World Forum on Global Stratege for Plant Conservation, 29 Oct 2019

种质资源库建设与受威胁植物的综合保护

Plant germplasm banking and the integrative conservation of threatened plant species

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Outline

- Threats to plant diversity
- Strategies for Plant Conservation
- Advantages of seed banking in plant diversity conservation
- The "Noya Ark" of Chinese wild plants
- Discussion and recommendations

Main Threats to Wild Plant Species

Over-harvesting

Climate Change

Invasive Species

Habitat Loss

“保护”

狭义：保存或维持部分或全部的生物多样性组成部分

“Preservation or maintenance of some or all of the components of biodiversity”

广义：对生物多样性的组成部分进行可持续的利用、恢复和重建

“Sustainable use of the components of biodiversity, or their recovery or restoration, or both”

Global Biodiversity Assessment, 1995

保护植物多样性的策略
Strategies for Plant Conservation

- **就地保护（*in situ*）**

-- 自然保护区, 国家公园
Natural reserves, national parks

-- 农林混作
Agroforestry

-- …

- **迁地保护（*ex situ*）**

-- 种子库 Seed banks

-- 植物园 Botanical gardens

-- 组织培养Tissue Culture

-- DNA库 DNA banks

-- 花粉库 Pollen banks

-- …

Strategies for Plant Conservation:

Natural Reserves\ national

Strategies for Plant Conservation:

Botanic Gardens

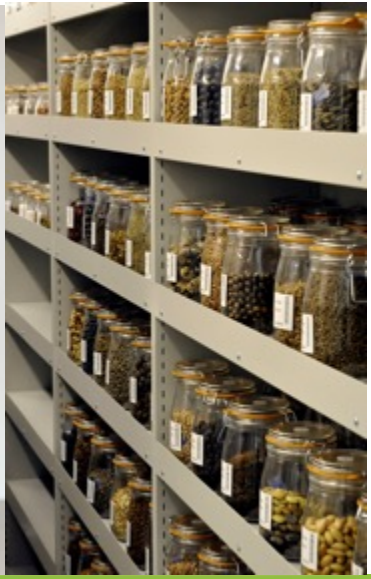
中国195个植物园，1285个植物活体组织收集中心，迁地

Strategies for Plant Conservation:

Tissue Culture

Strategies for Plant Conservation:

Seed Banks



种子库
Conventional seed bank conditions

- 相对湿度15％± 3%　—— 干燥

  Drying at 15％ ± 3% relative humidity（RH）

- 在-18℃ ± 3℃的冷库—— 储藏

  Stored at -18°C ± 3°C

(FAO, 2014)

Seed storage behavior

- **正常型种子（Orthodox seeds）/ 耐脱水种子:** i.e., storable by conventional seed banking (Roberts, 1973)
- **顽拗型种子（Recalcitrant seeds）/ 脱水敏感种子:** i.e., desiccation-sensitive and not storable by conventional methods;(Roberts, 1973)
- **中间型种子（Intermediate seeds）:**Between the Orthodox seeds and Recalcitrant seeds （Ellis et al, 1990）

种子可以存活多久?
How long can we keep seeds alive?

物种 Species	种子寿命（种子活力下降一个概率值的时间，比如，从 97.5%下降至 84%，或从 84%下降至 50%） Time for viability to fall 1 probit (e.g., from 97.5% to 84%, or from 84% to 50%)	
	昆明室外 Kunming outdoors（年均温 15℃，年均 RH 73%）	种子库 Conventional seed bank conditions（15%RH, -20℃）
玉米 Maize（*Zea mays*）	145 天 d	460 年 y
小麦 Wheat（*Triticum aestivum*）	99 天 d	786 年 y
水稻 Rice（*Oryza sativa*）	241 天 d	1,139 年 y
莴苣 Lettuce（*Lactuca sativa*）	71 天 d	372 年 y
大豆 Soya（*Glycine max*）	193 天 d	214 年 y
棉花 Cotton（*Gossypium hirsutum*）	1766 天 d（5 年 y）	17,076 年 y

Advantages of seed banking in plant diversity conservation

- 降低就地保护中潜在的风险：自然灾害、人类活动

Reduce the risk in in situ conservation: natural disasters, human activities

- c.80％植物的种子可干燥后低温保存：正常型种子

About 80% of plant can be covered:

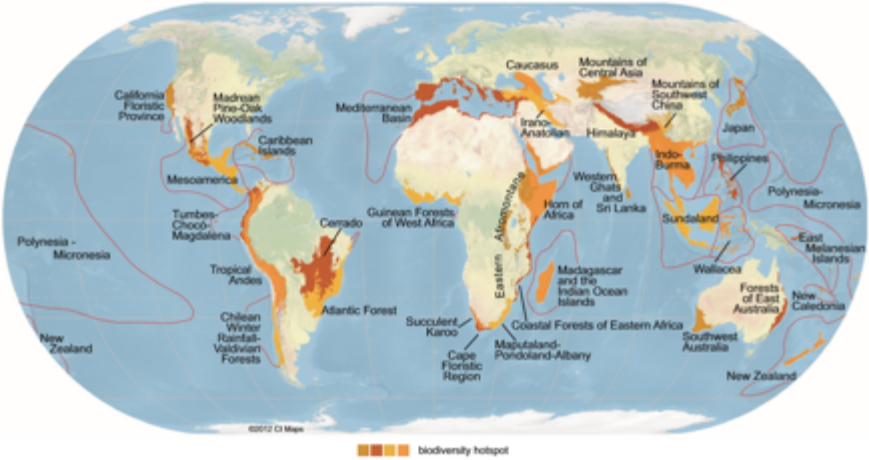
A National Effort for Biodiversity Conservation –

the Germplasm Bank of Wild Species (GBOWS)

中国西南野生生物种质资源库

Mongolia
Kyrgyzstan
Beijing
North Korea
Sea of Japan
South Korea
Japan
Yellow Sea
China
East China Sea
Nepal
Bhutan
Kunming
Bangladesh
India
Myanmar (Burma)
Laos
Philippine Sea
Thailand
South China Sea
Luzon
Vietnam

Map of 36 biodiversity hotspots



4 hotsopts in China: Indo-Burma ; Himalaya ; Mountains of Southwest China ; Mountains of Central Asia

中国科学院昆明植物研究所
Kunming Institute of Botany, Chinese Academy of Sciences

中国西南野生生物种质资源库
The Germplasm Bank of Wild Species（GBOWS）

- The project was approved by the national authorities in 2004 for:
  - Preservation and research
  - International collaboration
  - Public education
  - Restoration programme

- Construction of the Building completed in 2007
- Seed collecting started in Nov 2005
- Project began to run officially since Nov 2009

Target of seed bank at GBOWS

What Do We Do

1) Seed Collection

2) Seed Processing/Banking

3) Germplasm Utilization

4) Training

5) Research

确保采集野生植物种质资源的完整性

complete seed colleting items including:

Seeds

Voucher herbarium specimen

Collecting data

Dried leaves

Photos

Steps of seed banking: Collecting

Steps of seed banking: Drying

For every 1% reduction in seed moisture content, seed life span doubles.

Dry room（Temperature=15˚C，Relative Humidity=15 %）

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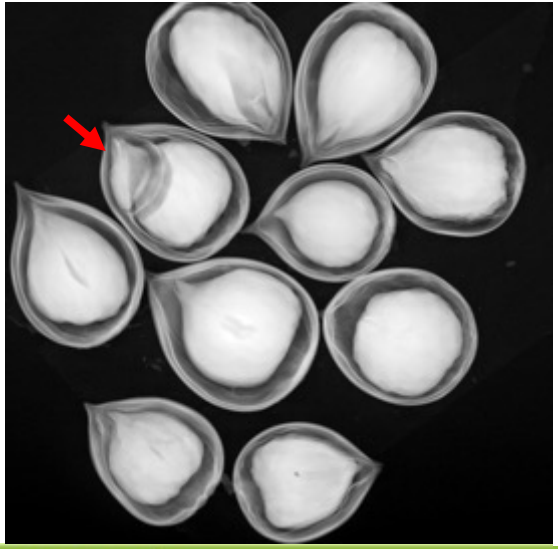
Kunming Institute of Botany

Steps of seed banking: Cleaning

Steps of seed banking: X-ray analysis

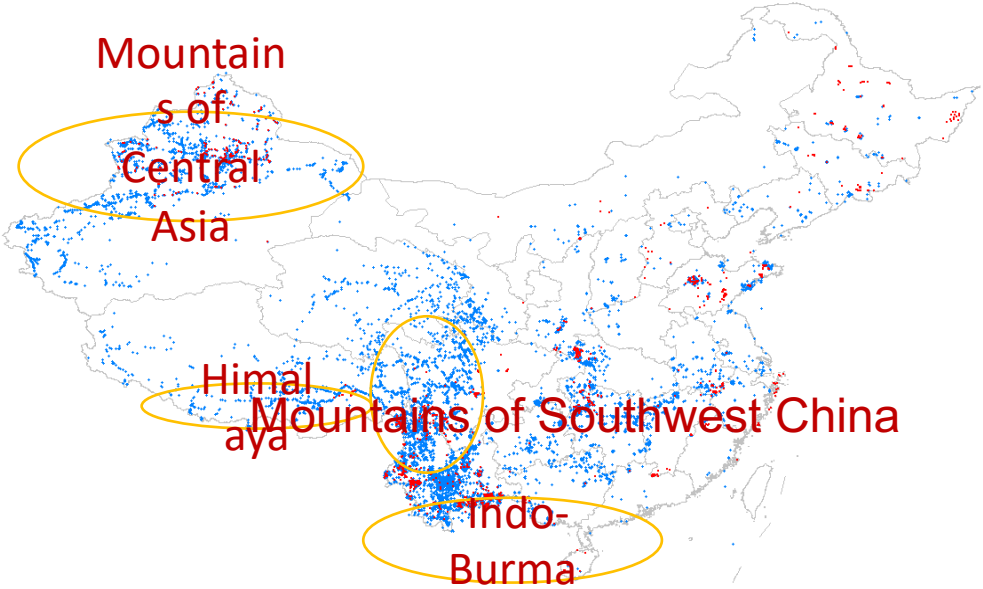


Steps of seed banking: seed counting

Steps of seed banking: Storage at -18ºC

Steps of seed banking:
Viability check /Germination testing

- **Germination tests of 57,666 accessions, 8,321 species ;**



34% of China's flowering plants saved

10,048 species (229 families, 2,004 genera), 80105 collections by Dec. 2018

Distribution：

Qiaojia, Yunnan

（29 individuals in the wild)

Protected status：

Protection List, II

Threatened factors：

Habitat Loss、Natural renewal difficulty

川东灯台报春 ***Primula mallophylla***

Distribution: Chengkou, Sichuan

Protected status：EX (CBPL, 2012)

水角 ***Hydrocera triflora***

Distribution:

Hainan

Protected status：

RE（CPRL, 2017）

水菜花 ***Ottelia cordata***

Distribution:

Hainan

Protected status：

RE（CPRL, 2017）

金铁锁

Psammosilene tunicoides

Distribution：

Guizhou、Sichuan、Xizang、Yunan

Protected status：

Protection List II

Threatened Plant Species at the GBOWS

Endemic: 4,035 native to China, 40.30% of all conserved species.

Threatened: 669 species of China's CR

中国科学院昆明植物研究所

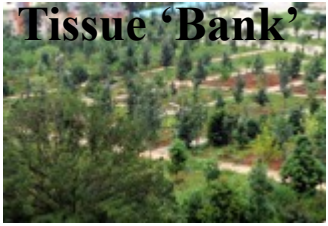
中国西南野生生物种质资源库
The Germplasm Bank of Wild Species（GBOWS）

▲ Seed Bank

▲Plant *in vitro* Tissue 'Bank'

▲ Animal Cell Lines (KIZ)

▲ DNA



▲ living plant nursery

▲ Microbial Culture (YN)

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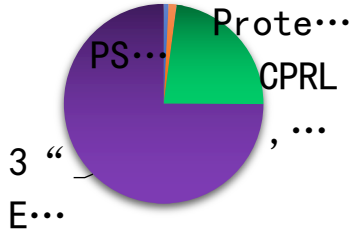
Which plant species might need in vitro tissue banking and cryopreservation?

Species that cannot be preserved long-term by seed banking:

- Desiccation tolerant but short-lived seeds .
- Recalcitrant (desiccation sensitive) seeds
- Species with strongly dormant seeds.
- Endangered species with few seeds, or the seeds are inaccessible.

Plant in vitro tissue ‘Bank’ at the GBOWS

- **2003 species/23500 collections tissue culture mateiral banked at the GBOWS;**



Plant cryopreservation facilities at the GBOWS

6 Air liquid cryotanks (-196℃， 500L， 16800 cryotubes/tank).

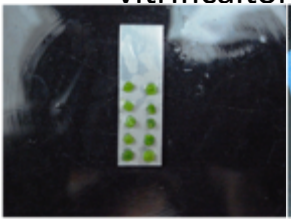
Plant cryopreservation research at the GBWOS

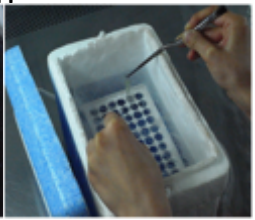
Species and explant type

1. Orchidaceae: seeds, protocorm-like bodies, shoot tips.
2. Gesneriaceae: shoot tips, leaf-discs.
3. Mangoliaceae: embryogenic cells .
4. Rutaceae: shoot tips.

Methods

- Vitrification
- Droplet-vitrification
- Encapsulation-vitrificaiton

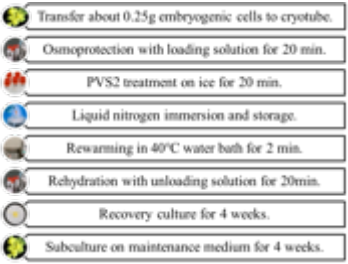




Development of a standard cryopreservation protocol for embryogenic cell lines of 13 Magnoliaceae species (regrowth 80-100%)

Somatic embryogenesis *of Michelia yunnanensis.*

The cryopreservation protocol of embryogenic cells of by vitrification



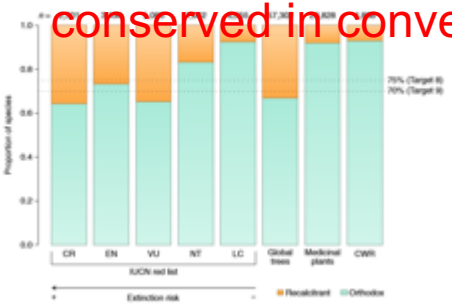
Somatic embryogenesis of cryopreserved embryogenic cells of *Magnolia officinalis*.

Plantlet produced by somatic embryogenesis.

Discussion and Recommendations

Red List species are likely to produce recalcitrant seeds and thus cannot be conserved in conventional seed banks?

FIG 1. the likely proportions of seed storage behaviours across different plant lists



- the IUCN red list
- the GlobalTreeSearch, a database of all known tree species
- the Royal Botanic Gardens Kew's 'Medicinal plants' database
- the Crop Trust's inventory of crop wild relatives (CWR)

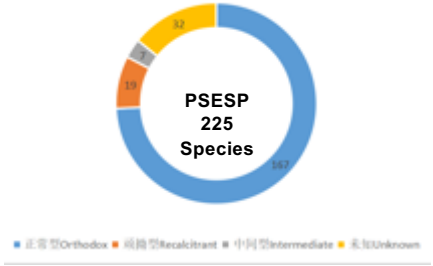
Wyse et al., 2018

- 36% of species in the 'Critically endengered';
- 27% in the 'endangered';
- 35% of species in the 'vulnerable';
- 33% of all tree species

- tropical moist forests
- mangrove habitats
- the highest

Discussion and Recommendations

Evaluation of seed Storage behaviors of Plant Species with extremely small populations in Southwest China (Yang X. Y. ,2018, Unpublished)



- **225 seed plants total;**
- **167 Orthodox（O），74.4%**

依据：http://data.kew.org/sid/sidsea rch.html

RESEARCH ARTICLE

Botany

Seed freeze sensitivity and ex situ longevity of 295 species in the native Hawaiian flora

Marian M. Chau, Timothy Chambers, Lauren Weisenberger, Matthew Keir, Timothy J. Krawczyk, Dustin Walker, Ray Kam, and Alvin Y. Nishimoto

Method: Using 20+ years of real-time seed storage viability data, tested freeze sensitivity for 197 species and calculated RCIs for 295 species. Using paired tests of accessions stored >2 yr at 5°C and −18°C

Conclusions:it is probable that species with freeze-sensitive seeds account for more than 25% of rare species in Hawai, but with the knowledge that many of them can be stored medium to long term at 5°C.

Chau et al, 2019

Discussion and Recommendations

- Seed storage is the most efficient way to preserve the threatened plant ;
- The “conventional seed banking” may not be the only form of low-cost seed banking that can provide for relatively long-term storage.;
- other potential storage methods, such as 5°C, −80°C or cryopreservation were recommended;
- Strengthening the research for threatend plant's seed storage behavior ;
- Intensifying research efforts into cryopreservation as a conservation tool ;

THANK YOU !

http://www.genobank.org/ ; http://seed.iflora.cn/

Seed distribution

- ✓ 74 scientific organizations
- ✓ 11,729 samples
- ✓ 444,165 good seeds

- 中国农科院作物科学研究所
- 中国中医科学院中药研究所
- 中国科学院上海生命科学研究院
- 中国科学院昆明动物研究所
- 贵州省山地资源研究所
- 云南省农业科学院高山经济植物研究所
- 云南省文物考古研究所
- 台州市农业科学研究院
- 浙江大学
- 兰州大学
- 浙江农林大学
- 江苏师范大学
- 南京农业大学
- 广东食品药品职业学院
- 国家地质实验测试中心
- 北京蓝标一成科技有限公司

Websites (Chinese version) http://www.genobank.org/
http://seed.iflora.cn/