

Research Progress in Breeding of *Lentinus Edodes* in China

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Abstract:

Lentinus edodes holds significant importance in China's edible fungi industry. This paper provides a concise summary of the primary breeding techniques and their progress in research application for *Lentinus edodes*. These techniques include artificial selection breeding, cross breeding, mutation breeding, protoplast fusion breeding, genetic engineering breeding, and molecular marker assisted breeding. Additionally, the paper offers insights into the future development direction of *Lentinus edodes* breeding, aligning with the current needs of the *Lentinus edodes* industry.

Keywords:

Lentinus Edodes, Breeding Technology, Breeding Trend

1. Introduction

Lentinula edodes, commonly known as winter mushrooms, is a large wood rot fungus belonging to the Basidiomycetes, Agaricales, Tricholomataceae, *Lentinula* family [13]. Despite being influenced by environmental and growth conditions, *Lentinula edodes* is widely distributed, particularly in Yunnan, Guizhou, Sichuan, Hubei, and other regions. China, with its rich history of mushroom cultivation, produces over 1,321,000 tons of mushrooms, accounting for 80% of global production [15,17].

Lentinula edodes is highly regarded for its tender flesh, rich aroma, and nutritional value. It is a well-known edible fungus, abundant in protein, dietary fiber, lentinan, vitamin D, and other beneficial substances that enhance immunity, prevent rickets, [1] and exhibit anti-tumor properties [14,11].

Lentinula edodes production ranks first among edible mushrooms in China and third in the world. It is an important species among edible mushrooms. The degradation of strains and the absence of factory-cultivated strains have resulted in unstable production and uneven quality. *Lentinula edodes* strain problem has become the most significant factor affecting the development of the *Lentinula edodes* industry.

The identification of excellent strains is conducive to the protection of China's independent intellectual property rights.

2. Breeding Method of *Lentinula Edodes*

The existing breeding techniques of *Lentinula edodes* mainly include artificial selection breeding, cross-breeding, mutation breeding, protoplast fusion breeding and genetic engineering breeding [11]. Table 1 indicates Advantages, disadvantages and applications of several main breeding techniques of *Lentinula edodes*.

Table 1. Advantages, Disadvantages of Several Main Breeding Techniques for *Lentinula edodes*.

Breeding methods	Advantages	Disadvantages
Artificial Selection Breeding	Simple operation, no equipment requirements	Long consumption time, multiple sample bases
Hybrid Breeding	With strong purpose and direction, the offspring have heterosis	The cycle is long and the process is complex, and distant hybridization cannot be achieved
Mutagenesis Breeding	The operation process is relatively simple and time-consuming	The frequency of mutations is low and the direction is uncontrollable
Protoplasmic Fusion Breeding	Low equipment requirements, short breeding cycle, and distant hybridization	The preservation time of protoplasts is short and the screening of prophase test is needed
Genetic Engineering in Breeding	Short time consuming, breeding orientation, ultra-distant hybridization	The technology is difficult, genome sequencing developed earlier, the technology is not perfect, and the research foundation is weak

2.1. Artificial selection breeding

Artificial selection breeding is to artificially select strains that produce favorable variations under natural conditions, aligning with Darwin's rule of survival of the fittest. It is the simplest and most effective method used in the initial breeding work as the basis for other breeding methods [11]. This method is characterized by the inability to change the genetic material of the strain, and the work is time-consuming and laborious with a wide range of constraints. Artificial selection breeding usually involves selecting and breeding strains that are suitable for local cultivation according to the characteristics of the local environment, including climatic and soil conditions. Some of the strains obtained through artificial selection breeding in China are shown in Table 2.

Table 2. Some strains obtained through artificial breeding in China.

Species name	Time of recognition	Breeding units	Variety source
Menglan 1	2023	Guizhou Provincial Crop Variety Resources Research Institute	The medium and low temperature varieties obtained from wild mushroom fruiting body in Qianxi County, Bijie, Guizhou province after many tissue isolation and systematic breeding
Yuxiang2	2021	Sanmenxia Academy of Agricultural Sciences	The wild <i>Lentinula edodes</i> fruiting bodies collected in the Funiu Mountain area of Lushi County, Henan Province were obtained through systematic selection
Lixiang 2	2018	Lishui Academy of Agriculture and	It was obtained by isolation and culture from the natural variant strain of

		Forestry Sciences	<i>Lentinula edodes</i> ‘Wuxiang 1’ and multiple artificial directional selection
Yuxiang 1	2018	Sanmenxia Academy of Agricultural Sciences	Wild <i>Lentinula edodes</i> strains collected from Funiu Mountain area of Lushi County, Henan Province were obtained through systematic breeding
Tianfeng 8	2016	Dazhou Academy of Agricultural Sciences	<i>Lentinula edodes</i> 808 was used as the starting strain and obtained after systematic breeding
Qingke 212	2015	Zhejiang Qingyuan County Edible Mushroom Scientific Research Center	The query was unsuccessful
Hunong 1	2004	Institute of Edible Mushrooms, Shanghai Academy of Agricultural Sciences	Conventional artificial selection breeding of imported varieties
9907	2000	Department of Bioengineering, Huaihua University	Wild <i>Lentinula edodes</i> fruiting bodies were collected from Huaihua City, Yuanling, Zhongfang, Mayang and other counties and obtained through systematic selection
Ningxiang . 8	2000	Fujian Ningde Academy of Agricultural Sciences	Polyspore isolation technique produces multiple new combinations obtained through domestication screening
Guangxiang 51	2008	Guangdong Provincial Institute of Microbiology	Domesticated from the wild <i>Lentinula edodes</i>
9101	1998	Institute of Mycology, Jilin Agricultural University	Among the wild <i>Lentinula edodes</i> fruiting bodies collected in Changbai Mountain, the shiitake mushroom strains suitable for the climatic conditions of Jilin Province were selected and bred by methods such as isolation, screening, biological determination, mushroom emergence test, and variety comparison test
Qinghua 6	2007	Shenzhou fungi Research Institute, Qingyuan County, Zhejiang Province	Strains bred by natural selection
Qingke 20	2005	Qingyuan County fungi Science and Technology Research Center, Zhejiang Province	Fruiting body meridians were collected from “Gyeongwon 9015” Weaving separation and other screening and breeding
Qiuxiang 607	2015	Huazhong Agricultural University, Hubei Changchang Bacteria Industry Co., Ltd	Excellent mutant individuals were selected from the fruiting body group of “Autumn Planting 6” and were separated by tissue and bred by directional selection

2.2. Cross-breeding

Cross-breeding usually refers to interspecies hybrids, which involve pairing two parents (monocots) with different and complementary genotypes to produce a hybrid that combines the advantages of the parents, which has hybrid advantage. Fisher-Mueller believed that the recombination of genetic material during sexual

reproduction leads to a new combination of alleles [13]. In the 1970s, China started the hybrid breeding of *Lentinula edodes* [2]. Li Xinlin used Xianggu 101, L18, L19, and SK11 as parent strains in 2022 to breed a new variety using multi-spore hybridization technology. The hybrid-bred “Nong Xiang 152” has advantages such as high yield and short cultivation cycle [8]. In 2021, the Hebei Institute used early-maturing hard Shiitake parent 0912, and single-crossed it with early-maturing, less original bridge parent B5. Through systematic breeding, they obtained the early-maturing high-quality Shiitake new variety ‘Ji Xiang E36’ [28]. Zhao Liqiang and others used the medium-low temperature, hard Shiitake variety L808 and the strong anti-trichoderma ability A288 as parent strains. Through protoplast regeneration, single-crossing, and backcrossing compound breeding methods, they selected the Shiitake “Shi Xiang No. 1” [29]. The author then summarized some of the *Lentinula edodes* strains obtained through hybridization methods in China, as shown in Table 3.

Table 3. Some strains obtained through hybridization in China.

Species name	Certification time	Hybridization method	Parents	Breeding unit
Nongxiang152	2022	Polyspore hybridization	<i>Lentinula edodes</i> 101, L18, L19, and SK11	Fujian Province edible fungus technology promotion station
L901	2022	Polysporulation	LTH	Horticulture Research Institute, Zhejiang Academy of Agricultural Sciences
Jixiang E36	2021	Single spore hybridization	0912 和 B5 0912andB5	Hebei Provincial Institute of Biology
Shixiang 1	2021	Single spore hybridization and Backcross compound breeding method	<i>Lentinula edodes</i> A288 and <i>Lentinula edodes</i> L808	Hebei Normal University
Ji xiang 15	2020	Hybrid breeding	868 <i>Lentinula edodes</i> and L808 <i>Lentinula edodes</i>	Institute of Biology, Hebei Academy of Sciences
Senli 17	2019	Single spore hybridization	<i>Lentinula edodes</i> 238 and <i>Lentinula edodes</i> 808	Shaanxi Provincial Institute of Microbiology, Ningqiang County Fungi Research Institute
Shenxiang 1513	2018	Only hybridization	Shenxiang 18and ‘L808’	Shanghai Academy of Agricultural Sciences, etc
Jixiang 29	2018	Hybrid breeding	<i>Lentinula edodes</i> '868'and 'L18'	Hebei Provincial Institute of Biology
Yufeng 1	2016	Single double hybridization	The query was unsuccessful	Dandong Municipal Research Institute of Forestry Science
Kunxiang 2	2016	Hybrid breeding	The introduction of new 808 varieties and Maoxian 2	Anhui Kunlin Biotechnology Co., LTD
Shenxiang 1644	2014	Polysporulation	Shenxiang 215	Shanghai Academy of Agricultural Sciences,

				etc
Liaofu no. 4	2013	Single spore hybridization	Hybrid strain 0517 of wild <i>Lentinula edodes</i> in Changbai Mountain and excellent cultivated variety L808 in China	Fushun City Research Institute of Agricultural Sciences
Fuxiang 2	2013	Single spore hybridization	Wild <i>Lentinula edodes</i> strain Yexiang 3 in Changbai Mountain and the distant parent of production application variety L66	Fushun Research Institute of Agricultural Science, Liaoning Province
SD-1	2009	Hybrid breeding	Xiang 62 and wild <i>Lentinula edodes</i>	Institute of Soil and Fertilizer, Shandong Academy of Agricultural Sciences
Xiangping 1	2004	Single spore hybridization	Ruidi No.3 <i>Pleurotus ostreatus</i> as female parent, 986 <i>Lentinula edodes</i> as male parent	College of Agriculture and Forestry, East Campus of Shandong Linyi Normal University
Fuxiang 1	2002	Single spore hybridization	Excellent wild <i>Lentinula edodes</i> strains and local cultivated varieties A3-3 in Changbai Mountain	Fushun Specialty Science Research Institute of Liaoning Province
Ningxiang 8	2001	Polysporulation	The fruiting bodies of wild <i>Lentinula edodes</i> collected from Xibingjiafu Forest Farm in Fu'an City	Ningde City, Research Institute of Agricultural Science, Fujian Province
1363	1998	Single spore hybridization	A total of 7 hybrid parents were selected by Shenyang Institute of Ecology, Chinese Academy of Sciences as the main varieties (CK) of semi-clinker sawdust cultivation in our province at that time.	Ningde City, Research Institute of Agricultural Science, Fujian Province
Senyuan 2	1998	Single spore hybridization	The query was unsuccessful	Hubei Province Yuan'an County Edible fungus Research Institute

Tingxiang 1	1992	Single spore hybridization	The query was unsuccessful	Fujian Shanghang County Tingjiang Edible fungus Research Institute
Zhongxiang 68	2006	Single spore hybridization	Parent 1 is the main strain Cr-08B for wood chips cultivation. Parent 2 is the main strain L-221 for wood segments cultivation.	Fujian Provincial Institute of Agricultural regionalization

2.3. Mutation breeding

Mutagenesis breeding is the application of physical or chemical methods to make the genetic material of the *Lentinula edodes* chromosome. These changes result in modifications to the traits of *Lentinula edodes*. The purpose of this breeding method is to exploit the variations in traits and subsequently identify strains with excellent mutations. The probability of mutation breeding is more than 100 times higher than that of natural mutation [12]. The most commonly used physical mutagenesis methods are ultraviolet mutagenesis, Co- γ , etc., and the commonly used chemical mutagens are nitrite, nitrogen mustard, etc. Most of the physical and chemical mutagenesis methods are simple, operable, short cycle and result diversity. However, improper control of mutagenesis time and mutagenic reagents make the germplasm resources that can achieve the desired purpose of the test are not many, and the stability of mutant strains in the cultivation is relatively poor [13]. Wu Xiaoyu et al [21] screened for new varieties of heat-resistant *Lentinula edodes* by using different UV irradiation times and intensities. They conducted a cultivation test for the mutant strains with better overall traits, and successfully bred a heat-resistant 38°C mushroom strain. High-temperature-resistant 38°C strain. Wang Fei and others used the mushroom strain “Qing 20” as a mutagenic material to study the effects of different radiation doses on the mutation rate and mortality rate of mushroom strains. They conducted antagonistic tests and SSR molecular identification on the mutant strains and control strains, resulting in 6 mutant strains that met the requirements [30]. Xu Shuai and others used ultraviolet radiation on the spore suspension of mushroom strain 808, preliminarily proving that the optimal duration for UV mutagenesis of mushroom spores is 60 seconds. This provides a technical basis for mushroom mutagenesis breeding [31]. Wu Yulong irradiated monokaryotic strains with ultraviolet light to create hybrid parents, conducted single-single and single-double hybrids, and then analyzed 6 mutant hybrid strains with good growth conditions through esterase isozyme electrophoresis and heat tolerance screening. The study finally obtained 2 excellent strains as excellent mutant hybrid strains [32].

2.4. Protoplast fusion breeding

Protoplast fusion breeding technology holds significant application value in genetic breeding of edible mushrooms. Protoplasts avoid physiological and physiological effects. They can overcome barriers between species and achieve distant fusion. Hybrid breeding of excellent parental strains is of great significance to the cultivation of shiitake mushrooms. This technology can combine the beneficial characteristics of different strains through fusion, thereby creating more advantageous new varieties. Through protoplast fusion breeding technology, the breeding process can be

accelerated, yield and quality can be improved, and the demand for edible mushrooms can be met [9]. The protoplasts required for protoplast fusion breeding can be mono- or binucleate, and the mononucleate mycelium obtained by this technique is not easily disturbed by the external environment. Xing Zhennan et al. used protoplast fusion technology and esterase isozyme as genetic markers to establish four new *Lentinula edodes* strains [39]. Liulu optimized the protoplast preparation and regeneration conditions of mushroom YJ-01 by single factor test and four-factor quadratic general rotation design [10]. Song Ying used protoplast mononuclear bodies to hybridize and breed excellent new strains of shiitake mushrooms. The hybrid offspring selected showed superior performance in mycelial growth, agronomic traits, and yield compared to the parents, indicating that protoplast mononuclear body hybridization technology is an efficient method for innovating shiitake mushroom germplasm resources [33]. In 2020, Li Liangmin and others established a new method for protoplast preparation, where after 3 days of culturing mycelia on a PDA plate lined with glass paper, the glass paper with mycelium was transferred to another sterile culture dish for direct protoplast preparation on the mycelium. This method has the advantages of shorter preparation time, simple operation, and reduced risk of contamination compared to commonly used protoplast preparation methods [34]. In 2021, Song Yitian optimized the conditions for shiitake mushroom protoplast preparation: enzymatic digestion temperature of 30°C, enzymatic digestion time of about 3.5 hours, and a stabilizer glycerol concentration of 0.6 mol/L [13]. In a recent study conducted by Jiang Shan, the research team investigated the relative dominance levels of different nuclei during the mononuclearization process of mushroom protoplasts. Their findings revealed the existence of a hierarchical relationship between dominant and weak nuclei, indicating that the behavior of dominant and weak nuclei in the phenomenon of biased segregation is consistent within heterokaryotic organisms [7].

2.5. Genetic Engineering in Breeding

Genetic engineering breeding technology is to extract the desired target gene from a donor organism with the help of artificial methods, and introduce it into the recipient cells for replication and expression under the condition of isolation, so as to select and breed new varieties. At present, many genes of *Lentinula edodes* have been cloned. For example, Yuan Xuewen identified, bioinformatics analyzed and cloned the heme peroxidase (LeHP) gene of *Lentinula edodes*, and studied the expression of each LeHP gene during postharvest storage of *Lentinula edodes* [36]. Han Yunyun identified the gene of glutathione peroxidase (GPX) and analyzed the cloning mode of coding sequence [37]. Zhang Wenjing cloned xylanase gene from *Lentinula edodes* by molecular biology method [25]. Duan Yingce et al. cloned LeOAH1 and analyzed its expression in vivo and in vitro [4]. Fu Yajuan et al. cloned the avidin gene (Leav1) of *Lentinula edodes* by RT-PCR, and compared the molecular characteristics and phylogenetic evolution of avidin proteins from different sources [5]. These have laid a solid foundation for the development of genetic engineering breeding of *Lentinula edodes*. ChunHangAu et al. identified A-type mating sites from the genome sequence of *Lentinula edodes*, and identified multiple alleles of different mononuclear strains. Two intron length polymorphic markers were developed to facilitate the rapid molecular determination of mating type A and greatly shorten the acquisition time of the target gene [1]. Genetic transformation as an important part of genetic engineering breeding includes electroschock transformation method, electroinjection method, gene gun method,

Agrobacterium-mediated transformation method, Table 4 compares the advantages of four different genetic transformation methods.

Table 4. Advantages of Four Different Genetic Transformation Methods.

Genetic transformation methods	Electric shock conversion method	Electroinjection method	Particle bombardment	Agrobacterium-mediated transformation
Advantage	High conversion efficiency, easy operation, and low dna content	Simple preparation and high repeatability	Wide application range, simple operation, high conversion frequency, and short time.	Repeatable, easy to operate, high conversion efficiency, and low cost

3. Commonly Used Screening Markers for *Lentinula Edodes* Breeding

Molecular marker technology is a DNA sequence that can be inherited and detected. The marker method based on the difference of nucleotide sequence reflects the difference of *Lentinula edodes* individuals. With the development and improvement of molecular biology and molecular cloning techniques, molecular markers used for genetic analysis at the DNA level have been unlocked. DNA level directly reflects the genetic polymorphism, and is less affected by the external environment. The molecular markers commonly used in the genetic breeding of *Lentinula edodes* are: simple sequence repeat interval (ISSR), random amplified polymorphic DNA (RAPD), related sequence amplified polymorphism (SRAP) [38].

3.1. ISSR (Inter simple sequence repeat)

ISSR provides more genetic information than RFLP and RAPD. Compared with other DNA molecular markers, ISSR marker technology is simple, fast, high polymorphism and low cost. It is a good marker technology for hybrid identification. As a common molecular marker technology, ISSR has been widely used in germplasm identification and genetic relationship analysis of edible fungi. The results of ISSR analysis of 40 wild *Lentinula edodes* strains by Guo et al. showed that wild *Lentinula edodes* strains had rich and diverse heredity [6]. Wang Hao et al. used 8 pairs of ISSR primers to study the genomic DNA polymorphism of 20 *Lentinula edodes* strains. The results showed that the genetic relationship between wild *Lentinula edodes* strains and cultivated strains was far. In order to quickly and accurately identify the diallel hybrids of *Lentinula edodes* [17], Wu et al. designed a new ISSR molecular marker technology based on distinguishing the dikaryons of donor parents. The new ISSR technology can identify the heterozygotes and non-heterozygotes of *Lentinula edodes* diallel hybrids more quickly, comprehensively and accurately, which can provide technical support for hybrid breeding and genetic analysis of *Lentinula edodes* [19].

3.2. SCAR (Specific Sequence Amplification)

SCAR is a molecular marker technology based on PCR, which has the advantages of high stability and repeatability, simple and efficient operation, and reliable results. However, there are few studies on SCAR in mushrooms.

Wu Shengjin 2022 used SCAR molecular markers to identify the double single hybrid offspring of *Lentinula edodes*, which provided technical support for molecular assisted breeding of *Lentinula edodes*. The specific bands of the two nuclei of the donor parent strain YX7 were obtained by ISSR analysis, and the SCAR primers were designed according to the specific band sequence. The SCAR-PCR identification of 40 hybrid strains obtained by hybridization of strain YX7 as donor and strain 808 spore mononuclear body as receptor was carried out, and verified by traditional microscopy and antagonistic test methods. SCAR molecular markers can efficiently, comprehensively and accurately identify heterozygotes and non-heterozygotes of *Lentinula edodes* di-monohybrid strains, which can be applied to molecular assisted breeding and genetic analysis of *Lentinula edodes* [20]. In order to combine the 10 SCAR markers, Zhao Wei completed the identification of 7 strains of the 47 main *Lentinula edodes* strains in China and the grouping of the other 40 strains in a short time. Some strains that are difficult to distinguish from morphological characteristics and have close genetic relationship have also been identified, indicating that the combination of SCAR markers can improve the efficiency of identifying or identifying *Lentinula edodes* strains [26].

3.3. RAPD (Random Amplified Polymorphic DNA)

RAPD and ISSR are the two most widely used molecular marker techniques in the screening and identification of hybrids in hybrid breeding of edible fungi. They are widely used in the identification and classification of germplasm resources, the markers of target trait genes, dominant markers, and cannot identify heterozygotes and homozygotes. RAPD has the advantages of short time, high efficiency and high sensitivity.

Ye Ming et al. used RAPD molecular marker technology to detect the genetic differences between six di-monohybrids and two dikaryotic parents of *Lentinula edodes*. The results showed that the genomes of hybrid strains and dikaryotic parents were quite different, and they were judged as true hybrids [23]. In 2013, Ye Changwen studied the RAPD of *Lentinula edodes* 135 with poor stress resistance and Qingyuan 9015 with natural variation resistance to find out the different genes between them, and cloned and sequenced them. Through a large number of RAPD experiments, five primers were screened, which could distinguish *Lentinula edodes* strains 135 and 9105 [22].

3.4. SRAP (Sequence-Related Amplification Polymorphism)

The marker has simple detection and good repeatability. Each pair of primers can produce moderate polymorphic markers, and some of them are co-dominant markers, which can easily separate the target markers and sequence. The effects of dNTP, Mg²⁺, template DNA, primer concentration and Taq enzyme dosage on SRAP (Sequence-related amplified polymorphism) reaction of *Lentinula edodes* were studied by single factor experiment in Yingzhenghe in 2006 in order to establish and optimize SRAP (Sequence-related amplified polymorphism) for *Lentinula edodes* [24]. In order to meet the urgent needs of factory annual cultivation of *Lentinula edodes*, a new variety of *Lentinula edodes* suitable for factory cultivation was selected. The antagonistic test and ISSR and SRAP molecular identification showed that the strain had molecular specificity. It is feasible to breed *Lentinula edodes* by multi-spore hybridization. 'Nongxiang 152' has the advantages of high yield and short cultivation period, which is suitable for factory cultivation [8].

3.5. *AS-PCR (single nucleotide polymorphisms)*

Currently, the mating type identification of mushrooms is usually done by pairing monokaryotic strains for observation under a microscope to look for clamp connections. This method is labor-intensive, time-consuming, and prone to human error. In 2019, Zhang Meiyu used the mushroom strain “Shenxiang 215” as an example. By resequencing the genomes of the dikaryotic parental strains that needed mating type identification, they obtained sequence alignment information for the A and B mating type factors in the dikaryotic strains. They performed a statistical analysis on the SNP sites within the mating type factor alleles, selected appropriate sites for primer design, and used allele-specific PCR technology to quickly determine the mating type of monokaryotic strains and exclude dikaryotic and heterozygous strains based on the amplification results. This method for identifying the mating type of mushroom monokaryotic strains has improved efficiency and accuracy. It can serve as a molecular marker-assisted breeding tool, providing new technical support for genetic breeding work in edible mushrooms [35].

4. Conclusions

Lentinula edodes has high nutritional value and good taste, and consumers are enthusiastic about it. The *Lentinula edodes* industry has become a pillar of agricultural production. Vigorously developing the mushroom industry and advocating the consumption of *Lentinula edodes* can improve the physical quality of our nationals. *Lentinula edodes* have been cultivated in China for 800 years, and people are constantly exploring breeding technology of *Lentinula edodes*. With the continuous development of science and technology, the development of *Lentinula edodes* industry has entered a new period. The author believes that although the period of hybridization breeding is relatively long, its breeding target is well directed and is the main way of *Lentinula edodes* breeding in the current and future period, while genetic engineering breeding will be the future trend. Mushroom germplasm resources from all over the country and the world were collected and carefully evaluated and analyzed, so as to provide abundant parental materials for the subsequent development of hybrid breeding. The combination of molecular markers and traditional selection and breeding techniques will promote the genetic breeding of *Lentinula edodes* more efficiently to cultivate *Lentinula edodes* with independent property rights, which is also the key to the development of *Lentinula edodes* industry in a big step forward.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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