Reproduction and Breeding 4 (2024) 126–133

Contents lists available at ScienceDirect

Reproduction and Breeding

journal homepage: www.keaipublishing.com/en/journals/reproduction-and-breeding

Improvement and application of genetic resources of grass carp (*Ctenopharyngodon idella*)

Yude Wang [a,b], Wuxia Liu [a], Zhipeng Li [a], Bin Qiu [a], Jian Li [a], Gen Geng [a], Biao Hu [a], Anming Liao [a], Yanping Cai [a], Ming Wen [a], Shi Wang [a], Qinbo Qin [a,b], Kaikun Luo [a], Shaojun Liu [a,*]

[a] *State Key Laboratory of Developmental Biology of Freshwater Fish, Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha, China*
[b] *Hunan Yuelu Mountain Science and Technology Co.,Ltd. for Aquatic Breeding, Changsha, China*

ARTICLE INFO

Keywords:
Grass carp
Germplasm resources
Genetic improvement
Disease resistance

ABSTRACT

Grass carp was an important economic fish for freshwater aquaculture. The exploring and utilization of high-quality grass carp germplasm resources were important for ensuring a domestic supply of high-quality aquatic protein. However, natural grass carp populations in China were facing severe threats because of the effects of frequent human activities, water environment damage, overfishing, etc. Against this background, high-quality grass carp germplasm resources were the foundation for the healthy and sustainable development of the grass carp aquaculture industry, so systematic collection, preservation, identification, evaluation, protection, and utilization of them carry great strategic importance. This paper summarizes major research results of grass carp in morphology, cytogenetics, molecular population genetics, etc. It not only introduced genetic improvement techniques such as gynogenesis, hybrid breeding, and polyploid breeding, but also discussed the existing research on the protection measures of grass carp germplasm resources. On this basis, the paper proposed new methods to improve the protection of grass carp germplasm and create improved varieties, which could provide high-quality resources for the sustainable development of the grass carp industry.

1. Introduction to grass carp

Grass carp (*Ctenopharyngodon idella*) was the most widely farmed fish globally, belonging to the genus *Ctenopharyngodon*, family *Cyprinidae*, and order *Cypriniformes*. It featured an elongated light green or white body. Grass carp was a typical herbivorous fish species living in rivers and lakes. It was of great commercial value for its fast growth, large body size, tender and delicious meat, and low intermuscular bones. Grass carp had been farmed in China for more than 1700 years and was commonly referred to as “wan”, “wanyu”. The name of grass carp can be traced back to Erya, the first Chinese dictionary written during the Warring States and Han Dynasty. In its chapter “Explanation of Fish”, grass carp was referred to as “wan”. During the Ming Dynasty, Li Shizhen recorded in Compendium of Materia Medica that “it was called ‘wan’ for its mild nature; it was commonly known as ‘caoyu’ because it feeds on grass.” Grass carp was also mentioned as a mixed breeding species in *Records of the Historian* about fish farming methods in ponds popularized during the Han Dynasty. Liu Xun recorded in *Records of Unusual Things in Lingbiao* that the Tang Dynasty opened a new era of taking grass carp as the main cultured fish. During the Song Dynasty, Jiujiang in Jiangxi Province became the main production area for grass carp fry. During the Ming and Qing Dynasties, a grass carp farming technology system with unique Chinese characteristics came into being.

Widely distributed grass carp had diverse species worldwide, such as those found in the Yangtze River, the Pearl River, the Heilongjiang River, and the Yuan River in Yunnan Province (excluding Tibet and Xinjiang sections), etc. In recent years, with the expansion of market demand, the aquaculture output of grass carp had been rising year by year, from 355,5963 tons in 2007 to 575,5095 tons in 2021, with a growth rate of 61.84 %, which had played a positive role in protein supply (Fig. 1).

* Corresponding author.
E-mail address: lsj@hunnu.edu.cn (S. Liu).

https://doi.org/10.1016/j.repbre.2024.04.003
Received 18 January 2024; Received in revised form 24 March 2024; Accepted 9 April 2024

2. The geographic distribution of grass carp germplasm resources

Grass carp was originally distributed in China, Russia, and Bulgaria. In China, it mainly inhabits the Yangtze River, the Pearl River, and the Heilongjiang River. As early as the Pliocene epoch, grass carp originated in the Yangtze River. Then when the Nenjiang River was connected to the Liao River and the Bohai Sea, grass carp entered the Heilongjiang River from the river plain area. The grass carp in the Pearl River system was believed to migrate downstream from the Yangtze River and Qiantang River during the glacial epoch when the sea level was 100 m lower than it was currently, and the Taiwan Strait was a low plain.

Grass carp had been introduced to 93 countries, with some countries directly importing it from China, including India, the United States, and Hungary, and others introducing it from countries that imported it, such as Sudan (importing from India) and Sweden (importing from Hungary). Malaysia was one of the earliest countries to import grass carp from China in the 1980s. Grass carp was introduced to the United States, Egypt, India, and other countries with the purpose of controlling undesired aquatic vegetation and aquatic breeding. Currently, grass carp populations had been successfully established in countries such as Malaysia and the United States, but not yet in some areas. This might be attributed to the absence of long rivers, as long river sections were essential for providing enough time for grass carp eggs to hatch, and for the hatched fry to seek food and grow in still-water bodies. In 1996, China imported gold grass carp from Russia and began promoting its farming domestically in 2001. With rich nutrition, it was used in the famous Cantonese dish "yusheng" by steaming with other ingredients, which was beneficial to the eyes (Fig. 2).

In recent decades, the wild grass carp resources in China had significantly decreased. A survey of grass carp resources in the Yangtze River, Pearl River, and Heilongjiang River conducted by Li Sifa et al., in 1990 revealed an overall declining trend in grass carp production from 1950 to 1990 in these three major river systems. From 2003 to 2006, Duan Xinbin monitored the total amount of the four major domestic fish in the reaches of the Yangtze River between Yichang and Chenglingji. The results showed that the total amount of the four major domestic fish was only 42.8 % of the average number from 1997 to 2002 [1]. Ding Longqiang et al. surveyed the four major Chinese fishes in the Anqing section of the Yangtze River from 2016 to 2018 and found that the grass carp populations had been declining in recent years, though its germplasm resources accounted for relatively larger proportion [2]. In 2019–2020, He Yujie conducted research on grass carp and found that it was being overfished [3]. In all, the improvement of grass carp germplasm resources was urgent, so it was necessary to regulate reasonably and use them.

3. Studies on grass carp genetic resources

3.1. *Grass carp studies in morphology*

Morphology was the basis for studying fish behavior, focusing on fundamental differences in the external arrangement of biological tissues. Due to long-term geographical isolation and evolution, grass carp populations in different regions had shown morphological differences.

In 1989, Li Sifa et al. found that 10 morphological characteristics of grass carp showed significant differences ($P < 0.01$) among different populations. Moreover, the morphological geographic isolation coefficients between the three populations were 1.00–2.85, indicating a positive correlation between the overall differences in morphological features and the distance of their geographical isolation [4]. In 1990, Tian Jianlong and Wang Dong discovered the measurable traits ratios of grass carp change to various extents with increasing body length [5]. In 2006, Zhang Zhiwei et al. conducted an experiment on wild and farmed grass carp populations via microsatellite markers. It showed severe differentiation between wild and farmed populations [6]. In 2011, Zhang Jianming et al. measured the morphological characteristics of 120 young fish of four major Chinese carps in the middle reaches of the Ganjiang River. The study found that there was a small intraspecific difference in four kinds of fish [7]. In summary, the overall shape of grass carp in different regions had a little difference.

3.2. *Grass carp studies in cytogenetics*

Cytogenetics focused on studying the structure, behavior, and inheritance of chromosomes, providing information for fields such as animal and plant biology, cell biology, etc. Studies on fish chromosome karyotypes had great significance for fish classification, evolution, genetics, and hybrid breeding.

The 48 chromosomes of grass carp ($2n = 48$), were determined

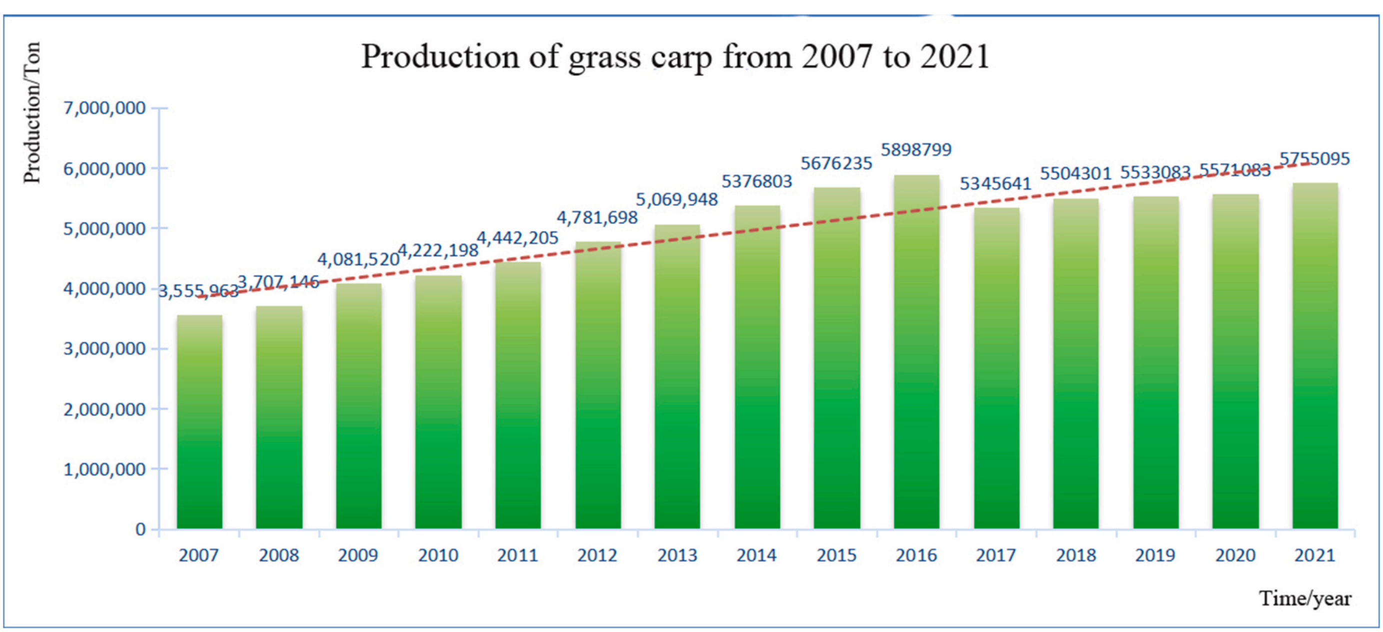

Fig. 1. Grass carp production map from 2007 to 2021.

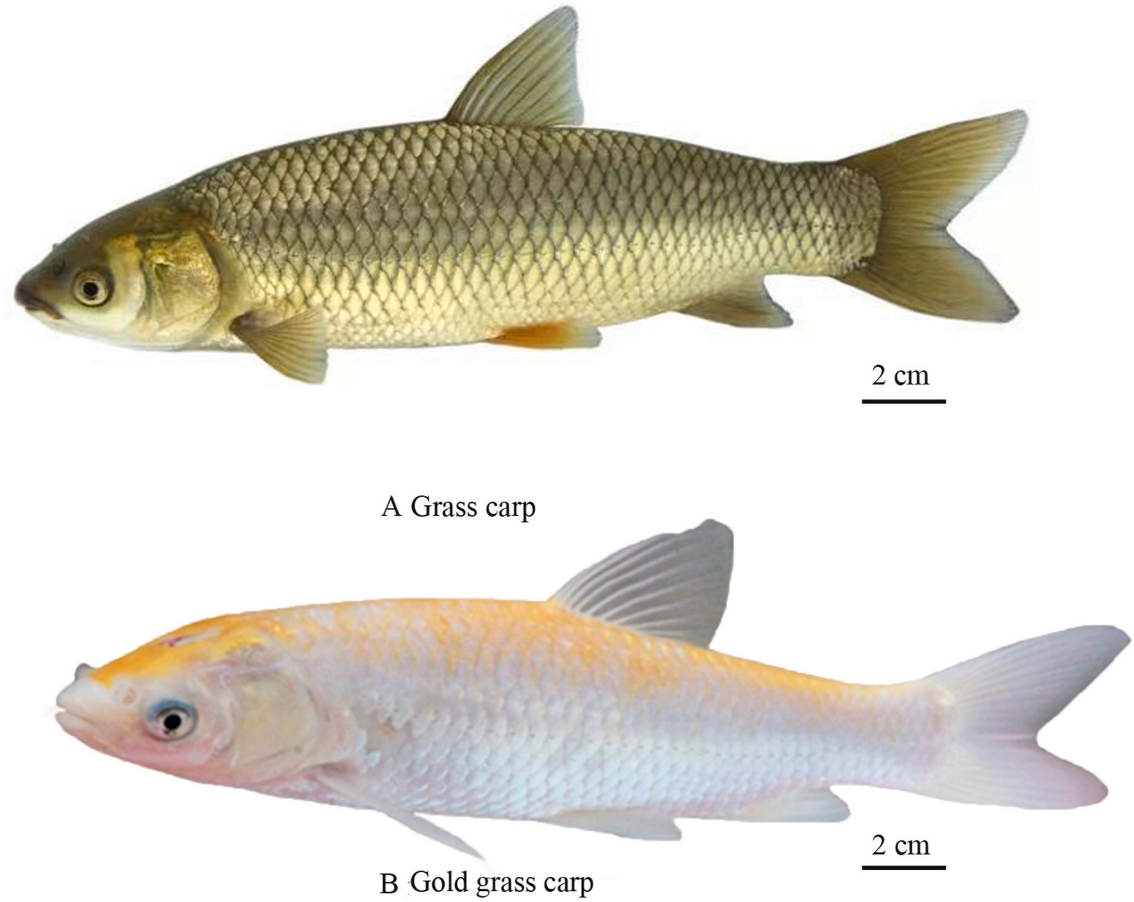

Fig. 2. Picture of Grass carp and Russian gold grass carp, Bar = 2 cm.

through extensive research and exploration. In 1981, Liu Lingyun conducted experiments using short-term in vitro culture of grass carp kidney cells, which showed that the grass carp karyotype was n = 8 m + 16sm, without "st" and "t" chromosomes [8]. In 1991, Yang Huiyi prepared slides with a pair of lymphocytes cultured for a short period, adopting C-banding and G-banding techniques respectively. The results showed that the grass carp karyotype was n = 8 m + 10sm+6th [9,10]. In 2007, Yang Xiaofen conducted karyotype studies on wild grass carp in Guizhou Province investigated by means of obtaining chromosome specimens from metaphase of kidney cells by vivo-injection of PHA and Colchicine, hypotonic-air drying, and Giemsa staining, respectively. The results showed that the grass carp chromosome number was 2n = 48, with a karyotype formula of 18 m + 22sm+8th, NF = 88 [11]. In 2014, Li Yuyuan used the intraperitoneal injection with PHA and short-term kidney tissues cultivation method to prepare grass carp chromosomes. After measurement, the diploid chromosome number of the grass carp was 48, and the larger chromosomes were all in the group of submetacentric chromosomes [12]. The karyotype of grass carp was found to be 2n = 20 m + 24sm+4th, NF = 92, without secondary constriction, satellite, or heterosome on chromosome [13]. In 2014, Shu Hu et al. prepared grass carp chromosome specimens by using a method of injecting with PHA and colchicines and hypotonic-air drying technique, and then analyzed the karyotype, silver staining (Ag-NORs), and C-banding of the fish. The results revealed that the karyotype of grass carp was 2n = 22 m + 24sm+2nd, NF = 94; Ag-NORs were observed in the metaphases, located on the tips of chromosomes; the centromere of most chromosomes showed different shades of the C-band [14,15].

The total and changing patterns of blood cell number in fish were indicators of its physiological and pathological conditions, and hence a technique of detecting changes in the peripheral blood cell number could be developed to get the health condition of fish. In 1996, Lin Guanghua studied the ultrastructure of peripheral blood cells in adult grass carp with transmission electron microscope. It turned out that peripheral blood cells could be classified into erythrocytes, lymphocytes, monocytes, neutrophils, eosinophils, and thrombocytes [16]. In 2012, Zhang Yan et al. conducted a study on the peripheral cells of four grass carp samples. The results showed that the number of erythrocytes per microlitre increased with growing individual size, indicating that the growth of grass carp individuals promoted the oxygen transport capacity as well as the overall metabolic capacity. At the same time, the number of leukocytes per microlitre volume also rose with growing individual size, suggesting that larger grass carp had stronger immunity [17].The accumulation of cytogenetic knowledge of grass carp provided an important basis for the breeding of grass carp.

3.3. *Grass carp studies in biochemical genetics*

Biochemical genetics focused on the physical and chemical properties of genetic materials and the regulation of protein synthesis and organism metabolism. Isozyme, as a kind of gene product, could work as an effective tool for analyzing the growth, genetics, system evolution, and physiological processes of organisms, including fish.

Research on fish isozymes began in the early 1960s. Current grass carp research concentrates on three isozymes - lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and esterase (EST). Wu Lizhao and Wang Zuxiong put forth that the isozyme system of grass carp had significant tissue specificity just like other bony fish (Osteichthyes): LDH-A and LDH-B subunits were expressed in the brain, eyes, heart, kidneys, muscles, and liver; LDH-C subunit was specifically expressed only in the liver; the LDH zymogram changed significantly during the early development phases [18]. However, the results of the experiment performed by Jiang Jianguo et al. differed from the above: there was no LDH-C subunit in the electrophoretogram of LDH isozymes in the liver tissues of grass carp [19].

MDH isozymes in grass carp were divided into two types: supernatant-type (*s*-MDH) and mitochondrial-type (*m*-MDH). S-MDH isozymes were encoded by two gene loci (s-Mdh-A, -B) and were distributed in the brain, eyes, heart, and other organs. M-MDH isozymes were encoded by two gene loci and were expressed in the brain, eyes, heart, and other organs. Moreover, the MDH zymogram did not change significantly during early development [18].

The zymogram of EST isozymes in grass carp was relatively complex. Various studies had reported different results. The experimental results of Wu Lizhao and Wang Zuxiong showed that 14 zymogram bands were detectable in different tissues of grass carp, which were encoded by at least 4 gene loci. Furthermore, they found that the EST zymograms of different tissues differed greatly from each other, with many low-activity bands [18]·

Isozymes had been taken as a research object in grass carp studies of population genetics. Wu Lizhao and Wang Zuxiong also conducted a genetic analysis of a natural population of grass carp from the middle reaches of the Yangtze River in Wuhan and discovered that it had 16.7 % of polymorphic loci and an average heterozygosity of 0.0739 [20]. Tan Shuzhen used isozymes to study the genetic differentiation of grass carp populations from Hanjiang and Jiujiang. It turned out that the proportions of polymorphic gene loci were 0.167 and 0.083 in Hanjiang and Jiujiang populations, respectively; the observed mean heterozygote were 0.0204 and 0.0064. These findings suggested that the genetic differentiation within both the Hanjiang and the Jiujiang populations was not significant, but the former population was slightly more diverse than the latter one [21]. Compared with most bony fishes, the isoenzymes LDH, *m*-MDH and ADH of grass carp had a special expression pattern: m-MDH and ADH were encoded by two gene loci. The isoenzymes of grass carp were studied to provide a biochemical genetic index for artificial offspring selection and directional breeding of grass carp.

3.4. Grass carp studies in molecular genetics

Molecular genetics was primarily concerned with the study of nature, function, and changes of genes. Research on the molecular genetics of grass carp began in the late 1990s and had systematically explored the genetic diversity and structure of grass carp populations with various techniques such as RAPD (random amplified polymorphic DNA), mtDNA (mitochondrial DNA), SSR (microsatellite DNA marker technology), and TRAP (target region amplified polymorphism). In 1998, Xue Guoxiong et al. used RAPD markers to evaluate the genetic distance of grass carp populations in three water systems. The study showed that the genetic distance between populations in the Yichang section of the Yangtze River and the Taihu Lake area was the smallest, followed by that between populations in the Yangtze River and Pearl River. And compared with other populations, the Heilongjiang population had the largest genetic distance [21]. In 2002, Zhang Siming et al. applied mtDNA and RAPD techniques to investigate the genetic diversity of grass carp populations from Jiayu of Hubei Province and Ruichang of Jiangxi Province in the middle reaches of the Yangtze River, as well as populations from the middle reaches of the Han River and the Xiang River. The study found that the genetic diversity from the highest to the lowest were Ruichang, Han River, Xiang River and Jiayu population, with low genetic differentiation among them [22]. In 2004, Zhang Dechun used PCR technology to explore the restriction fragment length polymorphism (RFLP) of mtDNA in four grass carp populations in the middle reaches of the Yangtze River. He concluded that the genetic diversity of cultured grass carp populations was significantly lower than that of wild populations, and there was a notable genetic differentiation between them [23]. In 2006, Song Xiao et al. and Chen Qin et al. analyzed the genetic variation within and among grass carp populations sampled from its native (China) and foreign colonized habitats from the mitochondrial D-Loop region and ISSR markers (a molecular marker technology), respectively. The results revealed that the genetic diversity of the native populations was more than twice as much as that of colonized populations. And the highest genetic diversity was observed in the Yangtze River population [24,25]. In 2009, Liu Feng et al. studied the genetic diversity and population relationships of nine grass carp populations, including several populations in the Yangtze River. Their study revealed that grass carp populations contained high genetic diversity and there was no significant genetic differentiation between different populations in the Yangtze River system [25]. In 2020, Zhai Dongdong et al. studied the genetic diversity and population structure of five grass carp populations in the upper reaches of the Yangtze River from mitochondrial Cytochrome *b* region (*Cyt b*) analyzation. The results showed that these grass carp populations contained low genetic diversity [26]. At the population genetic level, the population genetic diversity of grass carp in different river systems was the lowest in the Yangtze River system, followed by the Pearl River system, and the highest in Heilongjiang River system.

3.5. Grass carp studies in genomics

Systematic studying of fish genomes greatly promoted a deep understanding of fish biological characteristics, sex differentiation, reproductive modes, and regulatory patterns, and clarifies the genetic evolution of all vertebrates. The study results of Huang Weijie et al. showed that 677,363 microsatellite sequences were found in the 900.51 Mb genome sequence of grass carp, with a total size of 12.84 Mb, an average distance of 1329.43 bp, and a total relative abundance of 0.192. The copy numbers of microsatellites repeated in the entire grass carp genome range from 5 to 549, which showed a significant negative correlation between repeat motifs length and mean copy number($r = -0.8581$, $P = 0.0288$). By using PacBio third-generation sequencing and chromosome conformation capture technology, a grass carp chromosome-level reference genome was successfully obtained, 893.2 Mb in size, with a conting N50 length of 19.3 Mb and a scaffold N50 length of 35.7 Mb [27].

A genetic map could serve as an essential framework for the study of target gene isolation and analysis, gene structural and functional identification, as well as marking traits of the target molecular thus to approach comparative genomics. In 2009, Xia Junhong et al. constructed the first-generation linkage map of grass carp at the molecular level. It contained two different families and 24 SNPs and 279 SSRs with an average distance of 4.2 cM between each two of them. Furthermore, they conducted BLAST searches of 303 mapped markers, revealing substantial macrosynteny relationships and extensive colinearity of markers between grass carp and zebrafish [28]. In 2022, Guo Jiamin et al. constructed a high-density linkage map of grass carp with a total length of 1752.742 cM and an average marker resolution of 0.44 cM. This comparative genomic analysis showed that 23 out of the 24 LGs of grass carp possessed high synteny with 23 chromosomes of zebrafish [29,30].

The EST sequences, as an important genetic resource, were fragments of cDNA sequences generally generated from the 5′ or 3' ends of cDNA clones randomly selected from cDNA libraries, whose lengths usually range from 300 to 500bp. Currently, cDNA libraries had been constructed for various tissues of grass carp, including its head, kidney, intestine, and liver, etc. So far, about 7300 ESTs had been collected. Among them, nearly 140 genes were associated with immunity after identification, which provided significant resources for investigating the functions of disease-resistant genes in grass carp. More ESTs would be produced with the advancement of next-generation sequencing technology [31].

4. Studies on genetic improvement for grass carp

4.1. Studies on gynogenesis of grass carp

The study of gynogenesis in fish had high application value for current fish breeding to strengthen the advantages of hybridization of pure lineage and fish with recessive characters and for better breeding selection. Gynogenetic technology was used to destroy the sperm nucleus with ultraviolet rays, X-rays, or gamma rays and stimulate the ovum to develop into an individual with this sperm. Artificial induction of gynogenesis could establish fish purebreds in a short period. In comparison to traditional genetic methods, it saves time, labor, and costs, and was of great significance for fish genetic breeding, favourable variation screening, and good traits combination. This technology began to develop in foreign countries in the late 1950s. In the late 1970s, China began to study it and achieved certain results. Since the 1990s, Academician Liu Yun's team from Hunan Normal University had conducted a series of studies on the gynogenesis of grass carp. They activated grass carp ovum by inactivated heterologous sperms, and produced gynogenetic grass carp F_1 through cold shock or heat shock, and subsequently, they bred disease-resistant offspring by the backcrossing between F_1 and common grass carp. The team also confirmed the impact of allogynogenesis by conducting experiments with the ovum of grass carp and heterologous sperms, such as koi carp, red crucian carp, scatter-scaled mirror carp, etc. Fragments of these heterologous sperms in the genome of gynogenetic grass carp were detected by microsatellite maps and special molecular marker genes (*Hox* genes). Owing to the continuous efforts of Academician Liu Yun and Academician Liu Shaojun from Hunan Normal University, the artificial breeding platform for gynogenetic grass carp had been improved, then a large number of disease-resistant grass carp had been widely promoted, resulting in great economic, ecological, and social benefits. Wang Yude et al. conducted comparative research on common grass carp and gynogenetic grass carp, showing that the latter had stronger disease resistance, with a survival rate 50 % higher than that of the former, particularly against viral infectious diseases like hemorrhagic disease and bacterial infectious diseases such as gastrointestinal diseases [32].

4.2. Studies on hybrid breeding of grass carp

Hybrid breeding was an important method for the genetic improvement of animals and plants, as one of the most effective breeding ways to maximize growth potential with increasing yield and higher quality. At present, hybrid breeding played a significant role in hybrid variety improvement and variety cultivation in aquaculture. To enhance the grass carp's disease resistance and cultivate new hybrid varieties of grass carp, Chinese aquaculture researchers had conducted numerous artificial hybrid breeding experiments taking grass carp as the parents. According to records, these hybrid breeding experiments included three intergeneric hybridizations and seven intersubfamily hybridizations: two species in the subfamily *Hypophthalmichthyinae*, three species in the subfamily *Cultrinae*, and two species (varieties) in the subfamily *Cyprininae*.

4.2.1. Intergeneric hybridization

The Aquatic Science Research Institute in Nanchang, Jiangxi Province conducted hybrid experiments of grass carp (*Ctenopharyngodon idella*) (♂) and black carp (*Mylopharyngodon piceus*) (♀), and the hybrid offspring had a high growing speed but weak disease resistance [33]. However, hybrid experiments conducted at the Taihe Fishery in Qidong County, Hunan Province showed that the hybrid varieties not only grew fast, but also had strong disease resistance. Due to different experimental conditions and different research environments, the results of the same hybrid experiment varied greatly. The Aquaculture Division of the Agriculture Bureau in Liuyang County, Hunan Province conducted hybrid experiments on grass carp (♂) and *Squaliobarbus cursiculus* (♀), and their hybrid offspring grew slightly faster than grass carp [34]. These studies showed that the intergeneric hybridization of grass carp can effectively improve the growth rate.

4.2.2. Intersubfamily hybridization

(1) *Subfamily Hypophthalmichthyinae*

Guo Hanqing et al. conducted four hybrid experiments of grass carp (♂) and bighead carp (*Aristichthys nobilis*) (♀), with a high fertilization rate of up to 80 %, but a survival rate of only 55.1 %. The hybrid offspring had a similar diet to grass carp and grew fast [35]. After analysis of the hybrid offspring, the Biology Department of Hunan Normal University found that only 0.5 % of the individuals were hybrids, and only a few individuals had normal gonadal development. However, their growth rate was faster than that of the parents and the bighead carp-like offspring [36]. Hybrid experiments at the Institute of Hydrobiology of Hubei Province showed that the size and diet of the hybrid offspring were similar to those of grass carp [35]. However, the results of hybrid experiments of grass carp (♂) and bighead carp (♀) conducted in Hanshou County, Hunan Province showed that the size and diet of the hybrid offspring were intermediate between those of the parents [35]. Kilambi et al. analyzed the chromosomes of the hybrid offspring and found that they were triploid ($3n = 72$) [37]. In addition, the hybrid offspring of bighead carp (♂) and grass carp (♀) all died during the late development stage as fry [38].

(2) Subfamily *Cultrinae*

The Yangtze River Fisheries Research Institute bred a hybrid variety with a fast growth rate and strong disease resistance, with their morphology and diet being intermediate between the parents through hybrid experiments of grass carp (♀) and blunt snout bream (*Megalobrama amblycephala*) (♂) [39]. However, the offspring of grass carp (♀) and blunt snout bream (♂) bred by the Agriculture Bureau of Jiangxi Province showed similar embryonic development to grass carp [40]. The Institute of Hydrobiology of Hubei Province conducted low-temperature induction experiments on artificial triploids and tetraploids of grass carp (♀) and blunt snout bream (♂) [41]. The Fish Research Laboratory of the Biology Department at Hunan Normal University conducted hybridization experiments between grass carp (♀) and tri-angular bream (*Megalobrama terminalis*) (♂) and found that the hybrid F_1 was sterile and grew slowly. Subsequent chromosome testing revealed that F_1 was triploid ($3n = 72$) and completely sterile in both sexes [42].

(3) *Subfamily Cyprininae*

Artificial hybridization experiments of grass carp (♀) and common carp (*Cyprinus carpio*) (♂)were conducted in the Fish Breeding Farm in Wugang County, Hunan Province. The hybrids had lower fertilization and hatching rates than their parents, but they showed higher survival rates and had a herbivorous diet, with some disease resistance [43]. Wu Weixin et al. also reported that hybridization between grass carp (♂) and *cyprinus carpio*.L (♀) resulted in normal fertilization but low hatching rates. There were both common carp-like and grass carp-like hybrid offspring, but the latter could not survive. The survival rate of common carp-like offspring was only about 0.05 %, and all of them were allotetraploid [44]. The second generation of hybrids was successfully bred in 1982. In backcrossing experiments of F_1 (♀) (grass carp (♂) and Xingguo red carp (♀)) and grass carp (♂) conducted by the Hunan Fisheries Science Institute in 1983, the offspring contained two types - grass carp-like and common carp-like with respective habits. The backcross hybrids were triploid. In 1986, the second hybrid came into being.

All the intergeneric, intersubfamily, and interspecific hybridization of grass carp belong to distant hybridization [45]. While some carp of distant hybridization could develop normally, the overall hatching and survival rates were low. Fertilization cytological studies had revealed that intersubfamily hybridizations could undergo normal fertilization processes, such as those between bighead carp (♀) and grass carp (♂), or grass carp (♀) and blunt snout bream (♂). Distant hybridization could produce haploid, triploid, tetraploid, or rarely, androgenesis. From the perspective of morphology, grass carp hybrids from distant hybridization contained three types: expressing traits from both parents, mother carp-like or father carp-like. The growth rate of different hybrids varies, with different expressions: some showing potential for improvement, some similar to their parents, and some being intermediate between them. From the study perspectives of fertilization biology, embryonic development, and genetics, though heterogenous sperms could fertilize, distant hybridization of grass carp showed varying degrees of low fertilization rates, extended and abnormal embryonic development, low hatching rates, low and even zero survival rates of fry, which indicated nuclear-cytoplasmic incompatibility [46].

4.3. Studies on polyploid breeding of grass carp

Polyploid breeding was of significant importance in improving quality, accelerating growth rate, extending lifespan, increasing yield, regulating excessive reproduction, preserving species diversity, and developing new breeds of fish. Its application in aquaculture could bring substantial economic benefits [47]. In the mid-1970s, China initiated research on polyploid fish species as early as the mid-1970s. In 1976, the Institute of Hydrobiology of the Chinese Academy of Sciences reported the first successful induction of triploid and tetraploid grass carp through physical and chemical methods [48]. Later, researchers obtained triploid and tetraploid grass carp embryos by treating hybrids of blunt snout bream (♀) and grass carp (♂) and fertilized eggs of grass carp with colchicine and cold shock [49]. Xu Zhanning induced fertilized eggs of grass carp with heat shock treatment and obtained a certain proportion of triploid grass carp, but the overall proportion was not high [50].

5. Studies on germplasm identification techniques of grass carp

5.1. Morphological identification techniques

In 1998, Li Sifa et al. employed multivariate morphological analysis to identify the morphological characteristics of grass carp ranging from 24 to 88 cm in length. They selected 10 morphological parameters and established a discriminant program for populations of grass carp in the Yangtze River, Pearl River, and Heilongjiang River. The accuracy rate of discrimination was high ranging from 75.8 % to 100 % [4]. In 2005, Ding Shuquan et al. used traditional morphometric analysis to compare the morphological characteristics of four major Chinese carps and found that grass carp and other three fish showed small differences in the populations [51]. In 2011, Zhang Xi utilized discriminant analysis, cluster analysis, principal component analysis, and other polymorphic methods to analyze the possibility of large morphological differentiation among the populations of grass carp in six major water systems, including Poyang Lake, through the methods of Discriminant Analysis, Clustering Analysis, and Principal Component Analysis. The discrimination ratio was 76.4 %, and the morphological difference of grass carp populations in the six major water systems was primarily in the head and trunk [52].

5.2. Molecular identification techniques

Domestic aquatic researchers had used morphological markers, DNA molecular genetic markers, and protein markers to investigate the genetic structure and diversity of grass carp populations among the Yangtze, Pearl, and Heilongjiang Rivers and within the Yangtze River, including the wild and cultured grass carp populations. The findings were that the genetic diversity of grass carp was low, with wild populations having higher genetic diversity than cultured populations, thus providing a theoretical foundation for molecular-aided breeding of grass carp. In 2006, Zhang Zhiwei et al. compared the wild population of Hangjiang in Yangzhou and two cultured populations in Wuxi using TRAP technology, and discovered that the primer combination of the gene (Ga5-800-E5) showed a significant reduction of TRAP amplification in the cultured populations [53]. In 2008, Ding Weidong et al. used SRAP technology to study the wild population of Hangjiang in Yangzhou and two cultured populations in Wuxi, and found that SCAR1 was positively amplified in the cultured populations but not in the wild population, which provided a method for discrimination between wild and cultured populations of grass carp [54]. In 2020, Mao Zhuangwen employed microsatellite map technology and molecular markers, including *Hox* genes, to effectively distinguish between gynogenetic grass carp and common grass carp, thus providing a viable experimental approach for the identification of grass carp germplasm [55,56]. In 2022, a whole-genome bisulfite sequencing was performed for differential methylation analysis of 8 wild and cultured grass carp populations in different regions of Asia, thereby presenting new directions for the breeding of grass carp, including hybridization, disease resistance, and growth traits [57]. In 2022, Huang Weijie used polymorphic microsatellite loci for grass carp parentage analysis, which laid the foundation for further functional and applied research on SSR in grass carp and the breeding of grass carp [58]. In 2022, Wu Changsong proposed the innate immune phenotype of Phagocytic B cells and the possible regulatory factors that might be involved in their differentiation process through transcriptome, chromosome accessibility, and Genome-wide DNA methylation analysis, providing guidance for the improvement of genetic traits and disease prevention and control of grass carp [59].

6. Application of improved varieties of grass carp

High-quality varieties of grass carp were of significant economic and social value. The promotion of high-quality grass carp not only better protects aquatic environments, but also facilitates the sustainable development of the aquaculture industry.

As herbivorous fish, grass carp exerted a direct influence on aquatic higher plants in lakes. Its excrement could promote the proliferation of phytoplankton and cause changes in other environmental conditions of lakes, which in turn could impact the life of grass carp.

At present, high-quality grass carp were widely applied in the aquaculture industry. In 1986, Huang Xianzhi and Luo Shengping obtained high-quality grass carp varieties by hybridizing wild grass carp from the Yangtze River with grass carp from the Pearl River. The improved varieties were proven to have a high growth rate, strong disease resistance, and high yield, so they were widely promoted. According to data nationwide, they had high economic value [60]. In 2001, the Agricultural and Aquatic Station in the Shunde District of Foshan City, first introduced gold grass carp from Russia, which gained increasing market demand year by year with advancing breeding technology. As a result, many provinces also introduced gold grass carp. The gold grass carp was mainly made as sashimi for high profits [61]. In 2009, the College of Life Sciences of Hunan Normal University successfully bred a disease-resistant grass carp, characterized by strong disease resistance, rapid growth rate, fine meat quality, and a high survival rate, which won extensive recognition from fish farmers. The disease-resistant grass carp was subsequently introduced for large-scale aquaculture in Hunan, Shandong, Guangdong, and other provinces, showing a 50 % higher survival rate and a 10-15 % higher growth rate compared to common grass carp [62]. Experiments conducted by Wang Yude et al. revealed that improved varieties had stronger disease resistance than natural grass carp, and cultivating the disease-resistant grass carp could effectively reduce aquaculture costs and increase income. Comparative experiments on cage culture of the disease-resistant grass carp and ordinary grass carp, conducted by Wei Jianshan and Pan Jinxian, revealed that disease-resistant grass carp showed significant advantages in disease resistance and growth performance over ordinary grass carp, providing higher aquaculture benefits [63]. In 2010, Qinlu Aquatic Seedling Co., Ltd. bred pearl grass carp with hybridization technology, which was highly valued in the market due to its strong disease resistance, high survival rate, and ornamental value. Two years of experimental data on its culture showed that it had substantial economic benefits [64].

The ordinary grass carp, with its weak disease resistance and high breeding costs, had long been a concern for grass carp farmers. Therefore, breeding improved grass carp varieties not only was essential for promoting the development of the industry, but also holds enormous potential for economic benefits.

7. Conservation and utilization of grass carp germplasm resources

7.1. To strengthen research on grass carp germplasm resources

Germplasm resources of grass carp included common grass carp and golden grass carp. In addition to one kind of grass carp germplasm resources in China, there was one kind of gold grass carp in Russia, which belongs to Cypriniformes, Cyprinidae, Yaroideae and Grass carp. Because of its rapid growth, high yield, tender meat and delicious taste, it was used as a good material for sashimi in China, and could also be used as an ornamental fishery and recreational fishery, making it a very important germplasm resource. So far, the natural habitats of the grass carp had been disrupted and destroyed by human activities, including dam construction and overfishing, which had resulted in the degradation of their natural germplasm resources. Grass carp in the Yangtze River, in particular, tend to have good growth performance in optimal environmental conditions. The distribution of Yangtze River grass carp had become increasingly complex in various tributaries, yet there wasn't have systematic research on germplasm resources. Therefore, it was necessary to strengthen systematic domestic research on the genetic diversity and structure of grass carp populations in different waters, or even within the same water system. This could be achieved by employing various scientific theories such as resource ecology and management to undertake a comprehensive analysis. With a rising demand for grass carp fry, it was essential to conduct allopatric hybridization and breeding of grass carp from different water systems under rigorous experimental conditions to evaluate their farming performance objectively in varying ecological conditions.

7.2. To rationally utilize the germplasm resources of grass carp and outlook

On the basis of protecting the germplasm resources of grass carp, we need a timely and comprehensive understanding of the dynamic changes in the quantity and quality of the germplasm resources and suitable utilization methods. Fishing production required the management of fishing areas, the adoption of a reasonable fishing system such as a fishing permit system, and the implementation of a closed fishing season, to enhance administrative management and law enforcement in the fishery. In breeding production, we should select individuals or groups with good economic characteristics such as high growth rate and strong fecundity from populations in different water systems for breeding and culture, ensure the quality of the grass carp parent stock, maintain an adequately large effective in breeding population, prevent inbreeding and promiscuous hybridization, and preserve the ecological stability and genetic diversity of grass carp in different water systems. Furthermore, establishing natural reproduction reserves was also important in protecting the natural reproduction of grass carp. Notably, China, aimed at protecting germplasm resources, including grass carp, had implemented the 10-year ban on fishing for the Yangtze River and suitable fishing moratoriums in the basins of the Chinese top seven major inland rivers. In the next five to ten years, on the basis of the identification and traceability of grass carp germplasm resources, genome and transcriptome technology would be used to identify SNP markers of different varieties and parents, and carry out subgeneration protection for high-quality varieties, carry out reproductive performance assessment, ensure the continuation and utilization of high-quality germplasm, and promote the development of the grass carp breeding industry.

Funding

This work was supported by the National Key Research and Developmemt Program of China (2023YFD2401803), the Special Funds for Construction of Innovative Provinces in Hunan Province (2021NK1010), the National Natural Science Foundation of China (grant nos. 31430088 and 31730098) and the China Agriculture Research System of MOF and MARA (Grant No. CARS-45).

CRediT authorship contribution statement

Yude Wang: Writing – original draft, Funding acquisition. **Wuxia Liu:** Formal analysis. **Zhipeng Li:** Formal analysis. **Bin Qiu:** Formal analysis. **Jian Li:** Formal analysis. **Gen Geng:** Formal analysis. **Biao Hu:** Data curation. **Anming Liao:** Data curation. **Yanping Cai:** Data curation. **Ming Wen:** Data curation. **Shi Wang:** Formal analysis. **Qinbo Qin:** Formal analysis. **Kaikun Luo:** Data curation. **Shaojun Liu:** Writing – review & editing.

Declaration of competing interest

Shaojun Liu is the editor-in-chief for Reproduction and Breeding, Qinbo Qin is an editorial board member for Reproduction and Breeding and they were not involved in the editorial review or the decision to publish this article. All authors declare that they have no conflict of interest.

Acknowledgments

We would like to sincerely appreciate many researchers who helped complete this manuscript.

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