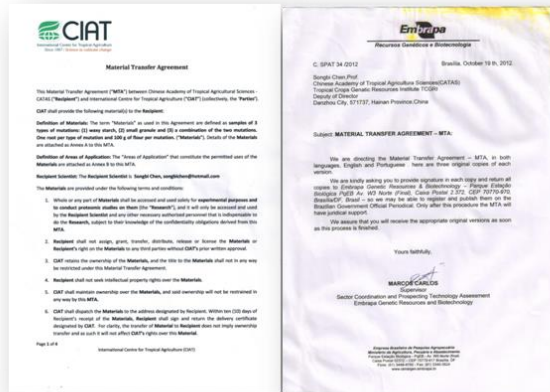
# 1. Accurate Evaluation of Cassava Germplasm Resources



# Cassava germplasm resources

- Through the project cooperation with Prof. Luiz Carvalho from Embrapa and Prof. Hernan Ceballos from Alliance of Bioversity-CIAT, the germplasm samples from CIAT and Embrapa were introduced.

MTA signed by Embrapa and CATAS



CIAT special cassava resources Brazilian precious cassava resources



Supported by NSFC-CGIAR cooperative projects (No. 31361140366) and NSFC (No. 31271776, No. 31371684)

# Collection and preservation of cassava resources



国家木薯种质资源圃



木薯野生近缘种W14



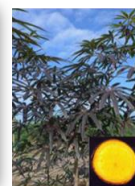
低淀粉、多纤维矮化木薯



高支链、淀粉含量低于1%黄心糖木薯



高番茄红素粉红木薯



紫叶黄心木薯



抗寒木薯品种SC124



抗细菌枯萎病木薯RXC9



感细菌性枯萎病品种桂热4号



高抗花叶病木薯I93与感病品种嫁接



耐PPD木薯SC14

Chinese National Repository of Cassava germplasm Resources have collected more than 3000 cassava accessions, and the genetic diversity index has reached above 0.5, which greatly meets the needs of genetic improvement of cassava in China.

# Accurate evaluation of cassava germplasm resources

- Generation of Phenotypic accurate evaluation model:** The accurate evaluation model of quality phenotype group of cassava germplasm resources was constructed by starch content, cyanide content,  $\beta$ -carotene content, resistance (low temperature, postharvest physiological deterioration (PPD), mosaic disease) and mean net photosynthetic rate of leaves; 15 cassava germplasm with excellent quality indexes were evaluated.

$$Q = \sum_{i=1}^n \binom{n}{k} x_i^m a^j$$

Q = 木薯种质资源品质评级

i = 1, ..., 5 (代表第 i 种表型特性的序号);

n = 5 (总共5种表型特性);

k = 1, ..., 5 (代表从第k种表型特性起开始累计);

$x_i$  = SC, 或HA, 或PPD, 或 $\beta$ C, 或PR (代表5种表型特性中的第 i 种特性);

$x_i^m$ : 代表第 i 种表型特性的数据分布型;

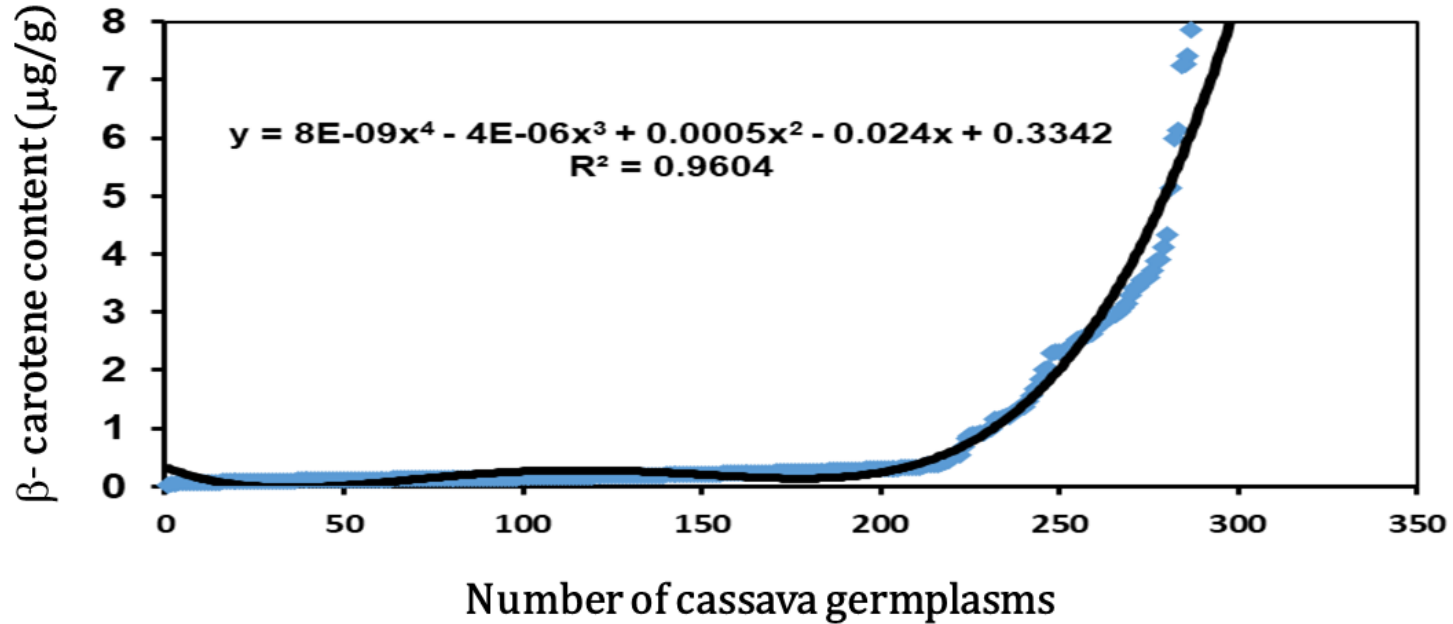
$a^j$ : 代表5种表型特性中的任意一种的权重系数及其数据分布型。

| 淀粉含量 (%) 分级<br>用橙色标注, 级差<br>为 4     | 氰苷含量 ( $\mu\text{g/g}$ ) 分<br>级用蓝色标注, 级<br>差如下 | 耐 PPD 分级用<br>红色标注, 级<br>差为 1 | $\beta$ -胡萝卜素含量<br>( $\mu\text{g/g}$ ) 分级用黑色<br>标注, 级差如下 | 叶片净光合速率<br>( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) 分<br>级用绿色标注, 级差为<br>1.5 |
|-------------------------------------|--|------------------------------|--|--|
| A 级, $SC \geq 38$                   | A 级, $HA \leq 10$                              | A 级, PPD=6                   | A 级, $\beta C \geq 3$                                    | A 级, $PR \geq 18$  |
| A <sup>-</sup> 级, $34 < SC < 38$    | A <sup>-</sup> 级, $10 < HA \leq 20$            | A <sup>-</sup> 级, PPD=5      | A <sup>-</sup> 级, $1 \leq \beta C < 3$                   | A <sup>-</sup> 级, $16.5 \leq PR < 18$  |
| B 级, $30 < SC \leq 34$              | B 级, $20 < HA \leq 30$                         | B 级, PPD=4                   | B 级, $0.3 \leq \beta C < 1$                              | B 级, $15 \leq PR < 16.5$   |
| B <sup>-</sup> 级, $26 < SC \leq 30$ | B <sup>-</sup> 级, $30 < HA \leq 50$            | B <sup>-</sup> 级, PPD=3      | B <sup>-</sup> 级, $0.2 \leq \beta C < 0.3$               | B <sup>-</sup> 级, $13.5 \leq PR < 15$  |
| C 级, $22 < SC \leq 26$              | C 级, $50 < HA \leq 100$                        | C 级, PPD=2                   | C 级, $0.1 \leq \beta C < 0.2$                            | C 级, $12 \leq PR < 13.5$   |
| C <sup>-</sup> 级, $SC \leq 22$      | C <sup>-</sup> 级, $HA > 100$                   | C <sup>-</sup> 级, PPD=1      | C <sup>-</sup> 级, $\beta C < 0.1$                        | C <sup>-</sup> 级, $PR < 12$  |

| Germplasm number | Score | Germplasm number | Score | Germplasm number | Score |
|------------------|-------|------------------|-------|------------------|-------|
| GPMS1029L        | 89.5  | MS000020         | 75.5  | MS000308         | 72    |
| MS000315         | 86    | GPMS0981L        | 75.5  | GPMS1009L        | 72    |
| GPMS0977L        | 79    | MS000436         | 75.5  | MS000223         | 72    |
| GPMS0980L        | 75.5  | MS000532         | 72    | GPMS1027L        | 72    |
| MS000545         | 75.5  | MS000514         | 72    | MS000241         | 72    |

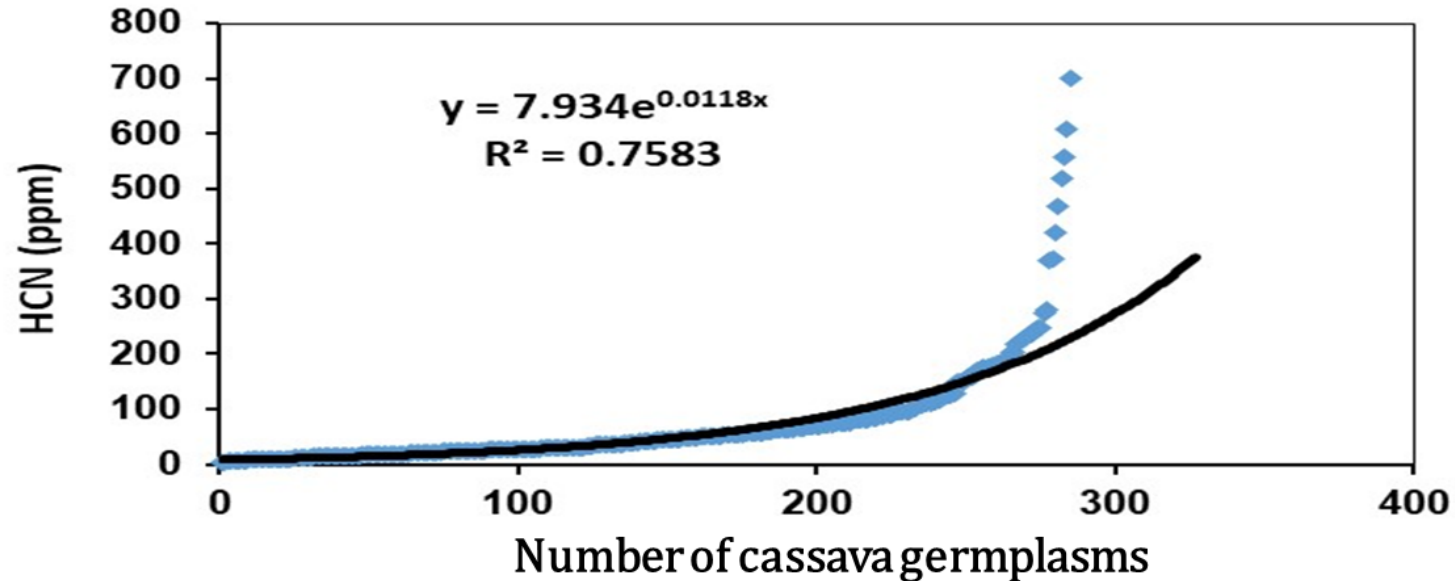


- The germplasm GPMS1024L β-carotene content was up to 7.853μg/g



Analysis of β-carotene content in cassava germplasm resources

- The germplasms in low cyanoside contents of tuberous root were excavated, as low as 4.161ppm



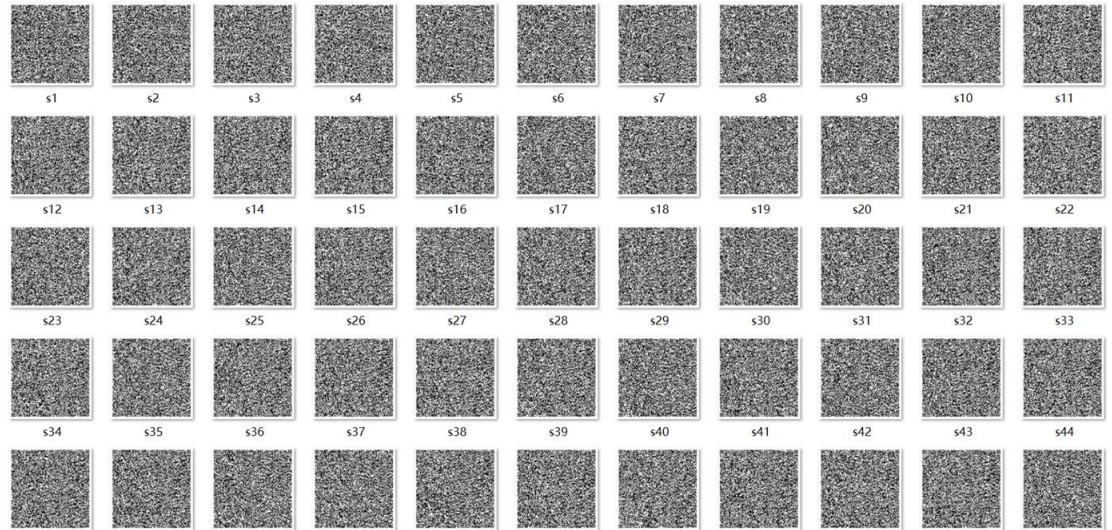
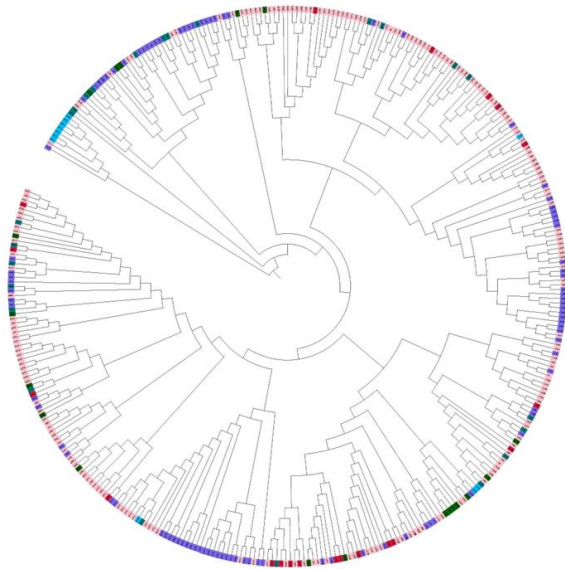
Analysis of cyanoside content in cassava germplasm resources



# Construction of cassava germplasm fingerprints

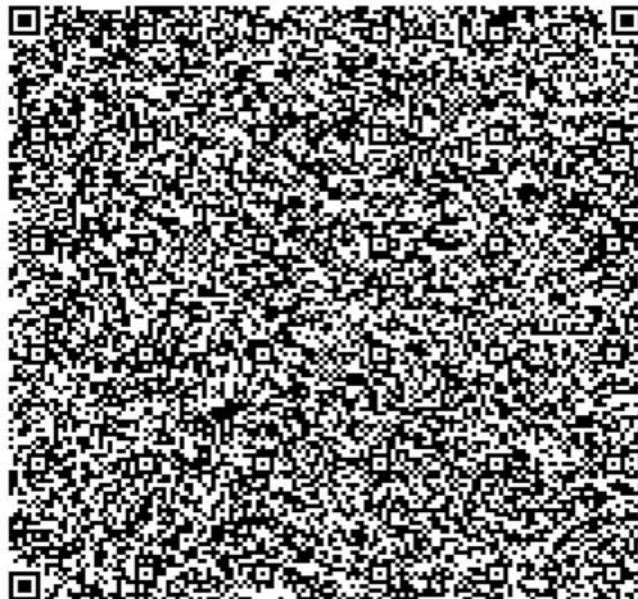
- Using whole genome resequencing, **503 loci** were filtered using minimum allele frequency, Hardy-Weinberg equilibrium, and linkage disequilibrium, etc to construct **368** cassava germplasm fingerprints and then get **368 QR code** of cassava germplasm molecular identity card.

Colored ranges  
■ DDPZ  
■ XYPZ  
■ YS  
■ PX  
■ YJPZ  
■ YJPK



# The excavation of backbone parent

- Cassava germplasm **GPMS1029L** with the highest score and its molecular ID card QR code and fingerprint map



names:172:Fingerprint\_code: G/A T/C G/G C/G A/G  
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A/A C/T G/T A/A T/T G/T G/A T/C A/T T/G A/A T/G C/T  
T/T A/A A/T C/T C/T T/C T/T T/G A/A C/T G/G C/C A/A  
T/T A/A C/T A/G T/T A/C T/G G/T G/C T/C G/T T/T C/C G/A  
-/ - A/A T/T G/A T/A T/T G/C A/C G/A C/T C/C G/G G/G  
G/A A/G C/C C/T C/C G/A T/C A/A G/A G/A G/C A/G  
T/C G/A A/T T/C G/G C/C T/A A/A A/A A/G G/G T/G C/T  
T/T G/G G/G T/T A/G G/T A/G G/G A/A A/A A/G G/T  
G/A G/G G/C G/G C/C G/A G/A C/C G/A C/C G/T C/C  
A/C C/T T/T T/A C/C C/C G/A T/C T/C A/G G/A T/T C/A  
T/A C/C C/T C/G A/G A/A C/C A/G T/T T/C G/G T/T A/G  
G/A T/A T/T T/T C/T A/G C/C A/T G/G T/C T/C A/G A/A  
G/C T/C A/A T/T T/G T/T T/C C/A A/A G/G G/G G/T C/T  
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T/T G/G G/C T/A T/T A/A C/C T/C T/A G/G G/A T/T  
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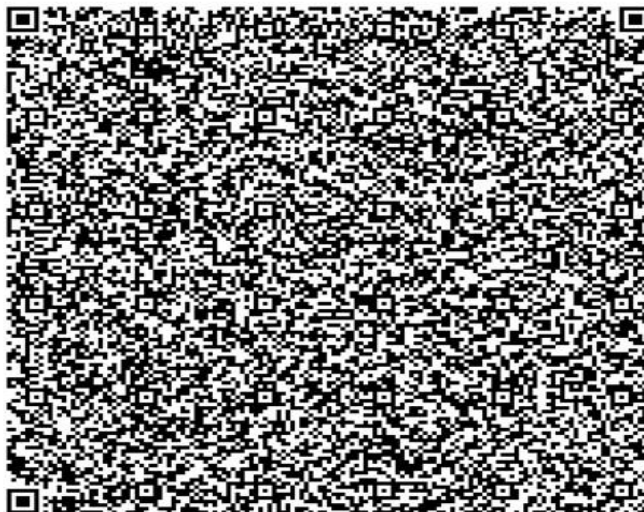
# The excavation of backbone parent

- High  $\beta$ -carotene content sugar cassava South China No. 17: In the tuberous root,  $\beta$ -carotene content is 6.5  $\mu\text{g/g}$  in (SC9 2.6  $\mu\text{g/g}$ ) and sucrose content is 28.66 g/kg (SC9 8.98 g/kg).



Cas36.17

华南17号

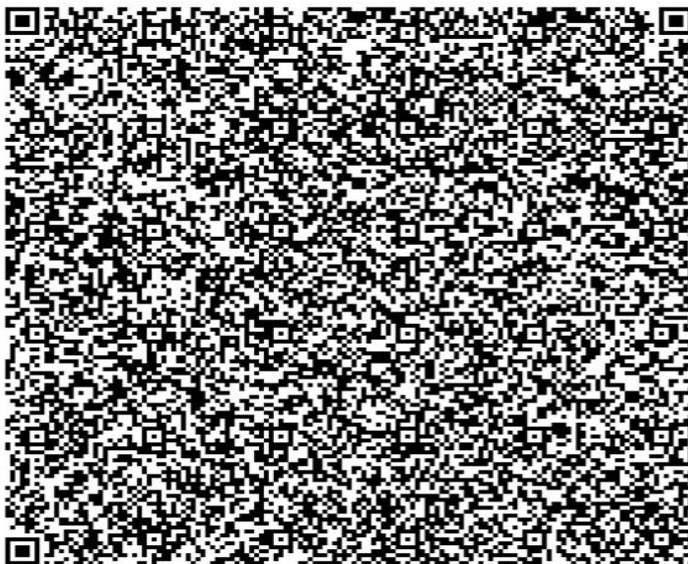


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SC 17 morphological characteristics, QR code and molecular ID card

# The excavation of backbone parent

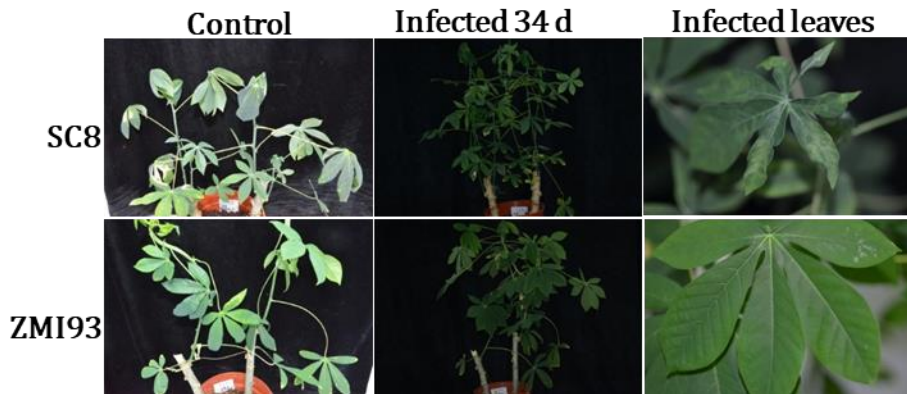
- Leaf high anthocyanin cassava South China No. 20.  $\beta$ -carotene content is rich in root, leaves are purple, and the anthocyanin content in leaves is 413 ng/g, much higher than SC9 (12.39ng/g)



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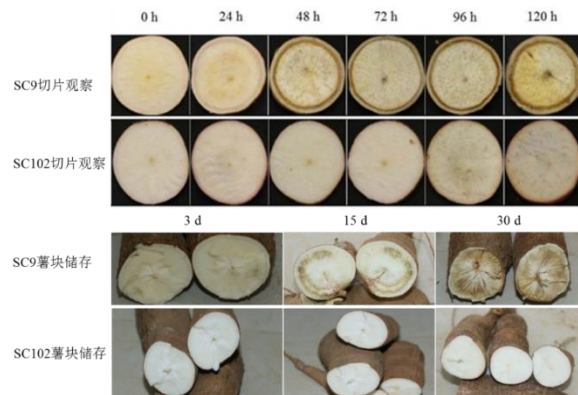
SC 20 morphological characteristics, QR code and molecular ID card

# The excavation of backbone parent



Plants and leaf phenotypes after inoculation with CMD

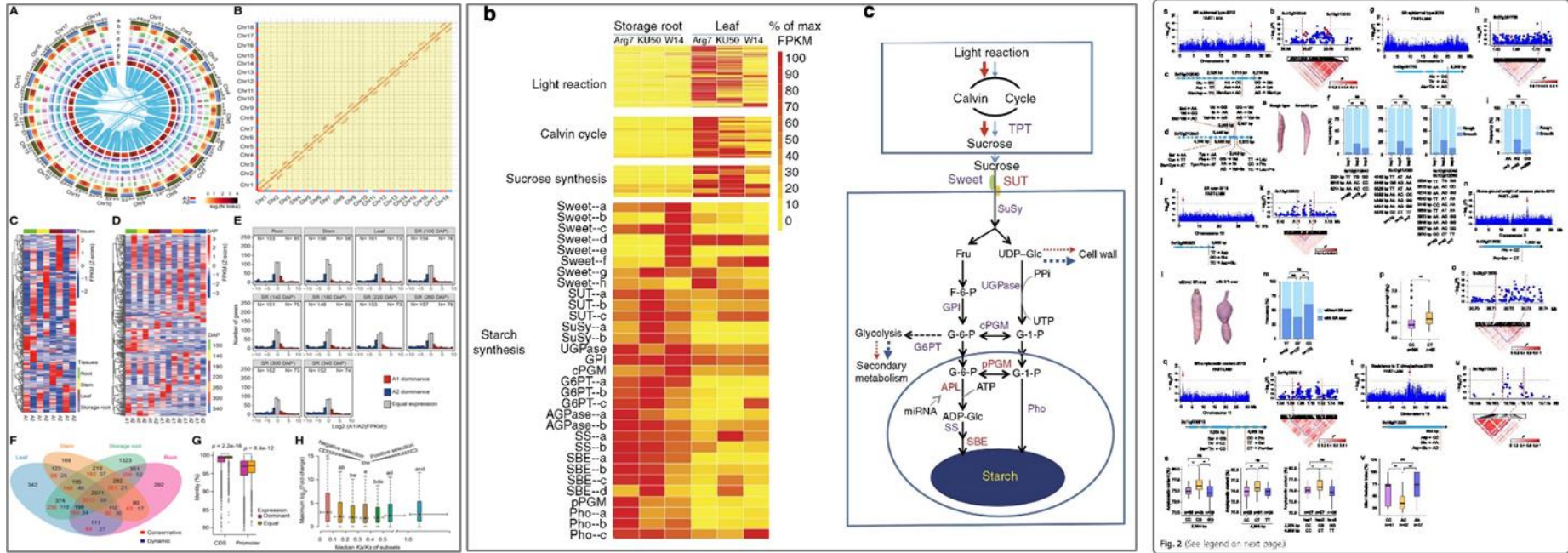
- ZMI 93 variety was planted in Cambodia from 2020 to 2022, and found that the SLCMV disease index was 0.



PPD observation of SC9 and SC102 tuberous roots after treatments

- Cassava SC14 and SC102 can tolerate PPD for more than 15 days, which can reduce the deterioration loss during transportation and processing.

# Cassava genome

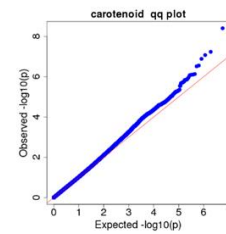
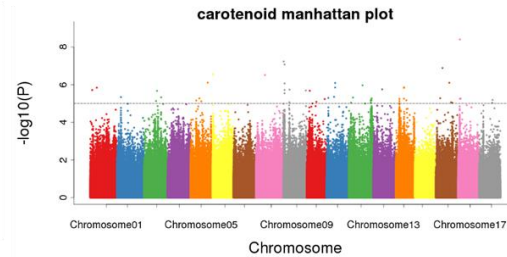
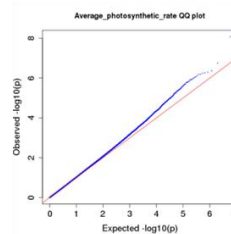
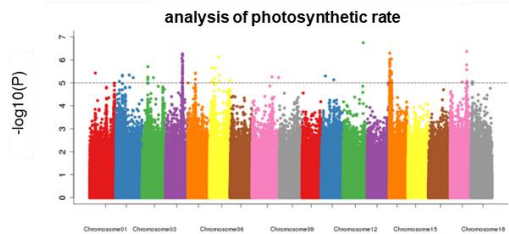
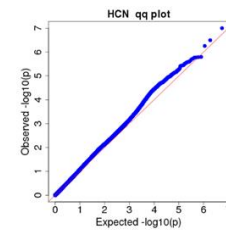
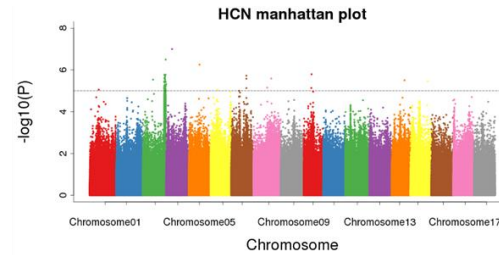
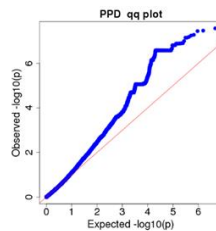
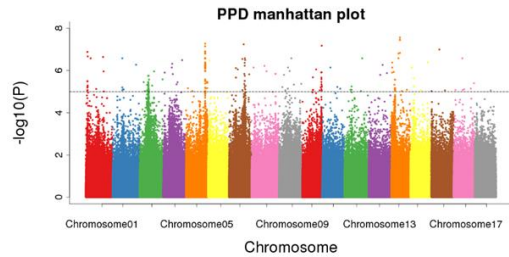


Completed cassava genome sequence; Proposed the models of transportation of cassava photosynthetic products, and carbon flow distribution and efficient starch accumulation; Allele-defined genome reveals biallelic differentiation during cassava evolution.

*Nature Communications (2014), Molecular Plant (2021), Genome Biology (2021)*

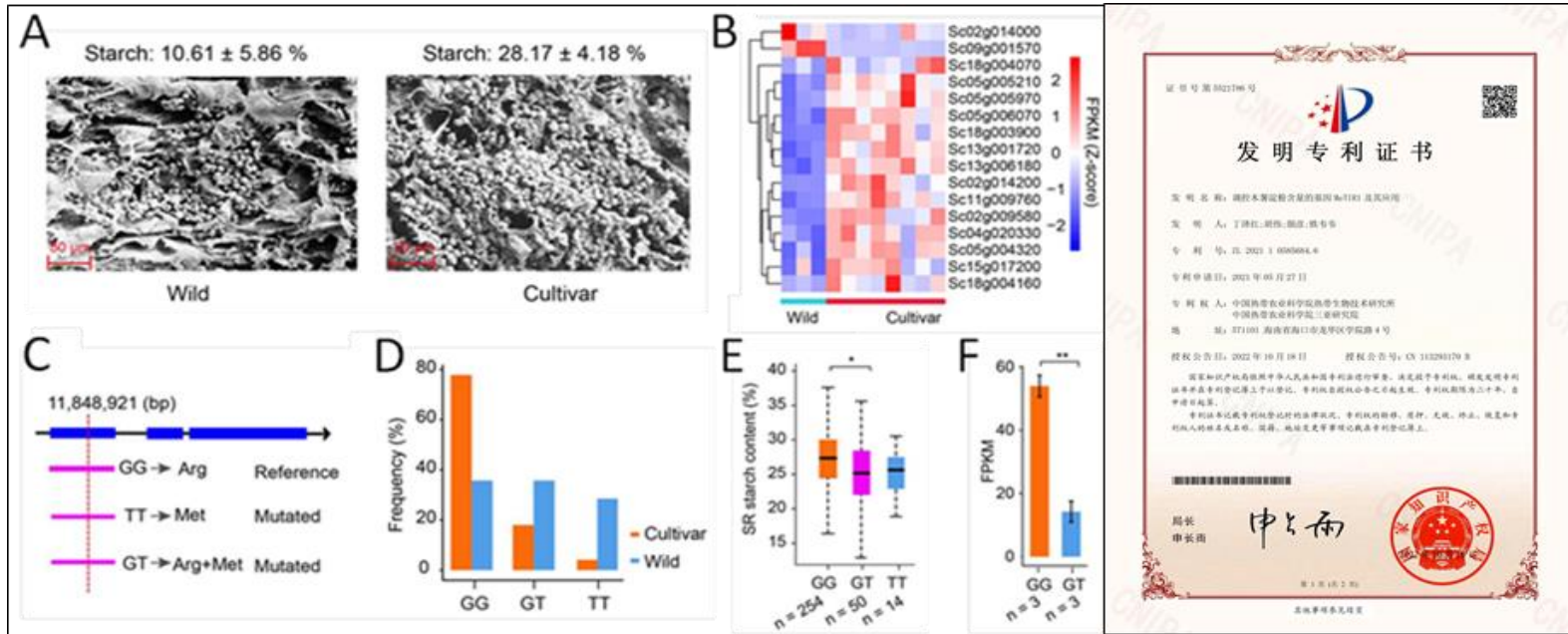
# Cassava gene mining

- Important agronomic traits and GWAS for key gene mining in cassava germplasms. 33 agronomic traits in 337 germplasms, including root starch content, HCN content, PPD and  $\beta$ -carotene content, performed GWAS of 33 agronomic traits based on 1,313,775 high-quality SNPs, and obtained 42 association signals for 21 agronomic traits.



Manhattan and QQ plots of HCN content, PPD, photosynthetic rate and  $\beta$ -carotene content with GWAS

# Cassava gene mining

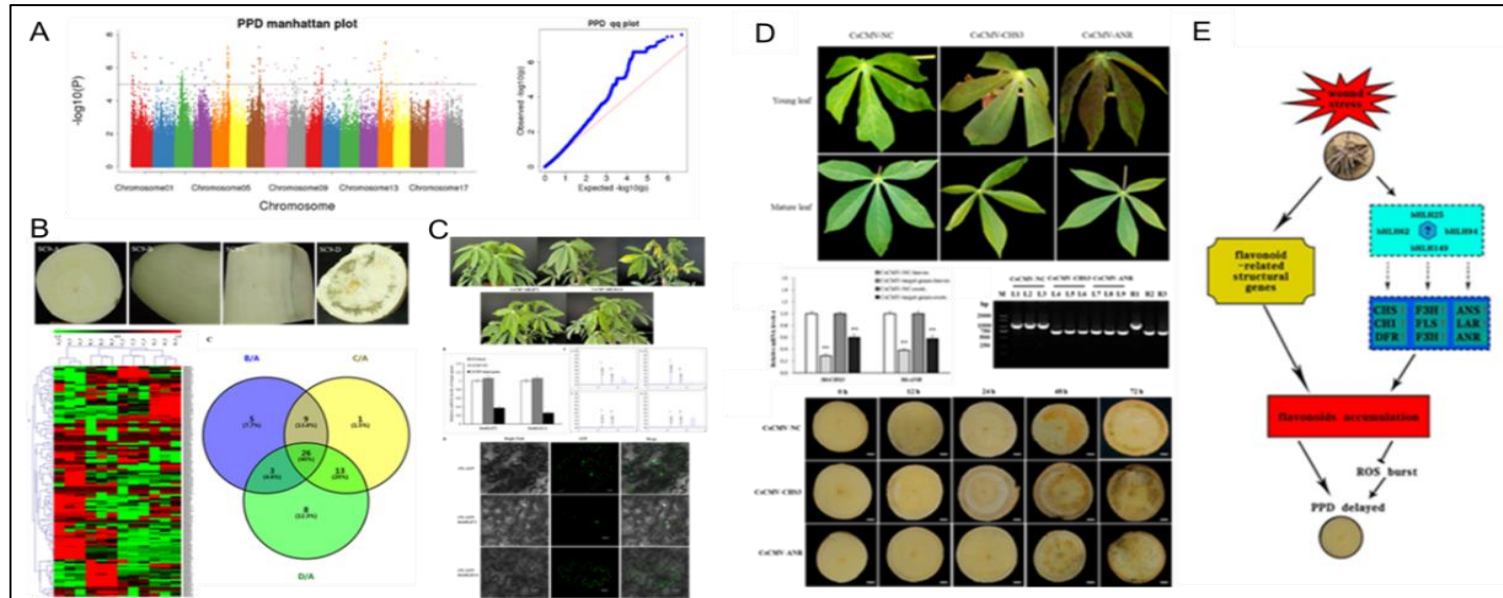


- Selection for homozygous GG allele in *MeTIR1* during domestication contributes to increased starch content. We have verified the positive roles of *MeTIR1* in increasing starch content.

Supported by National Key R&D Program of China: Accurate evaluation and gene exploration of germplasm resources of tropical crops (2019YFD1000500)



# Cassava gene mining



- Systematic Analysis of bHLH transcription factors in cassava uncovers their roles in PPD and cyanogenic glycosides biosynthesis. *Frontiers in plant science* (2022). The candidate genes *MeCHS3* and *MeANR* and metabolites in flavonoid pathways could participate in PPD regulation. *Postharvest biology and technology* (2023)

## 2. Cassava Breeding Program



# The direction of cassava breeding

## Industrial development request

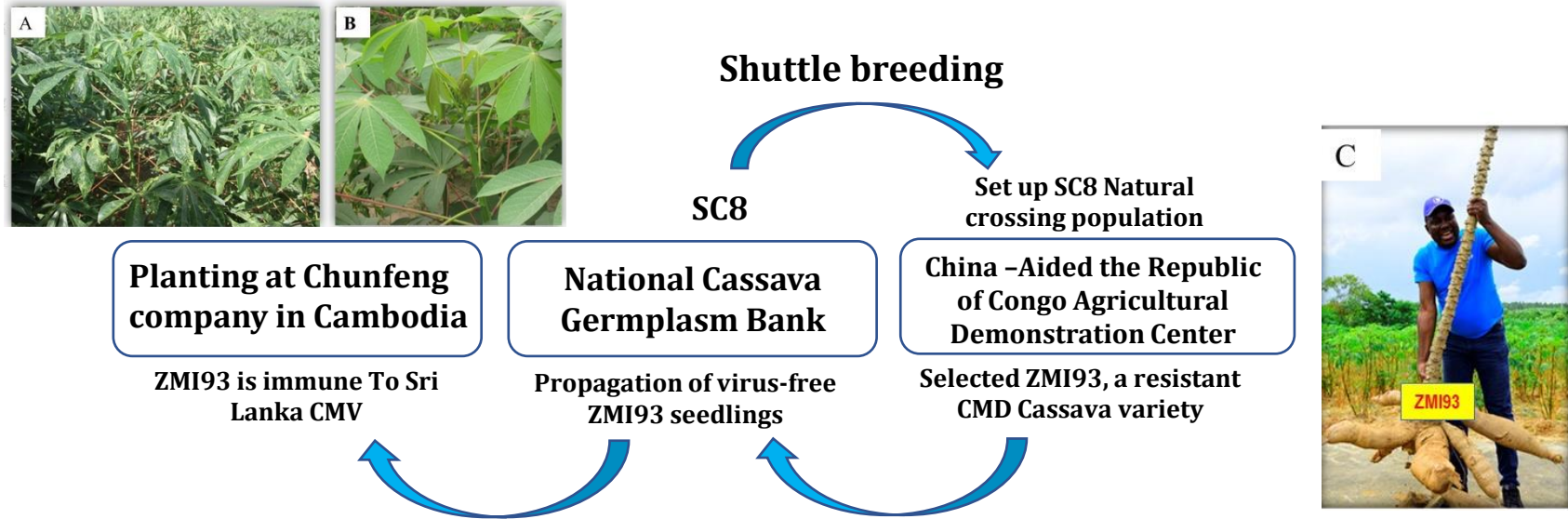
- Starch industrial raw materials: High photosynthesis, high starch accumulation, and high biomass.
- Food and feed: High carotenoids, high protein, tolerance to PPD and low cyanogenic glycoside content.

## Challenges to cassava breeding

- High heterozygosity in the cassava genome (Wild relatives W14: 3.9%, KU50: 3.5%).
- The generation separation is serious, and the cycle of breeding is about 7~9 years.
- PPD leads to the loss of 30 million tons of cassava per year.
- CMD and pests result in reduced root yield and a decrease in leaf quality.



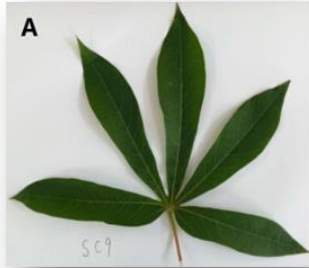
# Shuttle breeding technology



A: Cassava infected SLCMV in Cambodia; B: ZMI93, a resistant SLCMV variety; C: ZMI93 variety

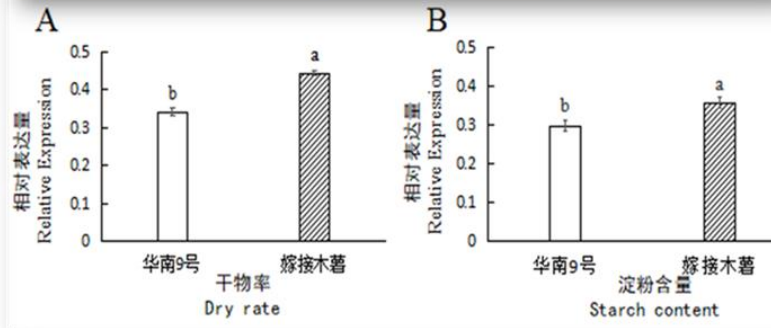
**The root yield of ZMI93 was 42t/ha in Chunfeng company, Cambodia**

# Cassava Grafting



A: SC9, B: Grafting SC9/Rubber cassava

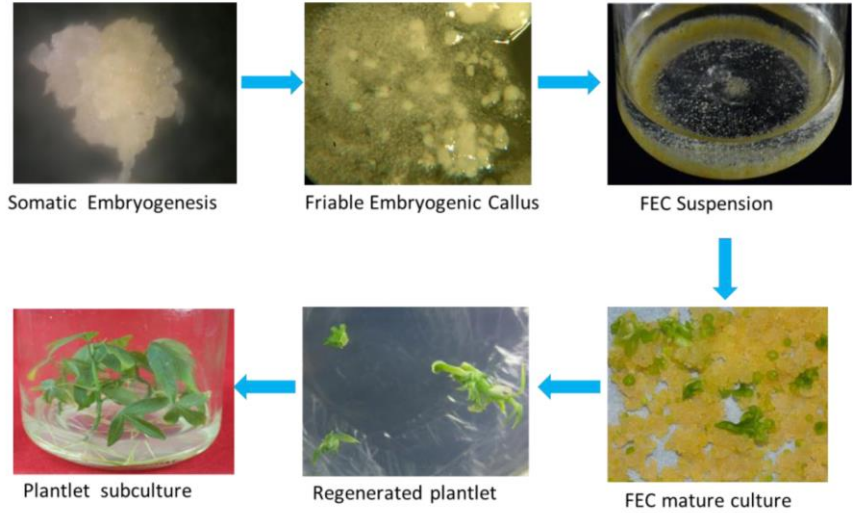
| 木薯品种              | 净光合速率(Pn)               | 气孔导度                 | 胞间 CO <sub>2</sub> 浓度                       | 蒸腾速率(Tr)           | 鲜薯单株产量                     |
|-------------------|-------------------------|----------------------|---|--------------------|----------------------------|
| Cassava varieties | Net Photosynthetic rate | Stomatal conductance | Intercellular CO <sub>2</sub> concentration | Transpiration rate | Fresh root yield per plant |
|                   | (Pn)                    | (Cs)                 | (Ci)  |                    | (Kg)                       |
| SC9               | 9.96±0.22a              | 0.28±0.01a           | 167.60±0.51a                                | 6.24±0.11a         | 4.520a                     |
| 嫁接木薯              | 7.25±0.33b              | 0.20±0.01a           | 166.85±2.13a                                | 4.94±0.24a         | 10.340b                    |



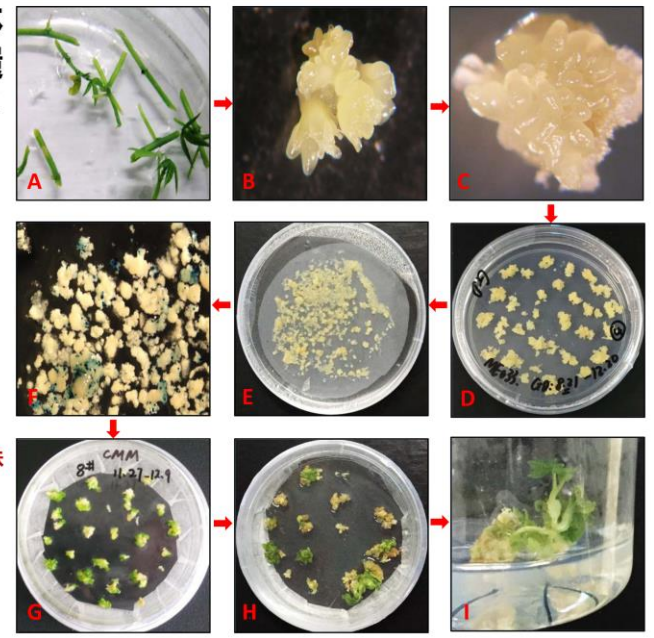
- The starch content of the grafted cassava was 35.72%, significantly higher than the 29.81% of SC9; The dry matter rate of grafted cassava was 44.29%, which was significantly higher than the 33.97% of SC9.

# Cassava regeneration & genetic transformation system

Optimization of the regeneration system and genetic system of cassava



华南8号木薯离体植株再生系统与遗传转化体系的优化

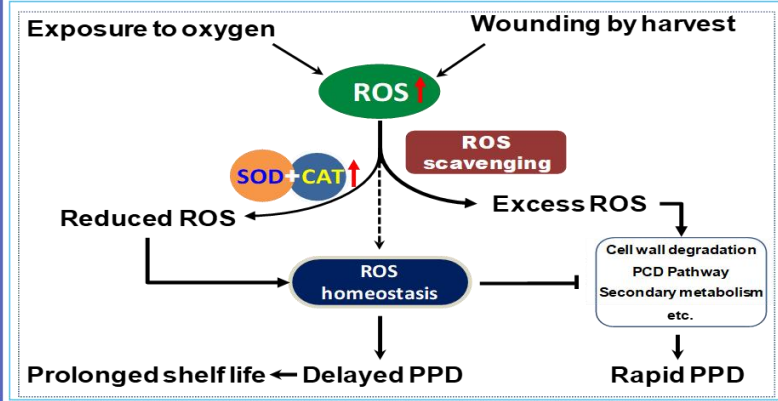
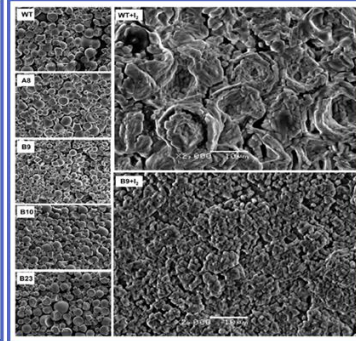
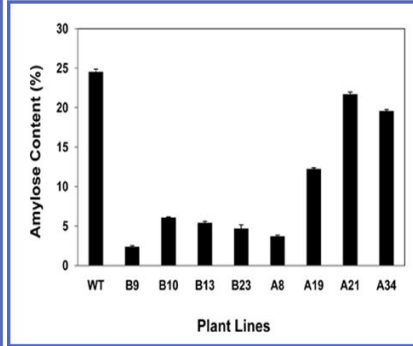
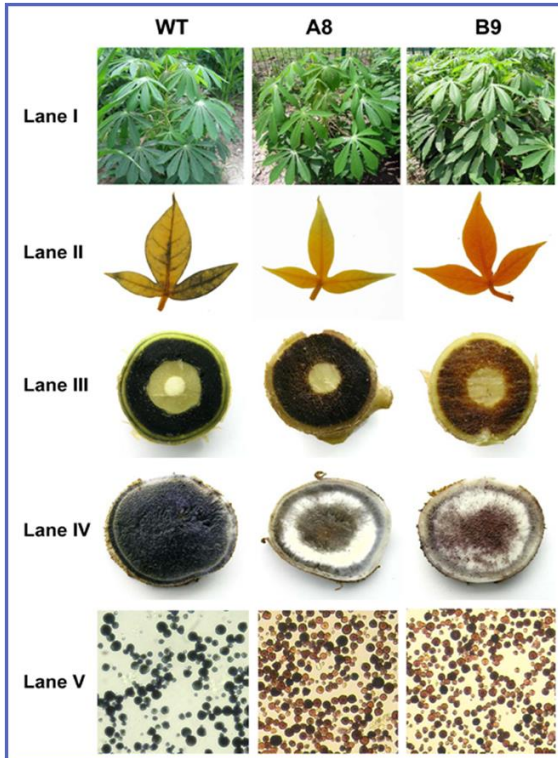


需要更多品种、更高效的植株再生和遗传转化体系

C3、SC8、SC205、SC9、巴西金、NZ199、KU50等

Plant regeneration and genetic transformation of cassava TM60444 plants

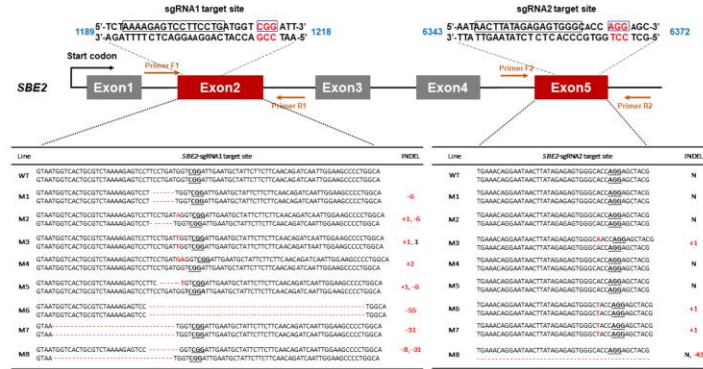
# Cassava transgenic technology



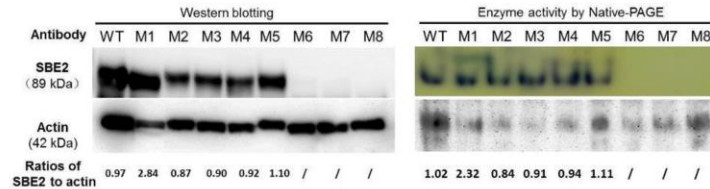
Using transgenic technology and genetic transformation system, a series of cassava germplasms have been created, such as waxy cassava, PPD tolerant cassava and high-yield cassava.

# CRISPR/Cas 9 gene editing

## Dual sgRNA-directed knock-out of the *MeSBE2* gene

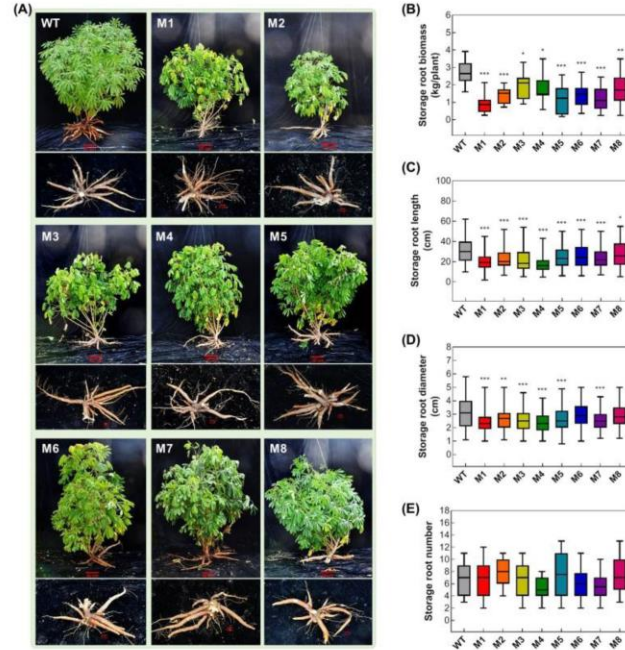


## Absence of MeSBE2 protein and enzymatic activity in homozygous lines



CRISPR/Cas9 基因编辑效率达93%，为培育非转基因高直链木薯提供了技术和材料

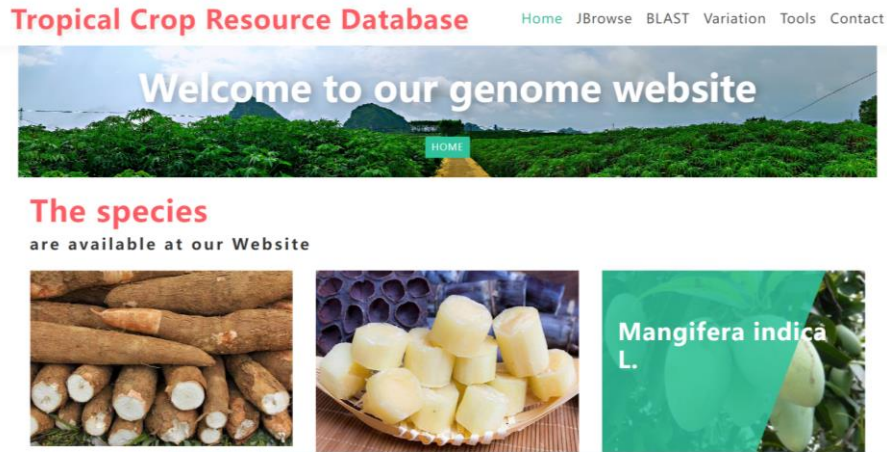
## Mutation of cassava *SBE2* affects plant growth



New varieties were created based on CRISPR/Cas 9 gene editing



# Cassava Database



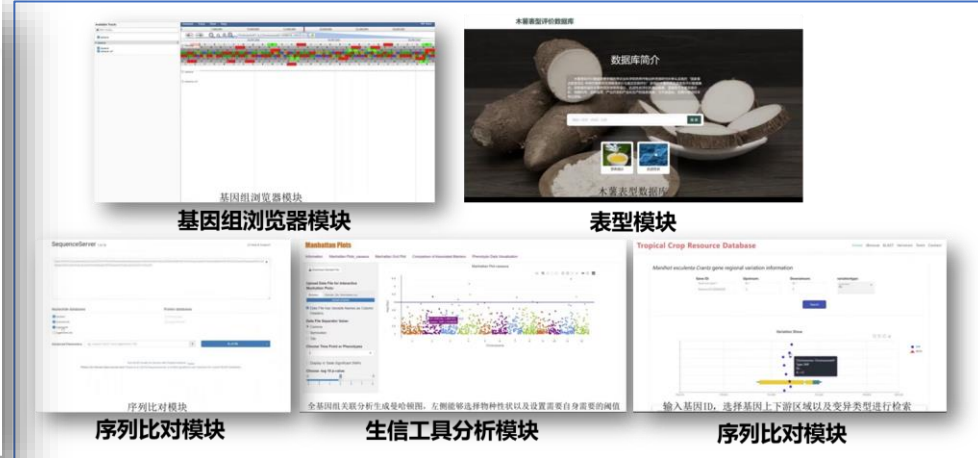
Tropical Crop Resource Database Home JBrowse BLAST Variation Tools Contact

Welcome to our genome website

HOME

The species are available at our Website

Mangifera indica L.



基因组浏览器模块

表型模块

序列比对模块

生信工具分析模块

序列比对模块

- The cassava resource database contains the sequence alignment module, the genome browser module, the variant module, the phenotype module, and the generation letter tool analysis module. Website: <http://www.tropical-resources.org.cn/>.

# Cassava Database Demo

Tropical Crop Resource Database Home Browse BLAST Variation Tools Contact



Welcome to our genome website

**The species**  
are available at our Website



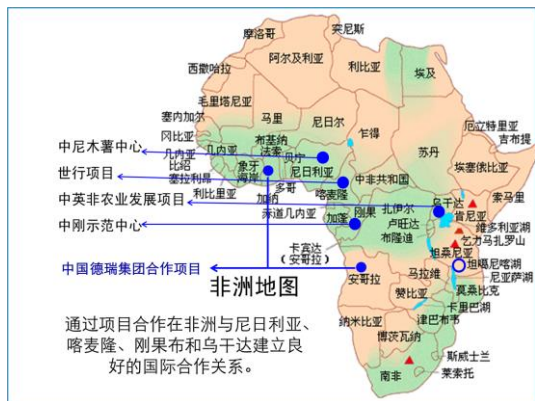
- Whole cassava roots
- Sliced cassava tubers
- Green cassava tubers on a plant
- Sliced cassava tubers with pineapple
- Cassava latex collection
- Cassava field



# 3. Vision for International Cooperative Project



# NSFC-CGIAR and AgriTT Cooperative Project will be a driving force for cassava international cooperation



- CATAS in cooperation with Alliance of Bioersivity-CIAT, IITA, University of Greenwich and Uganda Institute for Innovation implemented AgriTT project to work for food security in Africa countries.
- Six NSFC-CGIAR International Cooperative Projects supported cassava basic research area between CATAS and Alliance of Bioersivity-CIAT.

# Mining key genes from wild cassava and serving breeding



Angie Ayala



Milena Sepúlveda



Figure 2. *Manihot fallax*. **A** Habit **B** Leaves; note the spiral phyllotaxy **C** Inflorescence; note the showy bracts **D** Staminate flower, frontal view **E**. Pistillate flowers in the inflorescence, lateral views; note the free sepals **F** Fruit.



**MONTEZUMA CRUZ**  
 CAMPO GRANDE (MS) – A mandiocaba que os professores Songchi Chen e On seguram (foto) foi colhida depois de seis meses de plantada no sítio do técnico agrícola Flávio Ikeda, no município de Abaetetuba, a 101 quilômetros de Belém por rodovia, na região nordeste do Pará. Esses membros do Tropical Crops Genetic Resources Institute e do Chinese Academy of Tropical visitaram a região em maio, acompanhando o pesquisador da Embrapa Biotecnologia, Luiz Joaquim Castelo Branco Carvalho, a convite do Grupo Y. Yamada.

Conheceram a mandioca açucarada amazônica, genuinamente paraense, e também a degustaram, informa Ikeda. Lembra que a recente colheita de um pé de mandioca pesando 37,3 kg plantado três anos antes e com a raridade de três raízes em perfeito estado rendeu frutos. "Resolvi aproveitar todas as manivas que ficaram após a degustação dos cientistas chineses, para multiplicação, com tratamento vip a partir de agora", comenta o técnico.

A cada ano cresce o interesse chinês pelas experiências brasileiras no setor. A China tem procurado a Embrapa para estabelecer parcerias. Inegavelmente tem levado para suas academias os melhores resultados obtidos por pesquisadores. Em 2009, por exemplo, o professor Bin Liu, PhD assistente da direção da Chineses Academy of Sciences e do Beijing Genomics Institute, acompanhou a transformação da mandioca em álcool em Puanlinda (DF). Em troca do aprendizado acadêmicos chineses

Support cooperative projects such as “Mining key genes from wild cassava and serving breeding”

# Global cassava breeding program



# 40-year Cooperation between CATAS and Alliance



**Alliance won the International Science and Technology Cooperation Award** on 2021. This award was inseparable from the NSFC-CGIAR Cooperative Project grants; Dr Hernán Ceballos won 2022 Chinese Government Friendship Award.



**Thanks!**

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