RESEARCH ARTICLE

Establishment of collection and protection methods for local germplasm resources of tea trees-a case study of Anhui province

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Local germplasm resources are the main component of the germplasm resources of tea trees. However, the protection is limited currently in China for local germplasm resources of tea trees. There is no comprehensive and systematic method for collection and protection. This study has created a new method for collecting and protecting local germplasm resources of tea trees. According to the distribution and area of tea trees planted in each tea-producing area, we planned and arranged sampling points and one sample from each sampling point was collected and planted in a resource garden for preservation. As a case study of Anhui province, the first germplasm resource garden of tea trees was established comprehensively with the application of this method, at a systematic provincial level. There are 920 samples collected and preserved, nearly 100,000 individual plants. The proportion of samples is close to the proportion of local tea garden areas in various regions of Anhui province. By applying this method to establish a resource garden, the genetic diversity of local germplasm resources of tea trees is maximally preserved. The preserved resources are the material basis for the future breeding of new tea varieties, analysis of genetic relationships and population structure of germplasm resources and gene search.

Key Words: *Collection method; Protection method; Local germplasm resources; Tea trees; Anhui*

INTRODUCTION

Tea trees originated in the southwestern region of China [1,2]. Initially, tea was used as an herbal medicine during the Shang Dynasty (around 1600 BC-1046 BC) and gradually evolved into a beverage and became popular during the Tang Dynasty (618 AD-907 AD) [3,4]. Currently, tea trees are cultivated in more than 60 countries [4,5]. Tea trees are self-incompatible, cross-pollinated and have high heterozygosity [5,6]. After thousands of years of reproduction and evolution, tea trees have adapted to different ecological environments, forming different local tea varieties [7,8]. Different local varieties have their own characteristics and rich genetic diversity, making them the most important component of tea germplasm resources.

For the collection and protection of crop germplasm resources, national crop germplasm resource surveys and solicitations were organized three times in China, which were from 1956 to 1957, 1979 to 1983 and 2015 to 2020 [9]. The collection and protection of germplasm resources of tea trees were included in the last two actions. Since the 1980s, three national and more than ten provincial-level germplasm resource gardens of tea trees have been established [10,11]. These germplasm resource gardens preserve over ten thousand resources. However, the collection and protection of germplasm resources of tea trees are not systematic enough. Moreover, the collection for widely distributed local plant resources garden is limited to the collection after census and investigation only and there are no specific collection methods and indicators to refer to.

MATERIALS AND METHODS

Establishment of collection methods for local germplasm resources of tea trees

The primary purpose of collecting and protecting local tea varieties is to protect their genetic diversity. This requires the broad representativeness that the collected samples would have. For this purpose, we have created a method of collecting local germplasm resources of tea trees through the collection of network spots and the establishment of the resource garden. This method includes collecting seeds through network-spot layout and planting resource gardens through strip and block types.

Network-spot layout: Based on the distribution of local tea trees in the area, we divided the area into many small areas and set up a collection point (network-spot) in each small area. We collected a few kilograms of tea seeds as one sample at each collection point. The samples were nurtured and planted separately.

To facilitate, a county (city, district) with a certain number of local tea plantations will be a tea-producing area. The number of collection points is based on the local tea planting area and the distribution in these tea-producing counties (cities, districts). The more local tea planting areas, the more collection points. So, samples are representative of a wide range of local varieties.

Strip and block type: For the sensitivity to dehydration and low temperature the seed of tea trees is not suitable for long-term preservation. Therefore, the germplasm resources garden of tea trees is established for preservation [12,13]. Samples from a tea-producing county (city, district) were planted in a block area, where each sample was separated into rows. This is the germplasm garden of the tea-producing county (city, district). A provincial-level local germplasm resource of tea trees is formed with the gardens each other in one area (Figure 1).

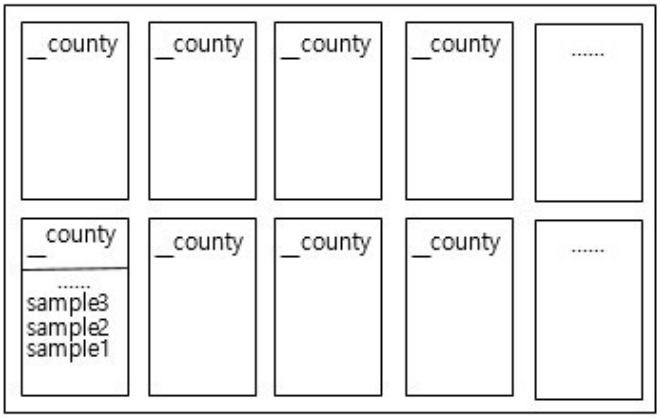

Figure 1) *Schematic diagram of resource garden layout*

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Setting of several parameter values

With the collection method established, several parameter values should be set, such as the number of samples, harvesting amount, planting amount, etc. The larger these values, the more secure the protection of genetic diversity. However, the smaller these values, the lower the protection cost. Therefore, it is necessary to set these values.

Collection area: Determine the collection area that needs to have a certain local planting area and introduction period. Known to all, it is a long-term process that the adaptability and characteristics of tea trees are formed in one place. The germplasm of tea trees in a local tea garden in a region must have originated from the introduction of other regions. After years of growth, there are many generations of tea trees in the garden, such as the tea trees with the introduction of contemporary, with contemporary fallen seeds and with offspring falling seeds. Based on this factor, we chose the county (city, district) as a collection area that planted tea trees before the founding of the People's Republic of China in 1949 (more than 70 years ago), where there are more than 666.7 hm^2 local tea plantations.

The number of samples: The number of samples collected in a region is mainly determined by the area of tea trees planted in that area. For the same area, there will be a few more samples if the distribution is relatively scattered. Based on this thinking, we set 10 to 30 samples for one collection area that has local tea plantations ranging from 666.7 hm^2 to 3333.3 hm^2. Similarly, 30 to 40 samples for 3333.3 hm^2 to 6666.7 hm^2, 40 to 50 samples for 6666.7 hm^2 to 10000 hm^2m^2 and 50 to70 samples for over 10000 hm2.

Collection quantity and planting quantity per sample: The amount of each sample collected depends on its planting amount. To preserve the germplasm of sexual tea trees, they should be planted 10 with a row spacing of 1.5 m-2.0 m and a plant spacing of 0.4 m-2.0 m. The requirements for tea trees experimental planting should be 112 plants with a length of more than 9 m in double rows, double plants. Based on these two references, we designed the local germplasm garden of tea trees with a length of 8 m in double rows and 100 plants. Taking into account factors such as the rate of seed germination, seedling and transplant survival, one sample should be about 1500 g of seeds which is from a local tea plantation with 666.7 m^2 ~ 6667 m2. There will be 500-1000 seedlings and randomly selected when planting.

RESULTS AND DISCUSSION

Preliminary verification-take Anhui province, China as an example

To protect the local germplasm resources of tea trees in Anhui province, we aim to collect germplasm resources of tea trees distributed throughout the province. Relied on the Tea Research Institute of Anhui Academy of Agricultural Sciences, we built an Anhui Provincial Local Germplasm Resources Protection Gardon of Tea Trees.

Determination of the collection area: The collection areas are 27 major tea-producing counties (cities and districts) in five cities, namely Huangshan city, Chizhou city, Xuancheng city, Anqing city and Lu'an city. Other cities which are located in the Yangtze plain such as Tongling, Wuhu, Ma'anshan, Hefei and Chuzhou are mainly engaged in grain production and there are only a few or scattered local tea plantations (especially before the founding of the people's Republic of China, few tea plants were planted). Thus, these cities are temporarily out of the collection area.

Development of the collection plan: We contacted the tea regulatory department, the leading enterprises or tea associations that are familiar with the distribution of local tea plantations in the determined collection county (city, district). We jointly planned the number of samples, divided the collection points and determined the collection personnel. To collect seeds before and after Frost's Descent in mid to late October and to indicate the relevant information of the collection location. The collected seeds should be delivered to the tea institute in time and the tea institute would arrange for the seedlings to be raised and planted in a nursery for storage.

Construction of the local germplasm resources garden of tea trees in Anhui province: Construction began in October 2019 and was initially completed in April 2023. There were 920 samples of the local tea germplasm resources collected and preserved, which are from 27 major tea-producing counties (cities and districts) in 5 major tea-producing cities in Anhui province, including Huangshan city, Chizhou city, Xuancheng city, Anqing city, Lu'an city, etc. Its distribution is shown in Table 1.

TABLE 1

The proportion of tea plantation area and the proportion of collected samples in Anhui province

	Huangshan	Lu'an	Anqing	Xuancheng	Chizhou	Total
The proportion of local tea garden area	26.86%	23.06%	18.35%	15.36%	10.41%	94.04%
Numbers of planned sample	280	250	190	170	110	1000
Numbers of actual sample	309	268	126	131	86	920
The proportion of actual numbers	33.59%	29.13%	13.70%	14.24%	9.35%	100.00%

Effectiveness of construction and outlook of utilization

Protected the genetic diversity of local tea plant germplasm resources: The success of collecting and preserving germplasm resources depends on the number and distribution of genetic variations present in the resource pool [14]. The Anhui Provincial Local Germplasm Resources Protection Gardon of Tea trees has been established with the method created in this study, which had 920 samples, with a total of about 100,000 individual plants covering 94% of the local tea planting areas in Anhui province. The proportion of actual samples is close to the proportion of local tea garden areas in various regions of Anhui province (Table 1). The samples are widely representative, preserving the genetic diversity of local germplasm resources of tea trees in various regions of Anhui province.

Beneficial to the analysis of genetic relationships and population structure characteristics in local germplasm resources: The genome size of tea trees is about 3 GB and their genetic diversity is very rich [4,15]. Classification and identification of tea trees were mainly based on morphological level early. In the 1990s, molecular markers began to be applied in tea plants [16,17]. Subsequently, molecular marker technology was widely used in tea plant genetic diversity, genetic map construction, Quantitative Trait Locus (QTL) analysis and other fields. Currently, Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNPP) have become the mainstream molecular marker technologies in tea trees [18-22]. Gathering samples from various regions in one place is beneficial to differential observation and research among the samples.

The base for breeding new varieties: The main goal of tea tree breeding is to select tea tree varieties with high yield, quality and high resistance to biotic and abiotic stress. At present, tea germplasm existing is one of the most valuable fundamental materials for tea breeding [9]. Among the registered tea tree varieties newly, 85% were applied for through systematic breeding based on local population varieties. Due to the rich genetic diversity, local population varieties have become the main source of tea tree variety breeding.

Gene retrieval: Protection of resources is not only about short-term but also long-term utilization. With the advancement of modern breeding technology and the application of new varieties, the genetic resources possessed by some local varieties were lost severely, which has been demonstrated in many crop varieties. Some of these lost genes have already been found in the resource pool [23]. The application of clone tea tree varieties will lead to a more severe loss of genetic diversity in local varieties. The local germplasm resources garden of tea trees can undertake this function-finding gene back.

Further applications: The collection method created in this study is also suitable for the collection of tea tree sexual-type resources such as wild species and closely related species. The method also provides a reference for the collection and protection of other crops, especially germplasm resources of horticultural fruit trees.

CONCLUSION

In conclusion, the protection of local germplasm resources of tea trees in China has historically lacked comprehensive methods, risking the loss of valuable genetic diversity. This study introduces a novel approach, establishing a systematic method for collecting and preserving local tea varieties. Through the creation of a resource garden in Anhui province, 920 samples encompassing nearly 100,000 individual plants have been preserved, representing 94% of local tea planting areas. This method ensures the maximal preservation of genetic diversity, serving as a crucial resource for future tea breeding, genetic analysis, and gene retrieval efforts. Moreover, the methodology developed in this study offers a blueprint for the collection and protection of germplasm resources in other crops, emphasizing its broader applicability in agricultural conservation efforts.

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