RESEARCH OF EDIBLE FUNGI IN SHANGHAI

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ABSTRACT

The modern edible fungi study in China was originated in Shanghai. The Edible Fungi Institute (EFI) under the Shanghai Academy of Agricultural Science has made many breakthroughs in the Chinese edible fungi study. EFI has succeeded in getting pure spawns, has diversified the cultivation varieties of edible fungi by domesticating wild mushrooms, and has developed the bag cultivation technology. The three essential scientific and technological innovations have allowed the Chinese edible fungi industry to leapfrog for three times. The IEF has been insisting on the researches on genetics and breeding of Xianggu. Up to now, the IEF has kept over 1000 wild Xianggu strains collected from domestic provinces and foreign countries, and has evaluated those strains by conducting fruiting tests and DNA Polymorphism Comparison Tests. According to the requirements of the Ministry of Agriculture of China, the IEF establishes the DUS (Distinctness, Uniformity and Stability) Testing Guidelines for new Xianggu varieties. In recent years, the IEF researchers have adopted RAPD, ISSR and AFLP to identify primary Xianggu cultivation spawns in China, and have been carrying on theoretical and technological innovation on breeding Xianggu mainly through inbreeding. Motivated by the new generation of high-throughput DNA sequencing technologies, more and more genome sequences are sequenced in fungi. The IEF has made use of the full genome sequence of Xianggu to promote the study on the genetic breeding technology. These researches include the development of molecular markers of Xianggu strains, the illustration of biological functions at the molecular level, the establishment of the linkage groups and the construction of QTL genetic map.

Keywords: The Institute of Edible Fungi (IEF); China; Xianggu

INTRODUCTION

Related research on artificial cultivation of the edible fungi in modern China can be traced back to 100 years ago. The earliest article about the cultivation techniques of the edible fungi was published in 1897 on Agricultural Study Newspaper sponsored by Shanghai Agricultural Society. From the late 1800s to 1940s, the elder generation of scholars including Zou Bingwen, Hu Changzhi, Pan Zhinong, Li Shiyi, Sun Yunwei, and Yu Xiaotie, etc. not only introduced many foreign techniques in edible fungi cultivation and disseminated scientific knowledge and cultivation techniques, but also held various edible fungi talents training courses, set up experimental bases, and conduct edible fungi cultivation experiments. Those have laid a preliminary foundation for the modernization of China's edible fungi cultivation techniques.

The modern edible fungi study in China was originated in Shanghai. After the founding of the Popular Republic of China, the Institute of Edible Fungi (IEF) under the Shanghai Academy of Agricultural Science has made many breakthroughs in the Chinese edible fungi research, mainly including the *Tremella* artificial cultivation technique, the hedgehog *Hydnum* artificial cultivation technique and the Xianggu artificial cultivation with crushed-wood material technique.

These inventions and innovations have provided solid technical support to the development of the edible fungi industry in China. The reform and opening-up policy starting in 1978 has provided a favorable environment for the development of the edible fungi industry in China. In the period of more than 30 years thereafter, the edible fungi industry in China has been developing rapidly, with the annual yield rocketing from 60,000 tons in 1978 to 20 million tons in 2009, and the proportion of the yield against total world yield growing from 5% in the past to more than 70% at present.

CONTRIBUTIONS OF EFI

In 1960, the EFI, which was founded on the former Edible Fungi Research Team of Shanghai Agriculture Experiment Station, became the first academic institute specialized in researching the science and technology of edible fungi. Thanks to great efforts of its researchers in the past decades, the EFI has made a series of remarkable achievements. Specifically speaking, the EFI has won over 50 scientific and technological awards at or above the municipal level, and what's more, it has gotten five out of eight national scientific and technological awards in the edible fungi field. The EFI has applied for 61 patents, and has received six healthy food certificates approved by the State Food and Drug Administration. 19 new varieties created by the EFI have been recognized by the Chinese government, and 39 recognized by Shanghai. More than 600 papers and over 10 monographs and popular science books have been published. The new scientific and technologic achievements derived from the EFI have generated considerable profits and ecologic benefits when they are applied at home and abroad. Obviously, the EFI has made indelible contributions to the scientific and technologic advances in Chinese edible fungi industry.

Pure spawns technology. EFI succeeds in getting pure spawns to lay a foundation for the edible fungi industry development. In 1956, the EFI researchers under the leadership of Chen Meipeng, the first chief of the EFI, adopted the tissue isolation and the spore isolation, and successfully attained pure spawns of over ten mushroom varieties, such as white mushroom, Xianggu, agaric, *Hericium erinaceus*, Lingzhi mushroom, button mushroom, and oyster-cap fungus [1-5]. Those pure spawns make the cultivation possible, laying a solid foundation for the mass cultivation of edible fungi in China.

Artificial domestication and cultivation. EFI has diversified the cultivation varieties of edible fungi by domesticating wild mushrooms. In 1959, Chen Meipeng and other EFI researchers attained the associate spawns of white fungus and *Hypoxylon* sp. On this basis, the artificial log inoculation experiment led to the white fungus fruit body [5-7]. Then, the cultivation technology of *Hericium erinaceus* was successfully developed [8].

The bag cultivation technology. EFI has developed the bag cultivation technology to promote the sustainable development of the edible fungi industry. A group of old researchers represented by He Yuansu succeeded in cultivating Xianggu with sawdust, rich bran and other necessary nutritious supplements rather than logs in the 1970s [9-10]. This technology expanded the cultivation area of the wood decay fungi from mountainous areas to different regions of China.

As three essential scientific and technological innovations, pure spawns and artificial breeding technology, artificial domestication and cultivation of edible fungi and the bag cultivation technology have allowed the Chinese edible fungi industry to leapfrog for three times.

RESEARCH OF XIANGGU

Up to now, the EFI has kept over 1000 wild Xianggu strains collected from domestic provinces, such as Hunan, Hubei, Yunnan, Gansu, Zhejiang, Jiangxi, and foreign countries, including Japan, Thailand and U.S. The majority of those strains were collected in the wild or exchanged over ten years ago. Since 2001, the EFI has evaluated those strains by conducting fruiting tests and DNA polymorphism comparison tests. In recent years, we are cooperating with relevant institutions to speed up collection, conservation and evaluation of wild Xianggu resources.

The Xianggu variety resources lay a solid foundation for the healthy development of Chinese Xianggu cultivation industry. Evaluating those resources in a correct, standard, reliable and fair manner is the basic premise to protect the intellectual property of those varieties.

DUS (Distinctness, Uniformity and Stability) Testing Guidelines. According to the requirements of the Ministry of Agriculture, the EFI establishes the DUS (Distinctness, Uniformity and Stability) Testing Guidelines for new Xianggu varieties, which are standards for the protection and recognition of new varieties [11].

Among the 239 Xianggu strains collected nationwide, 24 strains featuring clear source and traceable breeding history were selected as the standard ones in the DUS Testing. These standard strains are widely used [12], such as the Shenxiang series, No.1 Wuxiang, Cr04, 135, 9015, L26, and are provided by the EFI and other fungi institutes in Wuyi, Qingyuan of Zhejiang and Sanming of Fujian.

The Xianggu DUS Testing Guidelines, which are established on the basis of features and data in the Xianggu bag cultivation, include 36 testing specifications consisting of 34 compulsory traits (those must be tested) and 2 supplementary traits. The compulsory traits include 10 hyphae traits and 24 traits related to the bag cultivation. The hyphae traits are composed of envelope, density, growth rate, etc. The 24 cultivation traits cover those properties, such as pileus, stipe, context, scale, gill, fruit body. The supplementary traits are composed of content determination and DNA fingerprinting of special ingredients of fruit body.

DNA molecular identification of Xianggu spawns. Identification of Xianggu cultivation spawns has been the concern of EFI researchers all the time and a focus in the Xianggu genetic breeding study. Recognized as a quick, convenient, accurate and reliable method, the DNA Molecular Identification of Xianggu spawns can be divided into two kinds. One kind refers to the clustering analysis of the DNA sequence amplified by non-specific PCR primers to identify genetic polymorphism and kinship between spawns on the basis of Phylogenetic Tree, such as RAPD and AFLP markers. The other kind is to compare the DNA sequence amplified by specific PCR primers to create molecular markers which directly identify the distinctness of spawns, such as SCAR markers.

The EFI researchers have adopted RAPD, ISSR and AFLP to identify primary Xianggu cultivation spawns in China [12-14], and in addition have developed the SCAR markers for the identification of distinctness for Xianggu spawns in recent years [15]. Research results suggest that Xianggu cultivation spawn resources have small genetic differences, single parental source and narrow genetic background. All of these factors restrict the ability of Chinese Xianggu varieties to adjust to environmental changes, the potential to improve agronomic traits and the innovation in new varieties. Those result in restrictions for the Xianggu cultivation industry development from a long-term, sustainable and stable perspective. Therefore, it is necessary to make more efforts in collecting wild Xianggu resources, evaluating and utilizing the attained wild resources, and introducing more excellent strains to the cultivation resources.

SCAR stands for Sequence-characterized Amplified Regions. The SCAR Markers are suitable for the analysis of large samples because of its better specificity, stability and repeatability as well as its rapidness convenience and low cost. In recent years, a large number of SCAR markers have been developed in the molecular identification of Xianggu spawns. Some domestic

researchers have conducted development research to the SCAR markers of Xianggu in recent years. Xie Baogui Laboratory has successfully separated 14 SCAR markers from RAPD, SRAP and ISSR markers [16]. Ten SCAR markers separated by Wu Xueqian Laboratory can identify seven strains from 47 main cultivated Xianggu strains, while the other 40 strains can be divided into 11 groups [17-18]. Kwan Haishan Laboratory in the Chinese University of Hong Kong has improved the technical route of researching SCAR markers by high-throughput sequencing technologies and consequently enhanced the research efficiency [19].

Though the SCAR markers research of Xianggu has made some progress, the SCAR markers obtained through regular development technology are quite few that are worth using. In addition, most of these SCAR markers are single markers which cannot fully meet the practical needs. So separating SCAR markers through high-throughput sequencing technologies may be a novel technical route. The wide application of this route may produce plentiful SCAR markers which will pave the way for establishing a database concerning the SCAR markers research of Xianggu. As a result, the molecular identification scheme of Xianggu species can be really realized.

The breeding of Xianggu. Collecting Xianggu wild resources requires a sense of urgency. Developing the identification technology of Xianggu spawns demands a sense of crisis. The breeding of Xianggu needs the close integration of technicality, practicality and theory. Some new species have been selectively bred to meet the domestic Xianggu production in previous researches. These species are bred through technologies such as systemic breeding, symmetrical crossing and nonsymmetrical crossing [20-22]. The new development of Xianggu cultivation industry demands new varieties of Xianggu. For example, with the expanding growth area of Xianggu in recent years, it is increasingly urgent to develop ecological Xianggu species to meet local weather conditions, especially the high-yield variety adapting to the high temperature in summer.

The EFI has been carrying on theoretical and technological innovation on breeding Xianggu mainly through inbreeding in recent years. Inbreeding is the most common means to optimize and purify crossing parents. Inbreeding can improve the rate of homozygous genotype and the excellent qualities can be stable genetic as a result. In addition, the deleterious gene in parent material can be discovered. In a word, the pure lines obtained through inbreeding are of great importance to the application of hybrid vigor.

The research of inbreeding Xianggu is expected to achieve two goals: one is to get offspring strains having better characters and features which can be used as the parent for a new round of inbreeding; the other is to get relatively homozygous offspring strains having stable characters and features which can be used as parent for crossbreeding. According to the distribution of the growth rate of 931 strains' multispore self-bred progeny, the inbreeding hypha's growth rates are concentrated under 23 and 28. On the contrary, under 30, the hypha's growth rates become relatively scattered and the variation of traits of self-bred progenies was obvious. Two equal groups consisting of 20 offspring strains are established on the basis of growth rate's coefficient of variation. The growth rate refers to that of inbreeding strains in variable temperature test. The two groups are called heat resistant group (I) and the non-heat-resistant group (II). Group I displayed heat resistant characteristics in variable temperature test and can be used as the object of fruiting experiment later as well as the parent material for crossbreeding high temperature species.

The breeding of Xianggu is arduous, meticulous and long-term. It not only requires a great number of experiments and tests but also needs proper breeding theories as guidance. The EFI is determined to continue innovations concerning breeding technology and theory of Xianggu.

The prospect of Xianggu research. Motivated by the new generation of high-throughput DNA

sequencing technologies, more and more genome sequences are determined. Among these genome sequences, those belonging to basidiomycetes include *Coprinopsis cinereus*, *Laccaria bicolor*, *Schizophyllum commune*, oyster mushroom, white mushroom, *Tremella aurantialba*, button mushroom, *etc* [23-26]. Under this situation, Kwan Haishan Laboratory in the Chinese University of Hong Kong launched Full Genome Sequencing (FGS) of Xianggu and has made some progresses. As a participant in this plan, the EFI has made use of the full genome sequence of Xianggu to actively study the genetic breeding technology.

There are two markers which can be developed on the basis of the full genome sequence of Xianggu, namely, SSR markers and SNP markers. Attaining the full genome sequence of Xianggu provides a favorable premise for looking for SSR loci. By using the SSR primers (provided by Kwan Haishan Laboratory in the Chinese University of Hong Kong) designed according to the 80-pair genome sequence of Xianggu, the EFI tested the polymorphism of 36 cultivation and wild Xianggus. The research result revealed that 52-pair SSR primers demonstrated stable and obvious polymorphism. In addition, the test detected 351 polymorphic loci, that is, each pair of polymorphic primer generated 7.16 loci. Clearly, SSR primers possess high polymorphism in Xianggu, so they are relatively ideal molecular markers. A SNP, third generation molecular markers, has the advantages, such as large quantities, representativeness, genetic stability and suitability to the high-throughput test. The EFI compared the genome sequences of two protoplasted monokaryons with the coverage ratio of six times from 135 strains, and finally found that a SNP locus exist every 200bp DNA sequence on average. Such abundant SNP loci lay a foundation for the use of SNP as molecular markers.

Attaining the full genome sequence of Xianggu offers a rapid way to illustrate biological functions at the molecular level. As a result of comparing the full genome sequence and the biological functions, corresponding functional genes can be detected in the sequence. Eventually, the metabolism or response system will be constructed, such as lignocellulose degradation route, signal response process in the generative affinity match, the control procedure governing how the external factors stimulate Xianggu color change and fruit body development.

Based on the full genome sequence of Xianggu, the linkage groups analysis and the construction of QTL genetic map will dramatically differ from similar studies in conventional genetics. The Xianggu linkage group constructed on the basis of SSR or SNP markers corresponds to the full genome sequence. In the attainted linkage map, analyzing the sequence can identify the gene distribution near molecular markers, a good premise for the QTL analysis and positioning important trait genes of Xianggu. The further QTL analysis of the primary agronomic traits of Xianggu can identify major genes in the known DNA sequence linkage map, which provides the material foundation for further determination of major genes.

The new generation DNA sequencing technology will contribute to the genome information explosion of creatures. The Age of the Genome is coming, so is the age of Xianggu genome. How to make use of the full genome sequence of Xianggu to promote its genetic breeding research is a question that deserves our great attention. Scientific achievements in this area will propel the sustainable and stable development of Xianggu cultivation industry.

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