



Special article

A national strategy for sustainable utilization of TCM resources: Establishment of the state bank of Chinese drug germplasm resources

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Abstract: Aiming to achieve long-term preservation of TCM germplasm resources, a National TCM Germplasm Bank, the State Bank of Chinese Drug Germplasm Resources was established in 2017. This article introduces the background and the construction of the core warehouse, the tissue and DNA repository, the botanic garden, as well as the research on collection and preservation of Chinese medicine germplasm resources, seed storage and germination characteristics, seed longevity and safe storage period of the institution has been discussed which is the significant works for the conservation of biological diversity, genetic diversity and ecological diversity. We look forward to exchanges and cooperation with more institutions to contribute to the sustainable utilization of TCM resources.

Keywords: traditional Chinese medicine; germplasm resources; seed bank; long-term preservation; sustainable utilization

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Introduction

Traditional Chinese medicine (TCM) are widely used in China for thousands of years.^[1,2] Chinese medicinal resources are unique health resources, the most original scientific and technological resources, and cultural and ecological resources with great economic potential.^[3,4] Chinese medicine has been recorded the sources and applications with accumulated abundant clinical experience over a long period, that the earliest historical literature shen nong ben cao jing at Eastern Han Dynasty (25-220 C.E.).^[5,6] Currently, more than one-

third of clinic drugs are from Chinese medicinal plants in China, such as Compound Danshen Dripping Pills (CDDP). Even in face of the battle against the novel coronavirus pneumonia (coronavirus disease 2019, COVID-19), Chinese medicines also show potential medicinal value against the virus.^[7,8]

In terms of both economic value and volume of medicinal plants trade, the annual consumption of Chinese herbal medicine exceeds 400,000 tons in China^[9] which is also widely acknowledged to be the world's biggest exporter and importer

of medicinal plants. There are more than 11,000 kinds of Chinese herbal medicine which accounts for one-third of flowering plants in China also an important part of biodiversity in China even the the world.^[10] However, due to overexploitation, the sustainable development of medicinal resources has become an urgent problem. Many plant species were endangered which has been recorded at the Convention on International Trade in Endangered Species of wild fauna and flora (CITES),^[11] such as *Glycyrrhiza uralensis* (Gancao), *Panax ginseng* (Renshen), *Saussurea costus* (Yunmuxiang), *Cistanche deserticola* (Roucongrong), etc.^[12] On the other hand, the cultivation of Daodi medicinal materials for thousands of years also caused problems that cannot be ignored, such as breed confusion (caused by unwise introduction and lack of selective breeding), germplasm degradation (the reduced plant resistance of stress, disease, and insect pests, etc. or the unstable quality of drug) and succession cropping obstacle (cannot grow the plant in succession on the same piece of land). Both the problem of wild and cultivation TCM resources cause the quality decline of medicinal materials or counterfeit drug use rising which poses a risk of clinical application.^[13,14] That is the bottleneck we meet for sustainable utilization of TCM.

The preservation and research of TCM germplasm resources is the key step to solving this problem. With the preservation of germplasm resources became the common strategy around the world in modern time, both *in-situ* or *ex-situ* for maximum conservation of diversity, including medicinal species diversity, ecological type diversity, chemical type diversity, etc.^[15] *In-situ* conservation such as protected areas, nature reserves, and conservation corridors, etc. is considered to be significant ways to save. *Ex-situ* conservation such as botanic gardens, seed banks, and tissue culture collections

is widely used to protect wild plant species from extinction.^[16] *Ex-situ* conservation act as a backup within national and international facilities for thousands of species for global agriculture and plant diversity.^[17,18] The methods depend on the species and the desired storage time, generally that clonal crops in vitro library, and seed-bearing species in botanic gardens or seed banks. Because seeds contain relatively complete genetic diversity in species, are easy to collect and stored long-time in small space, in practice one approach, the seed bank is used for the maintenance of most *ex-situ* which is more efficient and economic estimated to cost as little as 1% of *in-situ* conservation.

The establish of the specialized national seed bank for TCM

In recent years, the CPC Central Committee attaches great importance to the development of TCM. In response to the State Council policies, two new seed banks were built by The Ministry of Health and the State Administration of TCM. They were designed to preserve the seeds collected from the Fourth National Resource Census,^[19] and further to support and promote the TCM industry for the national Medical and Health System. “The State Bank of Chinese Drug Germplasm Resources” located in the rich diversity place Sichuan is the largest one.^[20] (Figure 1)

According to internationally recognized standards of long-term storage of dry seeds, the germplasm repository was running in 2017 after five years of construction. It has been devoted to preserving Orthodox Seeds (increased longevity with increased drying at threshold water contents between 0.03 and 0.07 g H₂O⁻¹ DW) and Intermediate Seeds (desiccation tolerance between orthodox and recalcitrant seed or short lifespans no matter how they dried or cooled).^[21] Except for Recalcitrant seeds (cannot extend storage life by drying and the threshold



Figure 1. The pictures of the State Bank of Chinese Drug Germplasm Resources

water contents between 0.20 and 0.30 g H₂O⁻¹ DW) which conserved in the bank in tropical south Hainan using cryopreservation.^[22-24]

The core warehouse

The core warehouse is the key facility to realize long-term conservation of seeds which is located on the underground basement, up to 500 m². It has 3 long-term cold storage rooms (-18±2°C, Relative Humidity (RH)<45%),^[25] 2 medium-term cold storage rooms (-4°C±2°C, RH<45%), 1 short-term cold storage (4°C±2°C, RH<40%) room and 2 drying rooms (15°C±2°C, RH<50%). As different from -18°C from common banking conditions, the kinds of storage room can be used not only for seeds which will be researched and utilized in short-term but also for the preservation of the intermediate seeds which are not resistant to sub-zero temperature and dehydration. By November 2021, we have stored 13,263 seeds from 2,597 species of Chinese herbal medicines.

Tissue and DNA repository

DNA repository includes 8 ultra-low temperature freezers (-80°C) for DNA samples of different germplasm. Tissue library used

for propagation of tissue culture plants, for which long-term vegetative medicinal plants are polyploid cannot produce seeds, such as *Pogostemon cablin* (Guanghuoxiang),^[26] or have short-lived seeds, such as *Dendrobium officinale* (Tiepishihu), *Bletilla striata* (Baiji) from Orchid.^[27]

Botanic Garden

The botanic garden of Chinese medicinal plants which are located on the campus which is a part of the *ex-situ* conservation system. There are 105 families, 200 genera, and more than 1,000 species of medicinal plants grown in the garden. As a part of the National Medicinal Botanical Garden system, 109 species of the phenology of migratory plants are being observed and recorded. The germplasm nursery has also been established for endangered medicinal plants and cultivate excellent varieties.^[28-30]

Greenhouse

A greenhouse was constructed to precisely control the environmental cues, such as light intensity and quality, temperature, humidity, and CO₂ concentration during the plant growth period.^[31] In where we provided a platform to explore the underlying mechanisms of quality formation of traditional Chinese medicine regulated by environmental cues.^[32] In addition, the speed of breeding short-day traditional Chinese medicinal plants, such as *Carthamus tinctorius* L. from planting to harvest 3 months can be easy to implement.

The national research platform for TCM resources

In addition to preventing mildew and deterioration, seed storage requirements are higher than grain storage. The purpose of seed storage is to prolong the life of seeds, maintain vigor, and preserve the genetic integrity of the original germplasm. Based on the State Key

Laboratory of Southwest Chinese Medicine Resources and the National Top-class Discipline of Chinese medicine, an important national platform has been built for germplasm preservation and innovation.

As the first order of business, we are focused on the extensive collection of TCM germplasm resources. In addition to wild species of TCM, semi-wild species, proximal species, cultivated species, and new varieties created artificially in production should also include. This collection and preservation of the medicinal value of the various species and the potential genes for breeding.^[33]

Safe and efficient storage is the key to the seed bank, which is directly related to seed germination, seedling growth, yield, and quality after storage. There is not a lot of referential biological data of Chinese medicinal plant seeds. For the endemic species or the cultivated varieties, there are rarely reported on the stored seeds. Domestic researches mostly served agricultural production, lacking systematic researches on the physiology of dormancy type,^[34] germination characteristics,^[35, 36] storage behavior,^[37, 38] longevity,^[39] vigor testing^[40] and renewal for banking seeds.

Especially the storage characteristics, which are strategies to adapt to geographical and ecological environments during plant evolution, and cannot be classified according to plant taxonomy without a clear phylogenetic model.^[41] Generally recognizing, explore and defining the seed storage behavior can help us preserve correct seeds more efficiently.

Although the improvement of cryogenic storage technology and equipment, seeds can be stored in cold storage for a longer time, but still cannot completely stop the seed deterioration process, seed vigor will eventually decline and lead to genetic variation.^[42, 43] Therefore, the safe storage period is shorter than the seed longevity. At present, comparatively little

is known about how the influences of fundamental evolutionary forces of genetic drift, mutation, selection, recombination, and gene flow during the storage.^[44] We need to measure the germination rate at regular intervals (10 years or 5 years). FAO/IPGRI recommended that the germination rate of renewal standard should be 85% or 15% reduction for regeneration, and the minimum critical value should be 65% germination rate.^[45] For TCM seeds, some kinds have a low initial germination rate, the *Salvia miltiorrhiza* for example the germination rate only about 50% which is quite different from the crops. The physiological and molecular mechanisms of seed deterioration during storage are helpful to establish renewal standards by further research.^[46]

The significance of TCM banking seed

The practice of seed preservation began with the cultivation of crops, so the earliest seed banks were established for crops.^[47] Modernized seed bank was built a century and a half ago as a specialized and long-term seed storage institution, according to the general rule of Harrington: "When the storage temperature is reduced by 5°C in the range of 0~50°C, the seed life will be prolonged by twice. Every 1% decrease in water content between 5% and 14%, the seed will last twice as long".^[48] With the original purpose of protecting human agricultural civilization and food security, they practice different varieties of seed preservation began with the major food crops, vegetables, fruit, and other cash crops.^[49] For example, the Plant and Animal Genetic Resources Preservation Center, founded in 1958 in Fort Collins, Colorado, USA, is the world's first national modern cryopreservation seed bank. More than 500,000 crop seeds have been preserved. The Svalbard International Seed Vault was established in Norway in February 2008, which has amount total of 14,000 seed samples for

the future by now, responding to the growing impact of global climate change on world food production (<https://www.seedvault.no>). In China, National Crop Germplasm Bank was established in November 2002, aiming at mid-term preservation and seed supply distribution of food crop Germplasm resources.

With the aggravation of the irrational use of resources by human beings, the ecological environment is destroyed and more and more species are threatened from extinction.^[50,51] The Millennium Seed Bank (MSB) at Royal Botanic Gardens Kew, UK, was built to preserve wild plant seeds all over the world.^[52] It is the richest point in biodiversity which has conserved 39,841 species from 351 families by May 2020 (<https://www.kew.org>). In China, The Germplasm Bank of Wild Species (GBOWS) was established in Kunming in January 2007 by Academician Zhengyi Wu of the Chinese Academy of Sciences Kunming Institute of Botany. By September 2021, 85,046 portions from 10,601 species of plant seeds have been preserved, accounting for 36% of the total number of flowering plant species in China (www.genobank.org). They contributed positive work in promoting the wildlife germplasm resources conservation which species was endemic, endangered or economic, ecological and scientific value in China.^[53]

The State Bank of Chinese Drug Germplasm Resources is tailored specially for the TCM, which has a different purpose and meaning from the other existing seed bank. The hierarchical target is protecting the precious endangered and endemic species for biodiversity or genetic diversity, and building a national research platform for TCM germplasm resources. The ultimate goal is to ensure the production and supply of basic medicine materials and make the sustainable development of TCM resources. (Figure 2)

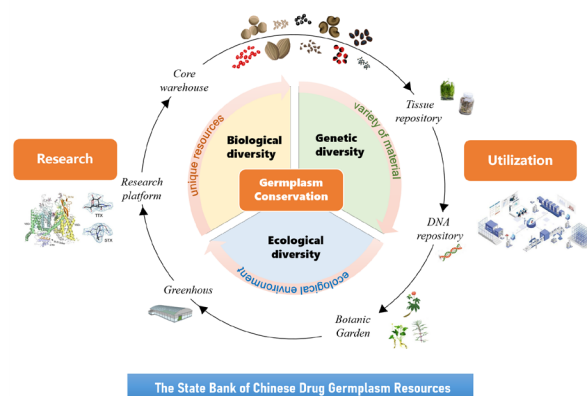


Figure 2. Content and significance of seed bank construction

Protect the unique medicinal resources for biodiversity

The State Bank of Chinese Drug Germplasm Resources particularly focus on the 800 kinds of common use and 200 kinds of Daodi medicinal materials among 11,000 medicinal plants in China, especially the most commonly used species as the National basic catalog of Chinese medicine which we focus on includes 604 species of 129 families of flowering plants. The overall target is to conserve 50,000 seeds in 10 years cover 90% of commonly used Chinese medicine and 30% of medicinal plants. With limited distribution, some genera are only distributed in Asia, such as *Bletilla striata* (Baiji) and *Pinellia ternata* (Banxia). Even some genera are only distributed in China, such as *Rehmannia glutinosa* (Dihuang) and *Eucommia ulmoides* (Duzhong), etc. Conservation of these seeds not only make an important role in medicinal value but also has great value in biodiversity.

Protect the variety of material for genetic diversity

Genetic diversity is the total of all genetic and chromosomal variations in all individuals within a species.^[54] It is manifested in the variation of genes or chromosomes in different

individuals of the same species, including the genetic diversity of different physiological, biochemical, growth, and development functions, maturity, resistance, as well as the karyotype of chromosomes, DNA base differences, etc.^[55]

The evaluation of the germplasm resources differences from that of crops and cash crops.^[56] In addition to yield and resistance, the content of effective ingredients, authenticity, and optimal harvesting time of medicinal materials should also be considered.^[57] Therefore, the collection and preservation of TCM germplasm bank should not only collect as many different species as possible but also carry out the extensive collection of genetic diversity of the same species. It has unique characteristics of Chinese medicine, a far-reaching impact on the development of TCM, and is imperative and irreplaceable.

Protect the ecological environment for ecological diversity

The characteristics of ecological environment such as climate, soil, topography, and community ecology are closely related to the growth and quality of medicinal plants.^[58,59] Medicinal plants were distributed in various ecological environments with rich ecological diversity.^[60] It is recorded in The Notes on Materia Medica Classic that “all medicines have their environment”, indicating that Chinese medicine has always attached importance to the correlation between ecological environment and the quality of medicinal materials since ancient times, and the concept of “Daodi medicinal materials” has a long history.^[61] The preservation and research on the germplasm resources of Daodi medicinal materials are beneficial to clarify the influence of ecological factors on the production process and secondary metabolism of medicinal materials,^[62,63] which has positive significance for improving the green cultivation technology of genuine medicinal materials

and protecting the ecological environment of genuine producing areas.^[64]

Outlook

The State Bank of Chinese Drug Germplasm Resources for traditional Chinese medicine (TCM) in high-profile contemporary arises at the historic moment. It's set up to protect endangered TCM resources and the irreplaceable important significance for genetic diversity. Maximizing the diversity of medicinal species is not just part of global biodiversity conservation, but also provides the material basis for good varieties breeding. It is the key to improving the quality of Chinese medicinal materials, solving the obstacles of the TCM industry, and then conducive to resources sustainable utilization.

The biological mechanisms of seed deterioration, genetic integrity, and critical value of reproduction and regeneration should be deeply explored in further research. The theoretical basis for monitoring and warning of seed vigor and technology of maintaining storage tolerance needs to be improved either. We will be unremittingly committed to these aspects of research and looking forward to more international exchanges and cooperation sincerely.

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บทความพิเศษ

ยุทธศาสตร์แห่งชาติเพื่อการใช้ประโยชน์จากทรัพยากรที่เกี่ยวข้องกับศาสตร์การแพทย์แผนจีนอย่างยั่งยืน: การจัดตั้งธนาคารเชื้อพันธุ์พืชสมุนไพรจีนแห่งชาติ

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บทคัดย่อ: เพื่อมุ่งเน้นที่จะเก็บรักษาเชื้อพันธุ์พืชสมุนไพรจีนได้อย่างยั่งยืน จึงมีการจัดตั้งธนาคารเชื้อพันธุ์พืชสมุนไพรจีนแห่งชาติในปี ค.ศ. 2017 บทความนี้จะแนะนำความเป็นมาและการสร้างโครงสร้างหลักในการจัดเก็บพืชพันธุ์ การจัดเก็บเนื้อเยื่อและสารพันธุกรรม การสร้างสวนพฤกษศาสตร์ เป็นต้น ตลอดจนงานวิจัยเกี่ยวกับการรวบรวมและรักษาแหล่งเชื้อพันธุ์พืชสมุนไพรจีน การวิจัยเกี่ยวกับอายุของเมล็ดพันธุ์และระยะเวลาที่ปลอดภัยในการเก็บรักษาเมล็ดพันธุ์ ซึ่งเป็นงานที่สำคัญในการอนุรักษ์ความหลากหลายทางชีวภาพ ความหลากหลายทางพันธุกรรม และความหลากหลายทางนิเวศวิทยา เราหวังเป็นอย่างยิ่งว่าจะได้สื่อสารและร่วมมือกับสถาบันต่างๆ มากขึ้น เพื่อเป็นการสนับสนุนการใช้ทรัพยากรทางการแพทย์แผนจีนอย่างยั่งยืน

คำสำคัญ: ยาสมุนไพรจีน; ทรัพยากรพันธุกรรม; ธนาคารเมล็ดพันธุ์พืช; การเก็บรักษาระยะยาว; การใช้ประโยชน์อย่างยั่งยืน

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特殊文章

一个中药资源可持续利用的国家策略：国家中药种质资源库的建立

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摘要：旨在实现中药种质资源的长期保存的国家中药种质资源库已于2017年建成。本文分析了国家中药种质资源库建设的背景，介绍了核心低温种子库、离体组织库与DNA库、药用植物园等建设内容，探讨了国家中药种质资源库在中药种质资源收集与保存、种子贮藏特性与萌发特性研究、种子寿命与安全贮存期等方面的研究等，以及这些工作对于保护生物多样性、基因多样性和生态多样性的重要意义。我们期待能与更多机构开展交流与合作，为了促进中药资源的可持续利用贡献力量。

关键词：中药；种质资源；种子库；长期保存；可持续利用

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