Advances in Genetics and Biotechnological Tools for Sustainable aquaculture: "A Review"

Abstract

India is leading country in promotion of biosciences, accelerating the pace of development in biotechnology in broad areas of agriculture, health care, animal sciences and environment. Worldwide, in food production there exists enormous challenges in agriculture and allied sciences to meet the demand of food for around 9 billion world's population. The deficit in world food production is met with alternative food resources to feed the billion mouths. While considering aquatic resources, the cultivated seafood production system has emerged as an alternative source of smart protein in food production system with quality fish production. Considering the advancements in aquatic food production system genetic application in fisheries and aquaculture plays a major role in vitalizing the blue revolution mission In India. The success has been achieved in fish breeding programme by selecting healthy and genetically superior broodstocks based on desirable traits such as growth rate, disease resistance, and fecundity. The induced breeding with use of hormones or other techniques lead to successfully breed the fish. In economically important species gene transfer work improved the possibility of producing rapidly-growing individuals through introduction of foreign growth hormones gene and production of genetically modified fish. The work on chromosome manipulation and gene transfer research witnessed the success in tissue culture system and production of new genetic material. The efforts in production of aquatic organisms over the last two decades through biotechnology is likely to switch over to commercial production in more valuable commercial species. The most commonly methods used in fish biotechnology are, the use of synthetic hormones in fish breeding, production of monosex, uniparental and polyploidy individuals to produce triploids, tetraploidy, haploids, gynogenic and androgenic using molecular biology and fish transgenics. The genetic engineering techniques lead to develop transgenic fish with high productivity and profitability. India is making accelerated developments in aquaculture production and sustainability improvement.

Keywords: Genetic enhancement, Biotechnology, Breeding, Fish, Molecular techniques, GMO

Introduction:

In recent decades, the global aquaculture industry has witnessed the remarkable growth, emerging as a vital source of protein with high quality products for a growing human population. The growth of the sector is due to technological advancements vitalizing the blue revolution in aquaculture and fisheries by developing high yielding fish and shellfish varieties. Aquaculture is the fastest growing farmed food production sector globally with remarkable growth and emerging as a vital food production system for growing human population (FAO, 2020. FAO, 2012). Aquaculture, the farming of aquatic organisms, is the fastest-growing food production sector globally and is critical for meeting the rising demand for seafood as wild fish stocks decline. Genetics and biotechnology provide powerful tools to enhance aquaculture productivity and resilience, making the industry more environmentally friendly and economically viable. The application of these technologies focuses on improving key traits in farmed species, optimizing nutrition, and reducing environmental impacts, thereby ensuring sustainable development. The selective breeding programs have successfully improved growth rates in species like Atlantic salmon and Nile tilapia by as much as 10-18% per generation. Modern techniques like gene editing using CRISPR-Cas9 allow for rapid and precise modifications of growth-related genes, such as myostatin (*mstn*), resulting in faster-growing fish that reach market size sooner. This leads to time reduction and resource utilization needed for production. In fish biotechnology genetic selection offer a more sustainable solution by breeding fish with natural resistance to common pathogens like sea lice or viral diseases. DNA vaccines, developed through biotechnology, also help prevent diseases, reducing the reliance on chemical treatments and improving fish health and welfare. Feed is typically the largest operational cost in aquaculture, and the reliance on fishmeal and fish oil from wild stocks is a major sustainability concern. Genetic selection for improved FCE means fish can convert feed into body mass more efficiently, requiring less feed to reach market size. Biotechnology also aids in developing alternative, plant-based feed ingredients and feed enzymes that enhance nutrient digestibility, thus reducing production costs and nutrient pollution from waste in water bodies. Genetically improved stocks can be developed to be more resilient to environmental stressors like temperature fluctuations or low oxygen levels, which are becoming more common due to climate change. Biotechnology helps prevent the potential ecological risks of escaped farmed fish by inducing sterility through chromosome set manipulation (triploidy) or gene editing, thus safeguarding wild populations and local biodiversity. Thus genetics and biotechnology are fundamental to the future of sustainable aquaculture. By enabling the production of healthier, faster-growing, and more resource-efficient fish, these scientific advancements allow the industry to meet the increasing global demand for seafood while minimizing its environmental footprint and ensuring long-term resilience and food security. The pervasive and enduring technical barrier to the sustainable growth of aquaculture is due to lack of improved strains capable of producing high-quality seed. The global aquaculture industry witnessed remarkable growth and is emerging as a vital source of protein source for growing human population. The genetic enhancement initiatives have gained prominence to improve the productivity, efficiency, and sustainability of aquaculture operations. By harnessing the power of selective breeding and genetic engineering, the initiatives made to develop strains of aquatic species with enhanced traits such as growth rate, disease resistance, and environmental adaptation in climate resilient food production system.

2. METHODS ADOPTED

Extensive reviews were made to understand fish biotechnology and aquatic sciences research practiced in India and various parts of the countries globally known. Fish biotechnology applications in genetic modification leading to increased productivity, marketability and customer acceptability reviewed. Conventional selective breeding, marker-assisted selection (MAS), quantitative trait loci (QTL) mapping, and environmental DNA (eDNA) assessed for optimization of production traits, and ensure the long-term sustainability. Various technologies enable the precise identification and manipulation of desirable genetic traits, such as disease resistance, growth performance, fillet quality, and environmental adaptability, leading to improved stock productivity and profitability examined in the review including work on chromosome manipulation research and development. The information gathered on data classification and grouping, ecological aspects, natural resource management, organic farming, and biodiversity. Qualitative descriptive methods were used to discuss the issues and relationships, and compared findings to understand the comprehensive and holistic approach in sustainable aquaculture development. Literature selection relevant to the research topic, data reliability, and topic-related journals, books, and research reports were referred.

3. DETAILED REVIEW

In Indian aquaculture system selective breeding lead to the development of strains with faster growth rates, improve production efficiency and reducing the efforts in husbandry practices. Fish genetics and biotechnology meets these challenges with new opportunities in cultivated seafood as an alternative protein source for human consumption and high value products. In recent decades, the global aquaculture industry has witnessed remarkable growth, emerging as a vital source of protein for a growing human population. The global aquaculture production has increased continuously over the last five decades. It has shown progressive growth and has become one of the fastest growing allied agriculture sector. It is estimated that about 16 million tonnes of fish will be required to feed the growing population of India by 2025 (Rasal et.al, 2017). Over the decades, the use of biotechnology overtook conventional techniques in commercially more valuable species. The most commonly used methods in fish biotechnology are the use of synthetic hormones in fish breeding, production of monosex, uniparental and polyploidy individuals which can be used to produce triploidy, tetraploidy, haploidy, gynogenic and androgenic fish. The techniques in molecular biology and transgenics supports the aquaculture nutrition and health management ultimately in the enhancement of aquaculture production and productivity. The sustainability in aquaculture has been evident with selective breeding with enhanced traits such as growth rate, disease resistance, and environmental adaptability for enhanced aquaculture growth in farmed food production sector globally (FAO, 2020). The yield of cultivated species and wild fish are dwindling in per unit of land and water due to overfishing and environmental degradation. Thus aquaculture has become increasingly important in meeting the growing demand for seafood overcoming with serious challenges of disease outbreaks, environmental impact, and improved genetic traits with growth rates and climate resilience. In India, Indian major carps are the most important common commercial fish species generating maximum market demand and acceptability as food by the consumers due to their taste and flesh. Among the Indian major carps, rohu is one of the most preferred species in the country and fetches a higher price in the market (Roy et al., 2013). The genetic improvement can contribute to more sustainable aquaculture practices by reducing the environmental footprint per unit of production requiring less feed and produce less waste, minimizing output in aquaculture operations (Gjedrem, 2000). The selective breeding programme in India could lead to the development of strains which grows faster, improve production efficiency and reducing the time required to reach the fish to market size. The improved genetic strains can also be utilized to improve the nutritional quality of farmed fish with increased levels of omega-3 fatty acids and other beneficial nutrients that can lead to healthier seafood products for the consumers.

The genetic enhancement can lead to develop fish strains that can be more resilient to changing environmental conditions, such as increased temperatures or fluctuating water quality parameters (Houston et al., 2020). It helps in genetic enhancement to develop strains with innate resistance or tolerance to common pathogens, reducing the impact of disease outbreaks, and minimizing the need of chemical treatments. The selective breeding programme improved tolerance to fluctuating water quality conditions equally important for maintaining stable production levels in aquaculture. In India carps are the main stay of freshwater aquaculture. Among the Indian major carps: catla, rohu and mrigal are contributing to around 70% of total freshwater fish production followed by silver carps, grass carps, common carp and catfishes forming a second important group contributing to 25% to 30% production (Chaudhari and Alikunhi, 1957). The genetically improved individuals developed efficient metabolic processes and stress tolerance thereby promoting resilience to ecosystem challenges. These strains have been well-adapted to their production environments by delivering optimal performance and productivity while minimizing the environmental stressors associated with the habitat by selecting individuals with greater adaptability to changing environmental conditions, In consumer market rohu is highly preferred species in India especially in Andhra Pradesh, West Bengal and Orissa states, practice carp polyculture with 90% rohu and 10% catla. Considering the consumer market rohu is selected candidate species under breeding programme. It has shown relatively good growth performance in multispecies carp culture system and low susceptibility. Many carp species have been widely cultivated in South and Southeast Asian nations among them rohu (*Labeo rohita*) in India (Gjerde et al. 2002; Mahapatra et al. 2007), silver barb (*Puntius gonionotus*) in Bangladesh and Thailand (Hussain et al. 2002) and common carps (*Cyprinus carpio*) in China, Indonesia, and Vietnam. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*) studies estimated with various genetic parameters as part of selective breeding programme (Ninh et al. 2011).The performance was started to genetically enhance rohu (*Labeo rohita*) growth at ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, Odisha, in collaboration with Institute of Aquaculture Research (AKVAFORSK), Norway in 1992. Five wild river strains selected and one locally farmed stock were chosen to create a base population (Reddy et al., 2002). The genetic selection in rohu as the model species exhibited slower growth in polyculture and was the most preferred fish species by consumers among other Indian Major Carps (IMCs). By understanding the primary goals of selective breeding and to acquire higher genetic diversity programme it was felt to demonstrate the survival rate with the breeding strategy adopted at various other field trial units. After eight generations of selection, the improved variety rohu, also referred to as “Jayanti," named in 1997 formally released on Swarna Jayanti, the eve of 50th anniversary of Indian independence. It has demonstrated a genetic gain of 18% per generation for growth traits tested extensively in different farms of rohu. The performance of Jayanti rohu was found better than the other strains used by farmers which resulted in superior performance in terms of body weight and survival rate. On an average farm, the improved carp strain showed 15% higher body weight at harvest in India and 36% higher in Bangladesh (Dey et al. 2010). The improved strains are found to be more profitable than the existing carp strains. This would increase the profitability of a hatchery which can afford to pay a higher price for genetically-improved brood stock (Kumar et al. 2008). The culture of Jayanti rohu is preferred over the normal rohu due to its higher growth performance and disease resistance (Das Mahapatra, et al. 2007). The performance of Jayanti rohu in monoculture system was 38.16% higher than that of normal rohu in monoculture system after the culture period of 11 months. The growth performance of Jayanti rohu in composite fish culture system was 17.72% higher than that of normal rohu in monoculture system (Sharma, 2015). The growth performance of ninth generation of Jayanti rohu studied in combination of three major carps catla, rohu and mrigal at different stocking densities indicates better performance of Jayanti rohu in terms of weight gain, daily growth rate, FCR, survival and production in comparison with non Jayanti rohu(Lucy Ingtipi, 2021). The net production of non- Jayanti rohu showed higher production in the control treatment which revealed that non-Jayanti rohu had no better growth performance over Jayanti rohu. In contrast to the above findings, Sah et al. (2018) reported that genetically improved rohu had a superior performance on growth and yield over the farmed rohu. Sharma (2015) reported that the growth of Jayanti rohu in both the culture conditions such as monoculture and polyculture with carps outperformed the local rohu. Genetic enhancement initiatives represent a cornerstone of modern aquaculture practices, offering a pathway towards increased productivity, profitability, and environmental stewardship. As these technologies continue to evolve the promise of reshaping the future of aquaculture (Chugambe et al., 2024). The improved G3 Rohu developed in collaboration with WorldFish through its Carp Genetic Improvement Program

(GIP) named generation-3 (G-3) Rohu in 2020 since 2012 by collecting wild seeds from the Halda, Jamuna, and Padma rivers at the initial stage. The brood stocks maintained at the privately owned hatcheries and reared in nurseries for assessment of growth performance. G3 rohu strain grows more than 37% faster than the conventional rohu strains is now available in the India and farmers are culturing in polyculture system of carp aquaculture. Progressive development observed in generation- of three multiplier (G3-multiplier) and control strains were spawned successfully at the WorldFish Carp Genetic Improvement Program (WFCGIP) facility, near Jashore, Bangladesh.

India's first genetically improved rohu, Jayanti- rohu with higher growth efficiency was developed with improved breeding programme of rohu with added disease resistance against *Aeromonas hydrophila* as a characteristic to sustain in adverse climatic conditions. According to Rahman et al. (2001), haemorrhagic septicaemia, dropsy, fin and tail rot, and ulcers are caused by the harmful rohu pathogen *Aeromonas hydrophila*. The expansion of aquaculture in India is likely to increase the cost of antibiotics and chemical with a result of the fish stock's increased disease resistance. The efforts of Department of Biotechnology in support of ICAR-CIFA collaboration could lead to the development of a vaccine named "CIFA-Brood- Vaccine that can prevent diseases and mortality of spawns and fry fish. The vaccine has been tested extensively in various hatcheries of Orissa and West Bengal confirming its efficacy in producing disease resistant spawns.

After carp, tilapia is the second most important freshwater fish in Asia. The efforts have been made to develop several better strains of tilapia. Fitzsimmons et al. (2011) stated that tilapia have the potential to overtake all other aquaculture species as the most significant species worldwide. The genetically improved farmed tilapia (GIFT) strain is an example that has been the focus of significant regional and international collaborations with organizations in Malaysia, the Philippines, Norway, and the World Fish Centre. ICLARM in 1988 in Malaysia started the first major Nile tilapia selection programme in the Philippines by renaming as GIFT, or Genetically Improved Farmed Tilapia. These fish were selected for high growth in the Philippines for six generations prior to 1996 (Bentsen et al. 2012). Early in the 1990s, the GIFT tilapia foundation population was established (Eknath et al. 1993, 2007; Bentsen et al. 1998). The GIFT strain was shifted to a Department of Fisheries, Malaysia, and research site in 2001 and since then, the selection programme has been continuing and by 2013, the GIFT fish had undergone 16 generations of selection. The GIFT strain is better than other strains that are available in India ((Nguyen et al. 2010). The GIFT strain's introduction is to provide high yields, quick growth, and other health conditions at a reasonable cost. Most of the Asian nations have widely used monosex GIFT strains of tilapia due to their value. The improved kind of Nile tilapia can withstand both in freshwater and saltwater without experiencing any negative effects on the fish's growth, FCR, or gill conditions. The GIFT tilapia can grow between 27 and 36% quicker than non-GIFT tilapia when mono and polyculture techniques are used. Furthermore, GIFT had survival rates 8.2–23.8% greater than local stocks and at present monosex (sex reversed or YY male) GIFT strain cultivation is commonly used under commercial production scientifically known as *Cyprinus rubrofuscus* (Dey and Modagdugu,2000)

Many strains of common carp have evolved due to a variety of factors, such as geographic isolation, adaptation, the accumulation of mutations, and pressures from both natural and human selection. Amur carp, one of the most extensively cultivated and highly domesticated fish species in the world. Among freshwater teleost, the largest family is Cyprinidae, which includes common carp is (*Cyprinus carpio*) followed by Amur carp known to be farmed for approximately 4,000 years in China, several hundred years in Europe, and then widespread throughout the rest of the world. Programme for selecting common carp resistant to dropsy, a serious infectious disease menace for farming was initiated and the breeding programme within the local and the Siberian wild carps from the river Amur, and crossing between them was made success in development of improved strain. DCFR imported improved strains of minor carps which has introduced in Indian waters are performing well with developed advanced feeds and recirculating aquaculture system (RAS) for sustainable development of coldwater aquaculture and conservation of hill stream fishes. A comprehensive review was conducted as part of the Department of International Development's (DFID) to evaluate the current genetic status of the common carp stocks and develop strategy for stock improvement by hatching and addressing the reproduction issues. The University of Stirling, University of Wales, UK, the former University of Agricultural Sciences, Bangalore, and the Karnataka Veterinary, Animal, and Fisheries Sciences University, Bidar, Karnataka collaborated in Asian carp breeding programme. The wild carp from Western Asian Rivers from Hungary were extended to UAS Hesaraghatta for assessment and breeding purposes, after seeing Amur common carp growing fast, accepts artificial feed, shown disease resistance and has similar food habit to that of existing stocks(Basavaraju et al,2003).

Indigenous efforts of CIFA has genetically improved strain of freshwater prawn '*Macrobrachium rosenbergii'* popularly known as Scampi. named as CIFA-GI Scampi after 14 years of generation from 2008-2022. This has enhanced the productivity and profitability of freshwater prawn culture. A collaborative endeavour of CIFA with WorldFish, Malaysia developed to address various aspects of aquaculture with major emphasis on disease obstacles. Freshwater prawn a significant crustaceans in inland aquaculture in the tropics is GFP is well suited for prawn polyculture system, commonly used by Asian smallholders to raise carp or tilapia (Zimmermann et al. 2010). The giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, was initially subjected to genetic improvement in 2007 in India following the increased recognition of the benefits of producing genetically modified strains of farmed carp and tilapia. Another approach of shifting on species cultivation in brackishwater was due to disease outbreak. This has compelled the importation of *P. vannamei* into Asia has been the apparent below quality performance, poor growth rate, and vulnerability to diseases of the two main native species of cultivated prawns, *P. chinensis* in China and *P. monodon*. The importation of *P. vannamei* into Asia has been the apparent to improve performance, poor growth rate, and vulnerability to diseases of the two main native species of cultivated prawns, *P. chinensis* in China and *P. monodon* in rest of the Asia. In India CIFA and fisheries research institutes giving emphasis on multiplier hatcheries that can produce around 400 million seed from the supplied brood seed. *Penaeus vannamei* breeding programmes initiated to increase disease resistance or tolerance sparked by increasing impact of disease to prawn farming globally(De Silva and Ranjula,2021). *P. vannamei* and *P. stylirostris* is providing benefits to Asian prawn farmer over *P. monodon*, regardless of the issues with disease spread. Genetic selection is seen to be a potential strategy against many diseases in *P. vannamei* and other shrimp species, as vaccination is not an option for shrimp. Prawns have successfully selected against the Taura virus and yellow Head Virus. The US Department of Agriculture (USDA) sponsored funds for the Oceanic Institute (OI) to run a selective breeding programme for Pacific white prawns from 1995 to 1998. *P. vannamei*, a specifically bred and pathogen-free (SPF) Pacific White prawn, is available in Latin America and the United States, legally imported SPF *P. vannamei* from overseas hatcheries in India produce seeds and sell them to farmers who cultivate shrimp by adhering to the regulations set forth by the Coastal Aquaculture Authority(CAA), a regulatory body in charge of managing the nation's shrimp industry. The majority of the prawns produced by the farmers, who cultivate them, are exported. The selective breeding program was initiated by AKVAFORSK in early 1970s by collecting fertilized eggs from Norwegian river population. The national breeding program on salmon and trout, salmon breeding shifted from growth rate to more complicated challenges, like disease resistance, in the 1990s with priorities of salmon breeders. This shift was driven by increased outbreaks of pancreatic necrosis virus (IPNV), disease resistance and challenge test against furunculosis. (Gjedrem, 2010)

Over 50 countries currently produce rainbow trout (*Onchorhyncus mykiss*), a fish that has been farmed for more than a century. Growth rate selection has proven to be quite effective; estimates of genetic gain per generation range from 10% to 13%. Over the course of two generations, a Finland breeding programme has selected for growth rate and early sexual maturation. Selection for a higher growth rate resulted in a 7% generation response. (John et al, 2006). Similar to Atlantic salmon, rainbow trout were shown to be negatively impacted by the IPN virus in some regions. Through selection process resistance of rainbow trout to IPN has been significantly enhanced in Japan. According to, a very sensitive strain had an average fatal outcome of 96.1%, whereas a resistant strain had an average mortality of 4.3%. Channel catfish breeding program has been developed at the USDA/ARS Catfish Genetics Research Unit at the Thad Cochran National Warmwater Aquaculture Centre in Stoneville, Mississippi (USA) lead to improve commercially important traits in channel catfish. It studies were focused on development of catfish germplasm with increased growth, feed efficiency, reproductive success, processing characteristics, and disease resistance. These efforts could achieve the production of female broodfish with spawning 30-50%, improving the reproductive efficiency to boost the percentage of spawning an important part of genetic improvement programme.

In aquaculture species, oysters are highest producing commodity globally, and they have a long history of being farmed in Asia, Europe, and America. The most widely farmed species is the Pacific oyster, or *Crassostrea gigas*. Selection has been done for higher live weight yield in a breeding programme on the West coast of the United States. Live weight yield is a function of both individual growth rate and survival. The yield improvements of families from selected broodstock compared to families from wild broodstock ranged from 0.4 to 25.6% in response to selection. Within the farming conditions of Sydney rock oyster populations (*Saccostrea glomerata*) in New South Wales, Australia, a parasite known as Marteilia sydney commonly causes severe mortality. In two generations of selection, the most improved breeding line had a reduction in mortality, which went from 85.7% in the control group to 63.5%. This indicates a 22% decrease in mortality following two generations of selection (Botta et al, 2020).

Although tools for selective breeding and genetic engineering have made advancement, it is still difficult to anticipate the long-term effects of genetic enhancement programmes in aquaculture due to scientific uncertainty. Earlier they used Zn finger nucleus (ZFNs) and Transcription activator like effect or nucleases (TALENS) have been used. but most recent ones have used CRISPR/Cas 9 editor proved provides a defence against bacteriophages and foreign DNA. The CRISPR from Streptococcus pyogenes and endonuclease Cas9 are introduced into a host cell with a synthetic small guide RNA (sgRNA) targeting a gene, creating a double-strand break in the DNA at a targeted site. All genome-editing tools employ site directed nuclease (SDN) technology to make a targeted DNA break. The host's non-homologous end-joining (NHEJ) or homology-directed repair (HDR) process relegates the double-strand break. Thus genetic enhancement in aquaculture brings continued innovation and refinement of genetic editing tools using CRISPR-Cas9, TALENs, and zinc-finger nucleases for precise manipulation of fish genomes. Novel genome editing techniques allow for efficient and targeted improvement of aquaculture stock and might be a solution to solve challenges related to disease and environmental impacts. These technologies are being used in all fields of science, including agriculture, animals, and fisheries. In humans, because of the completion of whole genome mapping work, efforts are being made on gene modulations to develop gene therapy technologies for treating severe, incurable disorders, including cancer. The application of gene editing CRISPER-cas technique has enormous potential in fisheries sector also following its applications in exploring in aquaculture and fish breeding. The gene editing tool could be used to enhance the traits in fish such as disease resistance, growth rate and fillet yield (Diwan et al, 2017, 2025). An innovative genetic technologies utilizing DNA-based tools are being developed for wider range of aquaculture species (Davis and Hetzel, 2000). Genome editing focus attempted on improvement of growth rate and disease resistance or achievement of reproductive confinement and other valued traits.

Marker-Assisted Selection (MAS) and Genomic Selection allow for the precise identification of superior traits, such as improved growth and disease resistance, accelerating breeding programs beyond traditional methods. In aquaculture MAS is the idea behind gene mapping in domestic animals and fish having possibility of employing gene maps to locate and map the genetic loci causing genetic variation in traits with significant economic implications. It is the process of using morphological, biochemical, or DNA markers as indirect selection criteria for selecting important traits in breeding programs in aquaculture(Sharma et al, 2024). This method for selection of better performing breeding individuals. MAS depends on identifying the link between a genetic marker and Quantitative traits loci. This technique identifies specific DNA sequences associated with desirable traits, enhancing the accuracy and efficiency of selection processes. Aquaculture industries can optimize production traits, ensure sustainability, and meet market demands more effectively, with enhanced productivity and profitability.

Quantitative trait loci (QTL) mapping:

Quantitative Trait Loci (QTL) mapping in aquaculture is another sophisticated genetic technique aimed at identifying regions of the genome associated with quantitative traits of economic importance. By analyzing the inheritance patterns of phenotypic variation across populations, QTL mapping enables the detection of genomic regions influencing traits such as growth rate, disease resistance, fillet quality, and stress tolerance. It plays a crucial role in accelerating genetic improvement programs in aquaculture by providing insights into the genetic architecture underlying complex traits, thereby guiding selective breeding efforts towards more efficient and sustainable production systems.

Environmental DNA (e-DNA) monitoring:

Environmental DNA (eDNA) analysis is an emerging molecular approach for species identification from samples containing cellular DNA and extracellular DNA sloughed off all living organisms. It has ability to identify the existence and dispersion of aquatic and terrestrial organisms revolutionized by environmental DNA, or eDNA. Environmental DNA (eDNA) meta barcoding is a relatively new monitoring tool featuring in an increasing number of applications such as the facilitation of the accurate and cost-effective detection of species in environmental samples. eDNA analysis has been successfully employed to detect and monitor eukaryotic macro- and microbial communities and populations and is a useful tool for early monitoring systems as it allows for more accurate and standardized detection of species that are cryptic, inaccessible and of low abundance. The main vision of aquaculture biotechnology is to achieve improvements of aquaculture stock, preservation of genetic resources, disease diagnosis, and control of microbial/microalgal genetic engineering (Nwokwa, 2012). It is also to ensure the safety, environmental sustainability, and ethical considerations effectively addressed in research and development. The application of genetic enhancement technologies in aquaculture require strong regulatory frameworks. DBT developed Biotechnology Strategy outlines India to strength and confidence in delivering a knowledge driven bio economy with a mission to make India globally competitive in biotechnology research. The efforts are also focused on aquaculture and marine biotechnology development (https://dbtindia.gov.in). The contribution of aquaculture to global food production and security has been widely acknowledged today. Global aquaculture production has increased continuously over the last five decades, and particularly in India, aquaculture is the fastest growing agri-sector supporting the Indian economy. At present, the most commonly used methods in fish biotechnology are the use of synthetic hormones in fish breeding, production of monosex, uniparental and polyploid individuals which can be used to produce triploids, tetraploids, haploidy, gynogenetic and androgenetic fish and transgenics. The advances in biotechnology research supports aquaculture nutrition and health management, gene banking including chromosome manipulation etc.

Worldwide, intensive breeding initiatives designed to improve desired features in cultivated populations. The decrease in genetic variety may make aquaculture systems less resilient to change over the long term by making them more vulnerable to infections and environmental stressors. Moreover, consumer acceptance, demand and market for genetically modified or improved organisms can be greatly influenced by consumer views. In order to increase customer trust and confidence in GMO aquaculture products, transparency, labelling, and education initiatives are required. GMO fish in India are strictly regulated under the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells, 1989" (Rules 1989) of the Environment (Protection) Act, 1986. The Genetic Engineering Appraisal Committee (GEAC) under the Ministry of Environment, Forest & Climate Change (MoEFCC) acts as the apex body for approval of any GMO activity, including import, transport, and commercial release. In Asian countries including, India research are engaged in transgenic fish by establishing the collaborative network to develop protocols to conduct sound and safe research on transgenic fish that would assure the benefits rather than having devastating effect due to gene transfer techniques(Pandian,2001). This would help in long term environmental impact that have undergone genetic modification or enhancement may have unintended creating the ecological impacts, affecting biodiversity, ecosystem dynamics, and non-target species. In India, the field of aquaculture genetics research is relatively new. The emphasis was on assessment of carp hybrids with exclusive focus on variety improvement including gene mapping, hybridization, selective breeding, and genetic characterization with marker assisted selection (Chugambe et al., 2024).

According to United Nations Food and Agriculture Organization global fish production continues to outpace world population growth, and aquaculture remains one of the fastest-growing food producing sectors. It is unlikely that the increasing demand can be met through increased natural harvest as many of natural Ocean and freshwater fisheries are being harvested to their limit (FAO, 2014). Aquaculture could help to meet increasing demand, and biotechnology can make a great contribution to improve aquaculture yields. The responsible development and practice of aquaculture can generate long lasting benefits for global food security and economic growth (Naylor et al., 2000, Pauly et al., 2002).Genetic improvement contribute to sustainable aquaculture practices by reducing the environmental footprint per unit of production. Faster-growing fish strains require less feed and produce less waste, thus minimizing the environmental impact of aquaculture operations with selective breeding programme adopted in aquaculture (Gjedrem, 2000. Gjedrem and Baranski, 2009). Genetic enhancement can also be utilized to improve the nutritional quality of farmed fish, such as increasing levels of omega-3 fatty acids or other beneficial nutrients. This can lead to healthier seafood products for consumers. Climate change and other environmental factors pose challenges to aquaculture. It also help develop fish strains that are more resilient to changing environmental conditions and water quality parameters (Houston et al., 2020).

Fish research includes genomics and genetic engineering to understand the genetic makeup of fish and develop genetic engineering techniques to enhance desirable traits such as growth rate, disease resistance, and tolerance to stress. This gene-editing tool enables precise modifications to fish genomes, allowing development of superior varieties with improved growth rates and disease resistance. Researchers are exploring the use of nanotechnology to enhance fish production and health management, including the development of nano-biosensors for detecting water quality and disease-causing pathogens to focus on understanding the genetic and protein composition of fish and their environments, providing insights into the complex interactions between fish, their microbiota, and the environment. The development of sustainable and nutritious fish feed that enhance feed efficiency and reduce feed costs also incorporating probiotics and prebiotics to improve fish health and productivity the research driven by institutions in India under ICAR setup. Overall, fish biotechnology holds great promise for improving the sustainability and productivity of aquaculture industry in the coming years.

Fish biotechnology has opened new windows for development of genetic resources in aquaculture. The genetic technologies can be utilized in aquaculture to improve production, cultivability and the conservation of natural resources (Moses et al., 2005). Biotechnology has the potential to enhance reproduction and the early developmental success of culture organism by extending tools necessary for artificial manipulation of genes and chromosomes in living organisms. The development of transgenic fish and shellfish created interest in aquaculture research due to huge improvement potential (Zbikowska, 2003; Dunham, 2004). The major areas of transgenic research in fish include use of growth hormones (GHs) to increase growth and feed conversion efficiency; use of antifreeze proteins (AFPs) for enhanced cold tolerance and freeze resistance; use of antimicrobial peptides for increased disease resistance; use of metabolic genes with promising success to promote low-cost, land based diets; and genetic methods for induced sterility. The success has been promising. The GMO salmon was developed by inserting genes from a Chinook salmon and an ocean pout into an Atlantic salmon to enable it to grow twice as fast as an Atlantic salmon. The amount of feed required to produce the same fish biomass is 25% less for the GM fish than a conventional Atlantic salmon.

The idea of producing transgenic animals became popular when Palmiter et al., (1982) produced transgenic mouse in the begging by introducing metallothione- in human growth hormone fusion gene (mT-hGH) into mouse egg, resulting in dramatic increase in growth. This triggered a series of attempts on gene transfer in economically important animals including fish. A foreign gene can be transferred into fish *in vivo* by introducing DNA either into embryos or directly into somatic tissues of adults (Hew, 1995). Direct delivery of DNA into fish tissues is a simple approach, providing fast results and eliminating the need for screening transgenic individuals and selecting germ line carriers. Gene transfer and expression following intramuscular direct injection of foreign DNA into skeletal muscles of fish has been successfully achieved (El-Zaeem, 2004). Although noteworthy progress has been achieved in several laboratories around the world, there are numerous problems to be resolved before the successful commercialization of the transgenic brood stock for aquaculture. Also to realize the full potential of the transgenic fish technology in aquaculture, several important scientific break-through are required. As an initial success in salmon the efforts need to be continued on commercialization process. The GM salmon has been introduced with great caution by taking care that the environment and human health to be protected as GE opponents spread falsehoods about the likely adverse health and environmental dangers with the growing fishing industry. The need of the hour is to adopt advanced GM technology as the fishing stocks have been suffering due to overfishing and pollution of the seas and due to global warming. As per future judgment by present trends of worldwide fishing, ocean fish stocks projected to be depleted by 2050. Although it appears safe to consume GM salmon, advance research is mandatory to ensure the safety of genetically modified fishes as GM food to meet the need of the promptly growing population of our earth planet (Benessia and Barbiero, 2015). India has actively developed transgenic fish, starting in 1991, with significant research at Madurai Kamaraj University and CCMB, Hyderabad. Experiments focus on faster-growing, transgenic, auto-transgenic rohu (*Labeo rohita*), zebra fish, catfish, and singhi, with some transgenic rohu growing 6–8 times faster than controls (Pandian, 2003).

Chromosomal Engineering

In India, chromosome sex manipulation techniques introduced to induce polyploidy (triploidy and tetraploidy) and uniparental chromosome inheritance (gynogenesis and androgenesis) extensively in cultured fish species (Pandian and Koteeswaran, 1998; Lakra and Das, 1998). These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control, improvement of hybrid viability and clonation. Induced triploidy widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management and the only practical means in which to sterilize large numbers of fish without using of potentially harmful chemicals or radiation (Benfey, 1989., Kizak et al., 2013., Lakra and Ayyappan, 2003). The culture of triploid fish noticed increased growth, increased carcass yield, better survival and flesh quality. Triploids would reach a larger size than diploids because of their larger cell size (Dunham, 2004).It can be achieved with temperature shock (hot or cold), hydrostatic pressure shock, chemicals (such as colchicine, cytochalasin-B or nitrous oxide), and the crossing of tetraploids with diploids. Tetraploids have a balanced set of chromosomes, which can result in viability and fertility. Tetraploidy in fish is commonly produced by disrupting the first cleavage with thermal or hydrostatic pressure shocks in eggs fertilized with normal sperm. Tetraploid breeding lines are of potential benefit to aquaculture by providing a convenient way to produce large numbers of sterile triploid fish through simple crosses between tetraploids and diploids (Guo et al., 1996., Pandian et al., 1999). Thus advances in chromosome manipulation alternative to GM has high potential for improving production in the aquaculture industry, particularly in the case of shellfish. The uses of polyploidy in aquaculture resulting sterility, along with enhanced growth and survival rates and increased quality of final products. This has improved color, growth and maturation in ploidy-manipulated fancy carp (Taniguchi et al, 1986) The development of improved fish seed stocks can contribute to increased fish production as one of the key solutions to meet the future food demands of the growing world population (Renu Singh and Babita Rani, 2020).

Sex control and hormonal Sex Reversal

The use of sex control techniques influence the characteristics of economically desirable teleost species to increase aquaculture production. Techniques that allow production of monosex population by sex manipulation are potentially useful in species where one sex is more useful than the other. There are basically two ways of sex manipulation i.e. hormonal and genetic. The production of single sex groups of fish can be accomplished by manipulation of the developing gametes and embryo (FAO, 2014). This method lies on the fact that at the stage when the fish larvae are said to be sexually undifferentiated (right after hatching up to about 2 weeks or up to the swim-up stage), the extent of the androgen (male hormone) and the estrogen (female hormone) pre-sent in a fish is equal The artificial elevation of the appropriate sex hormone is sufficient to overcome the natural hormone or gene product during the period of sexual differentiation and to dictate the sex of the individual. The production of single sex groups of fish can be accomplished by manipulation of the developing gametes and embryo (FAO, 2014). The principle behind this method lies on the fact that at the stage when the fish larvae are said to be sexually undifferentiated (right after hatching up to about 2 weeks or up to the swim-up stage), the extent of the androgen (male hormone) and the estrogen (female hormone) present in a fish is equal (Fuentes et al., 2013). The artificial elevation of the appropriate sex hormone is sufficient to overcome the natural hormone or gene product during the period of sexual differentiation and to dictate the sex of the individual (Dunham, 2004).

Hybridization:

Fish culture today is hardly possible without the artificial propagation of fish seeds of preferred cultivable fish species. Apart from being able to obtain quality seed, the artificial propagation technique can also be used to develop strains superior to their ancestors by the methods of selective breeding and hybridization (Akankali et al., 2011).Hybridization enhanced heterozygosity and improve growth and other desirable characters such as developmental compatibility, food conversion efficiency, and oxygen metabolism in a variety of species (Danzmann et al., 1985). Hybridization attempts to produce fish that combines valuables traits from more than one species or high heterosis (hybrid vigour) (Aluko, 1993). Hybridization evolve a hybrid or strain of superior quality than the parent species. In Nigeria, *Clarias gariepinus* and *Heterobranchus bidorsalis* have been crossed to produce a sterile hybrid which possessed the hardiness of Clarias and the fast growth of Heterobranchus. Hybridization attempts to produce fish that evolve a hybrid or strain of superior quality than the parent species. In Nigeria, *Clarias gariepinus* and *Heterobranchus bidorsalis* have been crossed to produce a sterile hybrid which possessed the hardiness of Clarias and the fast growth of Heterobranchus. The induced breeding methods constitute a major practicable means of providing enough quality seed for rearing in confined enclosure such a fish ponds, reservoirs and lakes (Charo and Oirere, 2000).In India the induced breeding of fish is successfully achieved by the development of Gonadotropin releasing hormone (GnRH) technology (Lakra and Ayyappan, 2003). It is a decapeptide with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH) and is the key regulator of reproductive cascade in all vertebrates. (Schally, 1973 Bhattacharya et al., 2002).

Molecular markers

Genetic markers can be used to identify individuals and family groups so that they can be reared together thus simplifying experimental designs. Recent advances in molecular biology have provided unlimited number of genetic markers which have multiple application in aquaculture and fisheries (Lakra, 2001). Mitochondrial DNA has provided a wealth of genetic markers to answer questions on the phylogeny, evolution and population structure of fishes. The application of the new DNA based technologies is to identify marker loci which are associated with nuclear loci that control economically important traits (quantitative trait loci or QTLs). These markers identified they can be used in selection programmes. An approach towards his marker assisted selection (MAS) in fish has been made in rainbow trout by Herbinger et al., (1995). The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishes, mollusks crustaceans for aquaculture.

Cryopreservation of gametes or gene banking

The problem of males maturing before females is a serious concern. Cryopreservation overcomes selective breeding and stock improvement and enables the conservation of genomes (Harvey, 1996). The emerging requirements for undertaking gene banking of aquatic resources is the need to build a genetic base collection that can be used by breeders for evolving new strains. Hormonal treatments that regulate the action of genes and modify the sex of offspring are now widely used in fish culture programs. Gene transfer work in many fish species, stimulated by the possibility of producing rapidly-growing individuals through the introduction of foreign growth hormone genes, has produced modified fish throughout the world. The production of cells containing new gene arrangements introduced by chromosome manipulation or by gene transfer, could be undertaken in tissue culture to give new combinations of genetic material. Furthermore, the organization of gene banks based on collections of frozen sperm and cells or on purified DNA molecules, obtained from different fish species and could play an important role in the future.

Strategic approach for development:

Fish biotechnology research and development in India has made significant progress in recent years, with a focus on improving fish production, productivity, and sustainability. In areas of research viz; genetic improvement of fish through selective breeding, hybridization, and genetic engineering to enhance growth rate, disease resistance, and tolerance to stress. The efforts lead to development of vaccines against major fish diseases, such as *Aeromonas hydrophila* and *Edwardsiella tarda*, to reduce disease outbreaks and improve fish health. Research on fish nutrition and feed development to improve feed efficiency, reduce feed costs, and promote sustainable aquaculture practices and development of diagnostic tools and management strategies for fish diseases, including molecular diagnostics and biosecurity measures. Further emphasis on research on aquaculture biotechnology, including the use of probiotics, prebiotics, and other biotechnological tools improving fish health and productivity.

The strategic development plan for fisheries development in India needed with improved fish strains and productivity with the following key components:

1. Introduction of Improved Fish Strains, genetically modified fish strains like GIFT (Genetically Improved Farmed Tilapia) to enhance productivity and disease resistance.

2. Develop capacity building programme to provide training and capacity-building for fish farmers, hatchery operators, and extension workers on modern aquaculture practices.

3. Strengthening Infrastructure Development by investing in infrastructure development, including hatcheries, feed mills, and cold storage facilities.
4. Adopt Sustainable Aquaculture Practices to promote sustainable aquaculture practices, including integrated multi-trophic aquaculture (IMTA) and recirculating aquaculture systems (RAS).
5. Establish genetic improvement programs for indigenous fish species to enhance their productivity and disease resistance.
6. Develop domestic and international markets for Indian fish and fish products.

Conclusion:

Biotechnology has the potential to enhance reproduction and the early developmental success of culture organism. A new avenue for the development of genetic resources in aquaculture has been made possible by genetic improvement approaches. Aquaculture is benefitting with application of genetic modification technologies for various reasons, including increased productivity, marketability, cultural ability, and resource conservation customer acceptability issues. The pivotal role in advancement addressing the challenges is being paid for maximizing production efficiency and promotion of industry. Through selective breeding, marker-assisted selection (MAS), quantitative trait loci (QTL) mapping, and environmental DNA (eDNA) monitoring, aquaculture stakeholders are empowered to expedite genetic progress, optimize production traits, and to ensure long-term sustainability. These technologies enable the precise identification and manipulation of desirable genetic traits, such as disease resistance, growth performance, fillet quality, and environmental adaptability, leading to improved stock productivity and profitability. Chromosome manipulation, resulting in sterile polyploidy individuals or monosex inbred lines, constitutes an important tool. DNA markers are being used to study stock identification and population differences, in gene mapping studies, and potentially as aids to selective breeding programs. The application of genetic engineering and molecular biology thus enhancing the production and productivity in successful aquaculture husbandry practices with performance improvement.

Highlights:

The review focusses on development of improved strains to enhance productivity and disease resistance.

Promote research and development for improving fish production, productivity and sustainability.

Strengthen genetic improvement through various approaches of selective breeding, MAS, QTL, chromosome manipulation and genetic engineering.

Identify desirable traits for higher growth, disease resistance and environmental adoptability.

Give emphasis on GE and molecular biology and biotechnology research for sustainable aquaculture development.

DATA SHARING

Data sharing is not applicable in this article as no new data were created or analyzed in this review article.

DECLARATION

The review has not been published previously/ under publication consideration elsewhere.

ETHICAL APPROVAL

This being a review paper and as such the clearance of ethical committee was not required.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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Competing interests

Author has declared that no competing interests exist.

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