ISSN: 2582-6980

AGRI MIRROR FUTURE INDIA

AIASA Agriculture Magazine

A Voice for Agriculture

Dr. M S Swaminathan, Chief Patron, AIASA
Bharat Ratna Award

Issue 01 / 2024

aiasanewdelhi aiasa5 AIASA https://aiasa.org.in/?page_id=2851

A Voice for Agriculture

AGRI MIRROR : FUTURE INDIA

Issue 01 | 2024

Article Id.	CONTENTS	Page No.

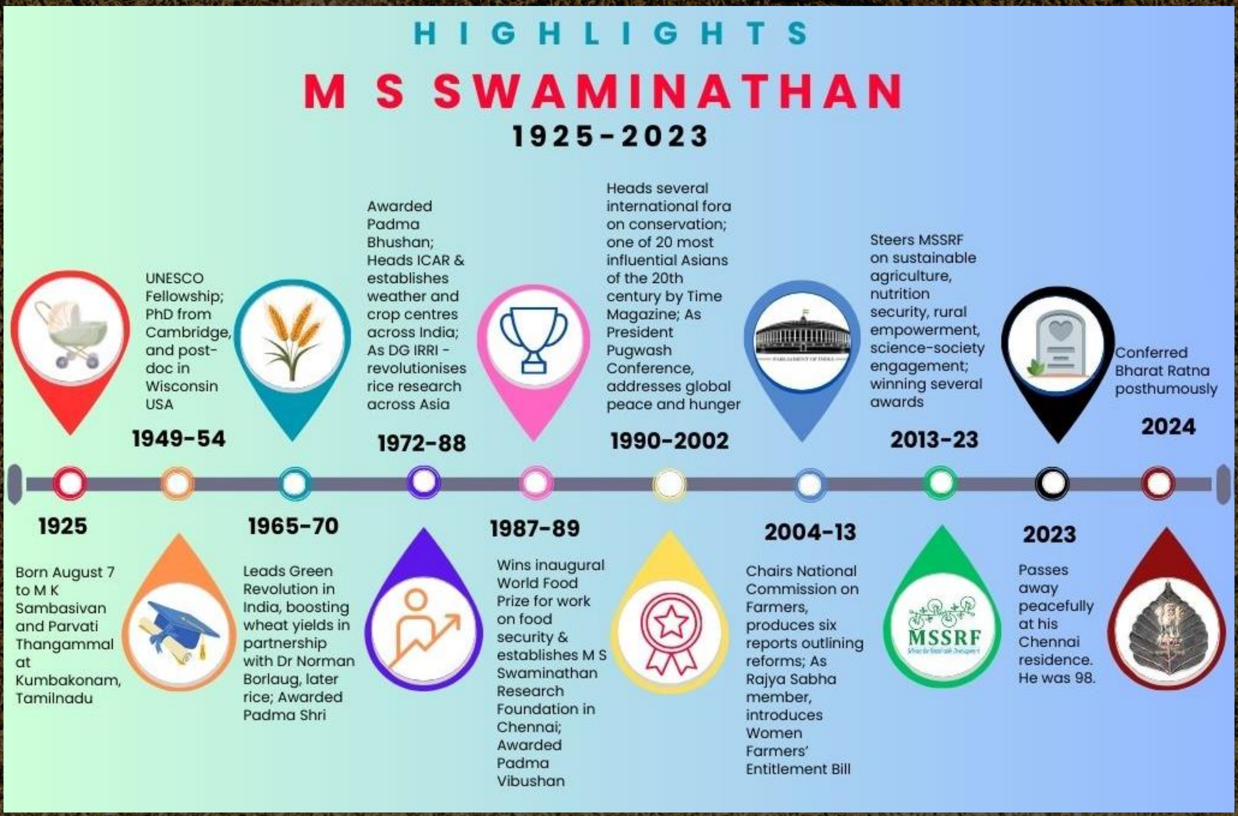
1925 

Remembering the Legacy of Prof. M.S. Swaminathan

 2023

Hon'ble Prime Minister of India, announced the well-deserved **Bharat Ratna** for Prof M S Swaminathan on February 9, 2024. It's a great recognition for his untiring work to ensure food, nutrition and livelihood security for all. Swaminathan was born in Kumbakonam, Thanjavur district (Madras Presidency) on August 7, 1925, to M K Sambasivan and Parvati Thangammal. M K Sambasivan was a general surgeon, and he passed away when Swaminathan was 11 years old. After his father's death, Swaminathan was looked after by his paternal uncle. Swaminathan was educated at a local high school and later at the Catholic Little Flower High School in Kumbakonam. Even as a child, he enjoyed spending time in the farm lands and with the farmers. He witnessed the impact fluctuations in crop prices had on his family, and the damage erratic weather caused on crops as well as the hardship farmers had to face because of poor incomes.

His family preferred that he take up medicine and be a doctor like his father, but Swaminathan took up Zoology at Travancore University in 1944. The shortage of food during World War II and the Bengal famine of 1943, had a tremendous influence on Swaminathan. He turned to agriculture, and earned his second B Sc from Coimbatore Agricultural College, Madras University in 1947.



If agriculture goes wrong, nothing else will have a chance to go right.

AMFI/1/2024

PROBIOTICS IN AQUATIC ECOSYSTEM

S. Saravanan, A. Uma and D. Kaviarasu
Department of Aquatic Animal Health Management,
Dr. M. G. R Fisheries College and Research Institute, Ponneri 601 204,
Tamil Nadu, India. Tamil Nadu Dr. J. Jayalalithaa Fisheries University.
Corresponding author: saravanan@tnfu.ac.in

Introduction:

Aquaculture has grown tremendously during the last few years becoming an economically important industry. With the increasing intensification and commercialization of aquaculture production, the disease is a major problem in the fish farming industry. The marine fisheries stocks have declined worldwide in general and provided an impetus for rapid development in fish and shellfish farming. The production process in shrimp aquaculture is determined by biological, technological, economic, and environmental factors. However, the utility of antimicrobial agents as a preventive measure has been questioned, given extensive documentation of the evaluation of antimicrobial resistance among pathogenic bacteria. On the other hand, antibiotics inhibit or kill beneficial microbiota in the gastrointestinal ecosystem but they also made antibiotic residue accumulated in fish products to be harmful for human consumption. Another important problem faced by our Indian shrimp culture farmer is excess ammonia in pond water and sediment which is caused by excess feed, faecal matter, and dead algae deposited in the bottom of the pond. Due to this, shrimps are exposed to toxic gases like NH3, NO2, and H2S further leading to eutrophication in the culture system and causing stress to the animal, and ultimately ends with microbial diseases and high mortality occurs.

One of the most significant technologies that evolved in response to disease control problems is the use of probiotics. The term probiotic means life; it was derived from two Greek words 'pro' and 'bios'. Probiotics are live microbes that can be used to improve the host intestinal microbial balance and growth performance. The development of probiotics in aquaculture management will reduce the use of antimicrobial drugs which were prophylactic alone and over-dependence in recent times poses potential hazards to the man who consumes them. This study summarizes the probiotic strains with relevance to algae and bioremediation processes and their significance in aquaculture. The alternative to antibiotics has prompted scientists to search for probiotics in controlling diseases in shrimp farms.

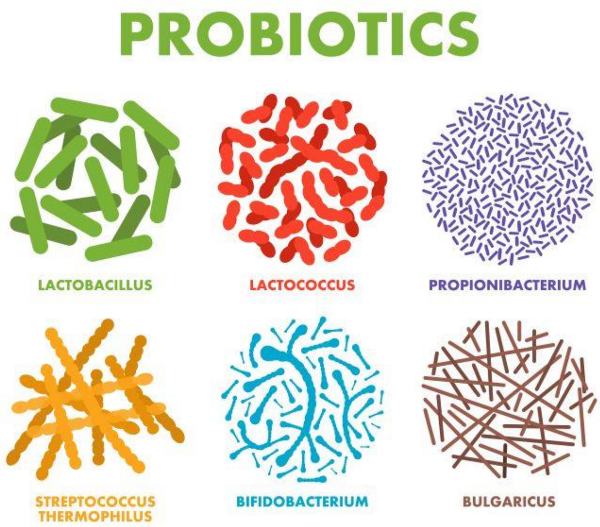


Figure 1. Example for probiotic bacteria in aquatic ecosystem

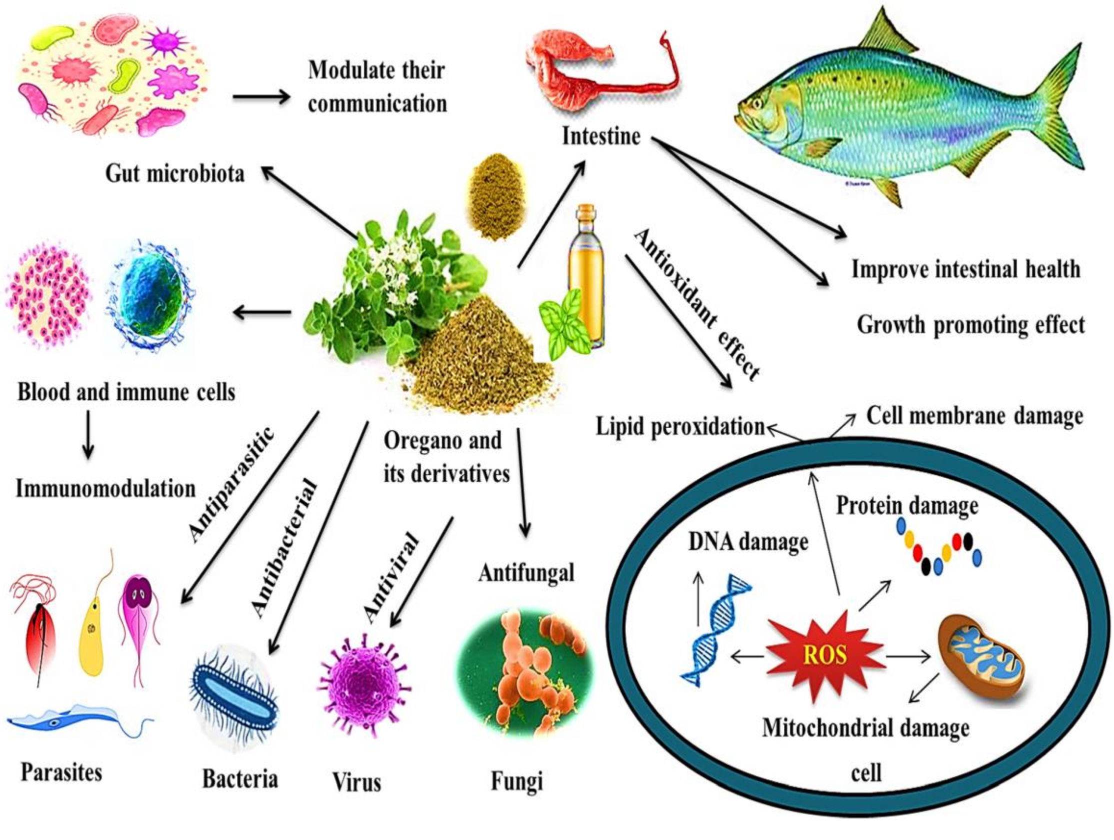


Figure 2. Importance of probiotic bacteria in aquatic animals

Aquatic Ecosystem and Probiotics:

Nowadays, probiotics are used in aquaculture to regulate micro dysbiosis, maintain microeubiosis, raise the health level of hosts, and promote proliferation enhancing their metabolic products in microecology. The other is to decompose organic matter (faeces, wastes, remnants, or leftovers of feed), to maintain dynamic ecological balance among organisms from the five kingdoms in nature and microbial ecological equilibrium in water and sediments, and to create a friendly environment for fish and shrimp growth. Microorganisms are broadly classified into microalgae, bacteria, fungi, and viruses. Photosynthesis by absorbing CO_2 therefore, supplying oxygen to aquatic animals is done by microalgae. The decomposition of organic matter is done by bacteria and fungi in sediments to maintain good water quality. However, due to the lack of oxygen, there might be anaerobic decomposition or fermentation in sediments, producing harmful gases such as hydrogen sulfide and methane. There are two food chains in the aquatic ecosystem, the detritus food chain and prey–predatory food chain.

Microbes are the predominant photosynthetic organisms in most aquatic environments. Algae, diatoms, and cyanobacteria predominate in aerobic conditions (e.g., Shallow water), whereas other photosynthetic bacteria are dominant in anaerobic conditions (Polluted or eutrophic waters), There is a big difference between natural aquatic ecosystems and man-made aquatic ecosystems, especially in intensive culture with high stocking density and high input. The water body in a high-stocking density fishpond often in a small area has a limited self-purification capability. The need to add probiotics in various stages is required for good water quality to reduce the prevalence of diseases. The infusion of probiotics in the water body primarily helps in the multiplication of beneficial bacteria and competitive inhibition of pathogens takes place which encounter the water system via various sources such as air, feed, faecal matter, etc.

Beneficial Bacteria:

The luminous bacteria *Vibrio harveyi has* been considered a devastating disease in aquaculture practices and is the largest economic loss in shrimp aquaculture due to mass mortalities. The role of probiotics emerges as prophylaxis to control the pathogens in the aquaculture system for its environment and eco-friendly nature. The growth rate and survival of shrimp are currently enhanced by using beneficial microbes which cater to a wide range of benefits. In general, the gastrointestinal tract (GIT) of aquatic animals is mainly composed of gram-negative bacteria. Researchers also have demonstrated the use of beneficial bacteria in aquaculture to improve the water quality and immune system by balancing bacterial flora in water and reducing the pathogenic bacteria load.

The attempt to isolate beneficial bacteria from various sources like soil, water, and animal gut to control disease-causing pathogens in aquaculture systems has been practiced. The wide range of beneficial bacteria used in the aquaculture system belongs to the taxa *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, Lake *Streptococcus* sp, and *Carnobacterium* sp. *Bacillus*, *Flavobacterium*, *Cytophaga*, *Pseudomonas*, *Alteromonas*, *Aeromonas*, *Enterococcus*, *Nitrosomonas*, *Nitrobacter* and *Vibrio* spp., and *yeast Saccharomyces*, *Debaryomyc*. While using some beneficial bacteria for fish, some might be highly pathogenic *Vibrio alginolyticus* and lead to destructive effects in the aquaculture systems. Therefore, it is necessary to take care of the choice of beneficial microbes before administration. The best-known beneficial bacteria such as *Bifidobacteria* species, *Lactobacillus* sp, and *Streptococcus thermophilus* are employed as dietary supplements with feed in the aquaculture industry and increased the efficiency and sustainability of aquaculture production. Beneficial microbes thus fulfil the concept of sustainable growth and production of shrimps by minimizing or de-promoting the growth of the disease-causing harmful pathogens.

Beneficial Microalgae:

The water quality of the pond environment is determined by the dominant presence of diatoms. The species such as *Pyrrophyta* sp., *Cyanophyta* sp., and *Cryptophyta* sp. dominate the pond water due to the increase in organic pollution compared to diatoms. The diatoms are considered the best nutritional requirement for the post-larvae of Penaeus shrimp and also possess antibacterial activity. The diatom *Thalassiosira* acts as a good nutritional source of food and the *Chaetoceros* sp can rapidly reduce ammonia nitrogen. To maintain good pond water quality infusion of diatoms before probiotic application yield good results. However, diatom inoculation is not recommended due to its specific growth requirements and requires specialized environmental conditions. The kind of ecological environment that is required to promote the growth of diatoms has not been reported so far. Austin *et al.* reported a kind of microalgae (*Tetraselmis suecica*), which can inhibit the pathogenic bacteria in fish. *Teraselmis suecica* was observed to inhibit *Aeromonos hydrophila*, *A. salmonicida*, *Serrstia liquefaciens*, *Vibrio anguillaram*, *V. salmonicida* and *Yersnia ruckeri* type I. When used as a food supplement, the algal cells inhibited laboratory induced infection in Atlantic salmon. When used therapeutically, the algal cells and their extracts reduced mortalities caused by *A. salmonicida*, *Serratia liquefaciens*, *V. anguillaram*, *V. salmonicida*, and *Yersnia ruckeri* type I. They suggested that there may be some bioactive compounds in the algal cells, and there appears to be a significant role for *Tetraselmis* in the control of fish diseases.

Biodegradation of Grow Outs:

Bioremediation using beneficial microbes either by single strain or combinations of strains is helpful to reduce the effluents from water bodies in the aquaculture system and other industries. Some of the advantages of bioremediation include 1. On-site process implementation, 2. Permanent excess waste elimination can be implemented and 3. Cost-effective due to its biological nature.

Aquaculture Grow Outs and Microbial Biodegradation:

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis B. licheniformis*, Nitrobacter sp., and Aerobacter and *Saccharomyces cerevisiae*. These water probiotics in tanks and ponds will play a crucial role in fish health by improving the quality of water by modifying the bacterial composition of the water and sediments. The degradation of organic matter by microbes determines the water quality in aquaculture systems. The carbon compounds in dissolved and suspended organic matter help the beneficial microbes to thrive and multiply by enzymatic cascade. The bacteria such as *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, and *B. coagulans* were used as suitable for the bioremediation of organic detritus. *Lactobacillus* sp. is also used along with *Bacillus* to break down the organic detritus. These bacteria produce a variety of enzymes that break down proteins and starch into small molecules, which are then taken up as energy sources by other organisms. The removal of large organic compounds reduces water turbidity. Many examples in which both singular bacterial strains and microbial systems have been successfully utilized to reduce and or transform selected pollutants into nontoxic molecules in laboratory conditions. Nitrogen applications over pond assimilatory capacity can lead to the deterioration of water quality through the accumulation of nitrogenous compounds (e.g., ammonia and nitrite) with toxicity to fish and shrimp. Bacteriological nitrification is considered beneficial for the reduction of ammonia from closed aquaculture systems and it is commonly achieved by setting up sand and gravel bio-filter through which water is allowed to circulate. The ammonia-oxidizing bacteria are *Nitrosomonas*, *Nitrosovibrio*, *Nitrosococcus*, *Nitrolobus*, and *Nitrospira* and the nitrate oxidizing bacteria are *Nitrobacter*, *Nitrosococcus*, and *Nitrospira* are the commonly used bacteria in aquaculture systems. Sulfur is of some interest in aquaculture because of its importance in anoxic sediments. Sulfide oxidation is mediated by microorganisms in the sediment, though it can occur by purely chemical processes. The photosynthetic benthic bacteria that break H_2S at the pond bottom have been widely used in aquaculture to maintain a favourable environment. These bacteria contain bacterio-chlorophyll that absorb light (blue to the infrared spectrum, depending on the type of bacterio-chlorophyll) and perform photosynthesis under anaerobic conditions.

Conclusion:

Increased use of antibiotics has led to a high proportion of antibiotic resistant bacteria which provide a threat to fish and man through consumption of the infected fish. Inefficiencies in antibiotic treatment of fish illnesses lead to significant economic losses. But the use of probiotics in aquaculture has been shown to have a beneficial impact on fish health and thereby the economic performance of fish farming. Shrimp aquaculture is burdened with environmental problems that arise from the consumption of resources, such as land, water, seed, and feed, and their transformation into products valued by society. In addition, after the culture period, effluents from aquaculture ponds contain living and dead particulate organic matter, dissolved organic matter, ammonia, nitrite, nitrate, phosphate, suspended soil particles, and other substances that can be considered potential pollutants. Hence, the application of probiotics in aquaculture systems has been rising over the years with increasing demand for more eco-friendly aquaculture practices. At the same time, the use of probiotics has also important environmental benefits. Therefore, the use of probiotics in fish and shrimp feed should also be seen as an important step in aquaculture sustainability.

References:

Austin., B and J.G. Day, 1990.Inhibition of prawn pathogenic *Vibrio* spp. by a commercial spray-dried preparation of *Tetraselmis suecica*, Aquac. 90:389-392

Gomez., B, Gil, A. Roque, J.F. Turnbull. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms, Aquac. 191:259-270.

Nitya Jeevan Kumar., P, S. Jyothsna, M. Hanuma Reddy, S. Sreevani. 2015. Effect of *Bacillus subtilis* and *Lactobacillus rhamnosus* incorporated probiotic diet on growth pattern and enzymes in *Penaeus vannamei*, Ind. J. Pure App. Biosci. 3(2): 250-480

Sahu., M. K, N.S. Swarnakumar, K. Sivakumar, T. Thangaradjou, L. Kannan. 2008. Probiotics in aquaculture: importance and future perspectives, Ind. J. Microbiol. 48:299-308.

Swapna., B, Ch. Venkatrayulu, A.V. Swathi. 2015. Effect of probiotic bacteria *Bacillus licheniformis* and *Lactobacillus rhamnosus* on the growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931), Euro. J. Exp. Bio. 5(11): 31- 36

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/2/2024

BIOSECURITY IS IMPORTANT FEATURE OF SUCCESSFUL AQUACULTURE

Ambrish Singh[1], Brajesh Kumar Pal[2] Mitrasen Maurya[3], Shivam Pandey[4] Surendra Kumar Maurya[5]*

Ph.D. Scholar, Dept. of Fisheries Resource Management, College of Fisheries, ANDUA&T, Ayodhya, U.P.

Teaching Associate, College of Fisheries Science and Research Centre Campus Etawah, CSAUA&T, Kanpur, U.P.

Ph.D. Scholar, Dept. of Aquaculture, College of Fisheries, ANDUA&T, Ayodhya, U.P.

Ph.D. Scholar, Dept. of Aquatic Animal Health Management, College of Fisheries Science, CCSHAU, Hisar, Haryana

Corresponding author: Ambrish Singh, singh7234aman@gmail.com,

Abstract

Aquaculture plays a vital role in ensuring global food security by producing a significant portion of the world's seafood. However, as the demand for seafood rises, it is crucial to maintain the health and sustainability of aquaculture operations. Biosecurity is a set of procedures that are essential for managing and preventing illnesses in aquaculture. These procedures include designing the location, disease control, health monitoring, and quarantine. Biosecurity acts as a preventive barrier to protect investments and production because disease outbreaks in aquaculture can have severe economic consequences. In addition to reducing the need for antibiotics, biosecurity also addresses environmental and public health issues, promoting sustainability. It improves the welfare of aquatic species in aquaculture by maximizing their development and reproductive success and reducing the risk of disease transmission between farmed and wild populations. This, in turn, promotes ecosystem health and biodiversity.

Introduction

Aquaculture is the controlled cultivation and harvesting of aquatic organisms. It plays a significant role in global food production, meeting a large part of the world's seafood demand while potentially reducing pressure on wild fish populations. However, intensive fish farming can lead to illnesses and environmental contamination, which can be harmful to sustainability. Biosecurity in aquaculture refers to measures and methods designed to maintain a disease-free environment. These protocols aim to prevent the spread of disease-causing organisms. Biosecurity practices reduce stress in animals, making them less susceptible to diseases.

Biosecurity Principles

1. Identify Hazards

Understand disease transmission

What are the risk factors for your farm?

2. Assess Risks

Impacts to your farm

3.Risk management

Prioritize

Major biosecurity goals are:

Animal management - obtaining healthy stocks and optimizing their health and immunity through good husbandry

Pathogen management- preventing, reducing or eliminating pathogens

People management- educating and managing staff and visitors

Farm management biosecurity procedures

Biosecurity in aquaculture is crucial to prevent and control the spread of diseases in aquatic organisms. Here are some key principles of biosecurity in aquaculture:

1. **Isolation and Quarantine:** New stock should be isolated and quarantined before introducing them to existing populations. This helps to prevent the introduction of potential pathogens. During quarantine, the health of the new animals is closely monitored.
2. **Site Selection:** Choose aquaculture locations carefully, considering the proximity to wild aquatic populations and the potential for disease transmission. Isolating aquaculture facilities from natural water sources can reduce the risk of disease introduction.
3. **Restricted Access:** Limit access to aquaculture facilities to essential personnel only. Controlling who enters the facility reduces the risk of contamination and disease introduction.
4. **Sanitation and Hygiene:** Implement strict sanitation and hygiene practices for equipment, personnel, and facilities. This includes the regular cleaning and disinfection of equipment, footwear, and clothing to prevent the spread of pathogens.
5. **Water Quality Management:** Maintain high water quality standards, as poor water quality can stress aquatic organisms and make them more susceptible to diseases. Proper filtration, aeration, and water treatment are essential.

6. **Stock Source and Health Certification:** Source animals from reputable suppliers with good health records and certification. Ensure that animals are certified as disease-free before introduction.
7. **Disease Monitoring and Surveillance:** Regularly monitor the health of aquatic organisms through observation and diagnostic testing. Early detection of diseases is crucial for their control.
8. **Vaccination and Immunization:** Where possible, use vaccines to protect against known diseases. Proper vaccination protocols can significantly reduce disease outbreaks.
9. **Selective Breeding for Disease Resistance:** Develop and use selectively bred strains of aquatic organisms that are resistant to common diseases, as this can reduce the need for treatment and antibiotics.
10. **Waste Management:** Manage waste properly to prevent the release of infectious agents into the environment. This includes responsible disposal of dead or diseased organisms.
11. **Emergency Response Plan:** Develop and implement a comprehensive emergency response plan to deal with disease outbreaks. This plan should include procedures for quarantine, treatment, and coordination with relevant authorities.
12. **Education and Training:** Educate and train aquaculture personnel about biosecurity measures and disease recognition. Knowledgeable staff are essential for effective disease management.
13. **Record Keeping:** Maintain detailed records of stock health, treatments, and surveillance results. This information is valuable for tracking disease trends and improving biosecurity practices.
14. **Communication:** Establish communication channels with neighboring aquaculture facilities, regulatory agencies, and researchers to share information on disease outbreaks and preventive measures.

Transmission routes within the farm

Farm operations involve various units with different health statuses, creating a risk of disease transmission. To address this, internal biosecurity measures should be implemented. This includes managing different areas of the farm separately according to established biosecurity standards to reduce the risk of disease spread within the facility (Kyule-Muendo et al., 2022).

Transmission routes from the farm

Transmission routes from a farm can have significant impacts on other farms, local water sources, and wildlife populations. Preventing disease transmission from the farm involves managing both incoming and outgoing pathways. To mitigate the risk, exit-level biosecurity standards should be applied to manage the routes from the farm effectively (Delabbio et al., 2005).

Biosecurity in shrimp farm management

Farmers implement on-farm biosecurity protocols to reduce crop loss, ensure cost-effectiveness, and maintain a healthy environment. These protocols include using specific pathogen-free (SPF) and high-yield fish stocks, implementing quarantine for incoming stock, analyzing diseases through techniques like PCR, treating incoming water sources, sterilizing and maintaining equipment, practicing personal hygiene, possessing knowledge about potential pathogens, utilizing SPR stock, and maintaining optimal environmental conditions. These measures collectively help protect crops and promote efficient and disease-free farming practices (Bera et al., 2018).

Approaches and implementation of biosecurity programme

STANDARD OPERATION PROCEDURES:

FAO technical guidelines for on aquaculture development for CCRF includes some standard operating procedure(SOP). Implementing a comprehensive biosecurity program in aquaculture involves numerous steps and components. This program encompasses risk assessment, careful site selection, physical barriers, stock selection, quarantine measures, water quality management, equipment and facility hygiene, disease monitoring, vaccination, selective breeding for disease resistance, staff training, emergency response planning, record keeping, regulatory compliance, effective communication, ongoing improvement, and fostering biosecurity awareness.

Following proper procedure for brood stock selection

HACCP APPROACH

It is the preventive risk management system based upon a hazard analysis and has been widely used to identify and control risk of some potential pathogens.

Principles:

- Perform systematic hazard analysis
- Determine critical control point
- Determine critical control limit
- Determine appropriate corrective measures
- Establish monitoring procedures
- Develop verification procedures
- Design record keeping systems

INFRASTRUCTURE

Effective biosecurity in aquaculture involves several key measures, including facility segregation for different stages of aquaculture, proper water management, dedicated areas for feeding and feed storage, specific packing facilities, and the isolation of different areas (Pruder; 2004; Delabbio, 2006).

WATER QUALITY AND TREATMENT:

- Physical filtration: UV, sand filter
- Chemical filtration: Chlorination, activated carbon, EDTA
- Biological filtration: Bed filter

BROOD STOCK QUARANTINE:

Effective biosecurity in aquaculture necessitates the isolation and containment of facilities from the culture area, ensuring there is no direct contact with the external environment. This involves implementing restricted entry and establishing an independent water supply with proper filtration for disinfection of incoming water and effluent purification. Water chlorination and de-chlorination are employed to maintain water quality (Adah et al., 2022). Additionally, the proper management of deceased animals through incineration and thorough disinfection of equipment and tanks after operations play a crucial role in maintaining biosecurity, preventing disease transmission, and safeguarding the overall health of aquaculture operations.

Advantages of Biosecurity

Biosecurity offers a multitude of advantages, including disease prevention in agriculture and aquaculture, increased productivity, protection of the environment, and public health. It ensures economic stability, promotes sustainability, facilitates global trade, enhances resource efficiency, reduces environmental impact, and boosts consumer confidence. Overall, biosecurity measures are indispensable in various sectors, contributing to healthier ecosystems and economic prosperity (Hasimuna et al., 2020).

Disadvantages of biosecurity

Using preventive techniques like pesticides to control crop pests can have unintended consequences on human consumption and the broader environment. This is because these chemicals may impact not only the targeted organisms but also non-target species, including animals and plants Peeler, 2005; Perera et al.,2020). Additionally, the need for thorough inspections of imported products from other countries is essential to ensure their safety. However, this can lead to increased resource demands and inspections, potentially causing inconsistencies in the products available for purchase.

Conclusion

Biosecurity measures in aquaculture may seem like an unnecessary financial burden, but they can have substantial financial gains and play a vital role in preventing disease outbreaks and limiting zoonotic transmission. Efforts should be made to raise awareness, provide training and support, and establish regulatory frameworks to promote the adoption of biosecurity practices in aquaculture, especially among smaller and less-resourced farmers.

Reference-

Adah, D. A., Saidu, L., Oniye, S. J., & Adah, A. S. (2022). An Assessment of the Impacts of Biosecurity Measures on Mortality of Fish from Fish Farms. *Aquaculture Studies*, *23*(5).

Bera, K. K., Karmakar, S., Jana, P., Das, S., Purkait, S., Pal, S., & Haque, R. (2018). Biosecurity in aquaculture: an overview. Aqua International, 18(12), 42-46.

Delabbio, J. (2006). How farm workers learn to use and practice biosecurity. *Journal of extension*, *44*(6), 6FEA1.

Delabbio, J. L., Johnson, G. R., Murphy, B. R., Hallerman, E., Woart, A., & McMullin, S. L. (2005). Fish disease and biosecurity: attitudes, beliefs, and perceptions of managers and owners of commercial finfish recirculating facilities in the United States and Canada. *Journal of Aquatic Animal Health*, *17*(2), 153-159.

Hasimuna, O. J., Maulu, S., & Mphande, J. (2020). Aquaculture health management practices in Zambia: status, challenges and proposed biosecurity measures. *Journal of Aquaculture Research and Development*, *11*(3), 1-6.

Kyule-Muendo, D., Otachi, E., Awour, F., Ogello, E., Obiero, K., Abwao, J., & Munguti, J. (2022). Status of fish health management and biosecurity measures in fish farms, cages and hatcheries in Western Kenya. *CABI Agriculture and Bioscience*, *3*(1), 18.

Peeler, E. D. M. U. N. D. (2005). The role of risk analysis and epidemiology in the development of biosecurity for aquaculture. *Diseases in Asian Aquaculture V. Fish Health Section, Asian Fisheries Society, Manila, Philippines, Queensland, Australia*, 35-46.

Perera, R. P., Jones, B. R. I. A. N., Beers, P. E. T. E. R., Kleeman, S., & McGladdery, S. (2005). Maintaining biosecurity in aquaculture systems: a constraint or a challenge. In *6th Symposium on Diseases in Asian Aquaculture*.

Pruder, G. D. (2004). Biosecurity: application in aquaculture. *Aquacultural engineering*, *32*(1), 3-10.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/3/2024

USE OF CHEMICALS IN AQUACULTURE

S. Saravanan, A. Uma and D. Kaviarasu
Department of Aquatic Animal Health Management,
Dr. M. G. R Fisheries College and Research Institute, Ponneri 601 204,
Tamil Nadu, India. Tamil Nadu Dr. J. Jayalalithaa Fisheries University.
Corresponding author: saravanan@tnfu.ac.in

Introduction:

Aquaculture has been practiced in India in both freshwater and coastal saline waters from time immemorial. These were characteristically low-input, low-production systems depending on natural seed collection from the wild, with stocking in natural ponds, or impounding in large water bodies without any further management measures. During the last decade, aquaculture has slowly but steadily transformed itself into a profitable business activity. In freshwater carp culture, production rates of up to 15 t/ha/yr have been attained, and in shrimp culture, yields of about 8 t/ha/crop have been achieved. In tilapia culture, production rates of over 400 t/ha/year in three crops have been reported. With the increase in area devoted to aquaculture and also the intensification of culture practices, environmental issues have come into focus, particularly issues concerning the planning and regulation of aquaculture in coastal areas.

Compared to coastal aquaculture, freshwater carp culture is widespread in the country, particularly in the states of Andhra Pradesh, West Bengal, Madhya Pradesh, Punjab, Uttar Pradesh, Orissa and Bihar. Sea-farming of oysters and pearls is still in the experimental stage in India, but interest is now growing for establishing joint-venture cage-culture projects with sea bass and grouper as candidate species.

Aquaculture production in the freshwater sector has steadily increased from a level of 7.9 lakh t in 1987 to 13.25 lakh t during 1994-95. The average has increased from 900 kg/ha in 1984-85 to 2130 kg/ha in 1994-95. Similarly, aquaculture production from coastal waters has increased from 35,500 t in 1990-91 to 74,850 t in 1994-95, with average productivity increasing from 545 kg/ha to 743 kg/ha.

Freshwater fish culture is primarily comprised of Indian major carps (Catla, Rohu and Mrigal), with the secondary species including exotic Chinese carps. In 1993, Total inland fish production of India (species-wise) was comprised of 54.7% major carps, 8.1% common carp, 6.1% other carps, 3.6% murrels, 2.2% *Hilsa* sp., and 25.3% other species (GOI 1993).

In traditional coastal shrimp farming systems, production is a mixture of several species of shrimp and fish. In the bheries of West Bengal, *Penaeus monodon* comprises about 18% of the total catch, while in the pokkali fields of Kerala, *Metapenaeus dobsoni* constitutes more than 50% of the total catch. *Penaeus monodon* is the major species cultured in new brackish water shrimp farming systems all over the country, with *P. indicus* in second place.

Basically, aquaculture in India is largely of the extensive type and primarily related to carp farming. However, there has been an emergence of large-scale commercial, semi-intensive culture of carps in a few states, especially in Andhra Pradesh, where nearly 50,000 ha of carp culture ponds and 35,000 ha of shrimp culture ponds are under commercial operation. With the increase in productivity in semi-intensive carp culture, semi-intensive or intensive shrimp farms, and related hatchery operations, there has been increased usage of artificial inputs in the form of chemicals. Fish farmers faced with serious fish health problems have resorted to management practices involving the use of chemicals and therapeutants. This has resulted in environmental problems that were not previously encountered. Although carp culture is practiced in self-enclosed systems with limited effluent release to the environment, coastal aquaculture farms need large volumes of water daily. Thus, the hazards posed by the release of effluents containing chemicals with residual effect and high nutrient loads into receiving waters are a new dimension that has unfolded in the aquaculture scenario of the country. The sudden change in culture practices that has occurred in India has caught the nation off-guard as regard to effluents affecting the environment.

As these environmental problems are of recent origin, not enough research has been done to assess the gravity of the situation. Since the outbreak of shrimp disease in 1994, the subject has drawn the attention of research and development agencies in aquaculture, and a number of studies have since been conducted on the environmental impacts of such commercial aquaculture activities. However, most studies have concentrated on the nutrient loading of recipient waters, heavy metal pollution, and changes in water and soil characteristics of adjoining areas. Hence, the present study is largely based on findings restricted to the above impacts of aquaculture. The impact of the use of therapeutants on the environment has yet to be studied in detail. However, some isolated studies on the use of chemotherapeutants in controlling fish diseases have been undertaken in various fisheries research agencies in the country.

Use of chemicals in Aquaculture:

The various chemicals used in grow-out farming and hatchery operations in both freshwater and coastal aquaculture in India can be classified into the following broad categories: water/soil treatment products, disinfectants, piscicides, herbicides, organic fertilizers, inorganic fertilizers, feed additives, therapeutants and anaesthetics.

Water and Soil Treatment Products:

In both freshwater and coastal aquaculture, it is common practice to treat the pond waters for mineralization of organic matter, for adjusting pH, and for disinfection. The chemicals used in this regard are lime in the form of lime stone ($CaCO_3$), slaked lime

$(Ca(OH)_2)$ or unslaked lime (CaO). Dried ponds are also sterilized using active iodine or potassium permanganate ($KMnO_4$). Soil conditioners that contain high numbers of sulfur-degrading bacteria and organic matter decomposing bacteria are also used by some intensive shrimp culture farms. Health stone, zeolite, or porous aluminium silicate is applied along with lime to re-activate the soil for stabilizing algal growth and absorbing fouling materials.

Disinfectants

Common disinfectants used are sodium hypochlorite, benzalkonium chloride (BKC), calcium carbide, Na-EDTA, and zeolite. These are used mostly in hatcheries and, to a limited extent, in grow-out ponds.

Piscicides

In both freshwater and coastal aquaculture, eradication of unwanted predatory fishes is a common pre-stocking management practice. The common fish toxicants used are mahua oil cake, tea seed cake, other plant derivatives and anhydrous ammonium substances.

Herbicides

Aquatic weeds are of common occurrence in fishponds in the country and are undesirable, as they pose serious problems by upsetting the oxygen balance and removing nutrients from the aquatic systems. The common herbicides used for controlling aquatic weeds are 2,4-D; Dalapon; Paraquat; Diuron; ammonia; and many others.

Organic Fertilizers

Use of organic manure in fish culture is an age-old practice. The manure used comes mainly from farm animals, the commonly used manures being cow dung, pig dung, poultry droppings, etc. Cattle manure and poultry droppings contain nitrates at the 0.5 % and 1-15% levels and phosphates at the 0.2 and 0.4% levels, respectively. The application of raw cow dung slurry helps to boost diatom bloom (Sarkar 1983). In modified extensive shrimp-culture ponds, 1,000 to 3,000 kg of cow dung/ha is applied initially. It is followed by application of two dosages of 200 to 400 kg of cow dung on the 8th and 14th d, respectively. Poultry droppings contain higher quantities of soluble salts, inorganic substances and organic products than does cow dung, ensuring quick zooplankton production. In semi-intensive carp culture, large amounts of cow dung are applied to increase the fertility and consequent natural productivity of the ponds. Natural food provides 50% of the food requirement in such carp culture ponds.

Inorganic Fertilizers

Considerable quantities of nutrients are removed from the pond ecosystem through the harvested fish crop. Hence, for proper management of pond soil and water, these elements need to be replenished from external sources. The fertilization schedule is prepared on the basis of the fertility status of the soil. Soils with available nitrogen of >50, 25-50, or <25 ppm; and available phosphate content of >6, 3-6, or <3 ppm are classified as "high," "medium" and "low," respectively. The application rate of the different nitrogenous and phosphate fertilizers varies with culture practice and the nature of the fertilizer used. The doses of fertilizers applied for both carp culture and shrimp culture are presented in Table 1.

Table 1. Use of chemicals in coastal aquaculture and hatchery system in India.

Items	Purpose	Dose	Mode of application	Remarks
1.Soil and water treatments				
Quick lime	Correcting pH, disinfectant	400-2000 kg/ha	Dissolved in water and broadcast over pond surface	Basal dose of 50%Remaining in equal monthly instalments
	Mineralization	400 kg/ha	Dissolved in water and broadcast over pond surface	Applied at the time of pond preparation
II. Disinfectants				
Bleaching powder	Disinfectant	25-30 ppm	Broadcasting/water Solution	Toxicity lasts 7-8 d
III. Piscicides				
Mahua oil cake (MOC)	Piscicide (4-6% saponin)	200-250 ppm	Soaked in water And spread over water surface	Toxicity lasts 15-20 d after which it acts as fertilizer
Teaseed cake	Piscicide	75-100 ppm	Same as MOC	Toxicity lasts 15-20 d after which it acts as fertilizer
IV. Herbicides				
2,4-D	Emergent weeds, grasses	5-10 Kg/ha	Foliar spraying/ root zone treatment Foliar spraying	*Hyacinth, Ipomea, sedges, lillies, Vallisneria, etc.*
Dalapan	Aquatic grasses	5-10 Kg/ha	Foliar spraying	Hyacinth
Simazine, Diuron	Submerged Weeds	4 kg/ha	Root zone treatment	Horn wort, *Hydrilla*, etc.

Items	Purpose	Dose	Mode of application	Remarks
Paraquat	Floating weeds	0.2 kg/ha	Foliar spraying	Pistia, Salvinia
V. Organic fertilizers				
Cow dung	Fertilization	15 t/ha	1. Pond bottom application, 20 % total dose. 2. Balance in equal amount of doses	Initial manuring done 15 d prior to stocking

Table 2. Use of Chemotherapeutant in fresh water aquaculture and hatchery system in India.

Items	Purpose	Dose	Mode of application	Remarks
A. Parasitic diseases				
Oxytetracycline	***Myxobolus*** *spp*	5gm/100 kg fish	Supplemented in the feed	Prevents secondary bacterial infection
Sodium chloride	***Epistylis*** *spp.*, ***Zoothamnium*** *spp.*	20-50 kg/ha	2-3 instalments at 4-d interval	Pond water application
Malathion/ Dichlorvos	***Dactylogyrus*** *spp.* ***Gyrodactylus*** *spp* ***Argulus*** *spp.*, ***Lernaea*** *spp.*, ***Ergasilus*** *spp.*	0.2 ppm	2-3 instalments at 4-d interval	Pond water application
B. Bacterial/fungal diseases				
Sulphadiazine + Trimethoprim	**Surface ulcerative and systemic type** (*Aeromonas hydrophila*)	5gm/100 kg	Applied for 7 d	Water dispersible powder

Items	Purpose	Dose	Mode of application	Remarks
Chloro tetracycline		7gm/100 kg	Applied for 7 d	Supplemented in feed
Oxy tetracycline **Nitrofurans,** **Trimethoprim,**	**Columnaris Disease,** **Microbial gill disease**	**7-10 gm/100 kg** **10 gm/100 kg** **5-7gm/ 100 kg**	**Applied for 10 d**	**Supplemented in feed** **Immersion treatment**
Copper Sulfate	***Saprolegnia*** *spp.,* ***Branchiomyces***	**0.2-0.5 ppm**	2-3 instalments at 3-4-d interval	**Immersion treatment**

Table 3. Use of chemicals in coastal aquaculture and hatchery systems in India

Items	Purpose	Dose	Mode of application	Remarks
I. Soil and water treatment				
Quick lime (CaO)	Correcting pH	270-1130 kg/ha	Dissolved in Water and broadcast over pond surface	Basal dose of 50%; remaining dose given in equal monthly instalments
Slaked lime (Ca(OH2)) Lime stone (CaCO3)		340-1610 kg/ha 380-1690 kg/ha		Dose depends upon pH of soil and water
Gypsum	Reduces turbidity	300 kg/ha	Applied before manuring	
II. Disinfectants				
20% active iodine $KMnO_4$	Sterilization	1-2 ppm	Applied in dried ponds	
III. Soil reformers				
Sulfur bacter	Reduces soil pH	75-120 kg/ha	Applied in wet soil and sun dried 2-3 d	

Items	Purpose	Dose	Mode of application	Remarks
Health stone/zeolite	Reactivates soil/promotes algal growth/ absorbs fouling materials	**250-1000 kg/ha**		Dose depends on soil pH
IV. Piscicides				
Mahuo oil cake	Piscicide (4-6% saponin)	200-250 ppm	Soaked in water and spread over water surface	Toxicity lasts 15-20 d, after which act as fertilizer
Teaseed cake	Piscicide (10-15% Saponin)	75-100 ppm	Soaked in water and spread over water surface	Toxicity lasts 10-12 d, after which act as fertilizer
Derris root powder Calcium hydroxide + Ammonium sulfate	Piscicide	5-10 ppm	Soaked in water and spread over water surface	
V. a. Organic fertilizers alone				
Cow dung	Fertilization	Primary dose 1-3 t/ha Secondary dose 200-400 Kg/ha	Applied when water depth is 10 cm Two doses, one at 30 cm water depth and another at 100 cm water depth	
Poultry dropping	Fertilization	Primary dose 100-500 kg/ha Secondary dose 25-100 kg/ha	Applied when water depth is 10 cm Two dose one at 30 cm water depth another at 100 cm water depth	
b. Organic fertilizers along with inorganic fertilizers				
(i). Cow dung	Fertilization	200-500 kg/ha	Applied initially by broad casting	
Urea, Single super phosphate	Fertilization	150kg/ha	Applied in 2 doses	Following application of cow dung

Items	Purpose	Dose	Mode of application	Remarks
(ii). Poultry dropping	Manuring	100-250 Kg/ha	Applied initially by broad casting	
Urea, Single super phosphate	Fertilization	20 kg/ha	Applied in 2 doses	Following application of cow dung

Feed Additives

With the intensification of aquaculture practices, there is a shift from using supplementary fish feeds comprised of agricultural wastes and by-products to using complete feeds developed to meet the complete nutritional requirements of the species cultured. These feeds usually have other additives in the form of pigments, vitamins, chemo-attractants, and preservatives, like Mold inhibitors and antioxidants.

Therapeutants

There is an increasing occurrence of disease caused by parasitic, fungal, bacterial, and viral infections in both hatcheries and grow-out farms. In the recent past, India has witnessed three major outbreaks of disease. The first to strike was the dreaded epizootic ulcerative syndrome (EUS) in freshwater cultured species. This was followed by yellowhead-virus disease and white spot-virus disease in shrimp farms. The outbreak of these diseases rendered severe blows to the aquaculture industry in the country, creating the need to use therapeutants in both the freshwater carp industry and in shrimp culture.

Anaesthetics

The use of anaesthetics in aquaculture in India is rather limited. They are sparingly used, particularly in the long-distance transport of broodstock and fish seed.

Information on the uses, methods of delivery, doses, frequency of application, and other aspects for chemicals commonly used by the Indian aquaculture industry is presented in Tables 1-3. Information is presented separately for freshwater aquaculture and coastal aquaculture. Information on the use of chemicals other than therapeutants in the freshwater sector is presented in Table 1, whereas that for therapeutants is given in Table 2. The use of chemotherapeutants in freshwater aquaculture is rather limited and has gained significance only since the outbreak of EUS a few years ago. In carp hatcheries, their use is also limited.

Supply of Chemicals

The various chemicals and antibiotics used in aquafarming are mostly available locally, and there is no control on their sale and usage. Most of the farmers use veterinary-grade antibiotics for application in the fishponds. Certain products are imported from companies such as Aurum Aquaculture Ltd (USA), J.V. Marine (Taiwan), Argent Chemical Laboratories (USA), and Pfizer Food Science (USA). Information on the quantitative usage of these chemicals is generally not available and no agencies mandated to regulate usage are in existence at the moment.

FARM MANAGEMENT AND USE OF CHEMICALS

Preventive Methods

Most therapeutic agents have residual effects on the tissues of the candidate species. In addition, antibiotics may also leach into natural habitats, leading to modification of native bacterial flora and the emergence of antibiotic-resistant strains. The persistence of therapeutic agents in the aquatic environment may also cause adverse effects on the ecosystem (Anon 1988, Choo 1994). The best strategy in the management of aquaculture enterprises is to prevent the occurrence of disease. The industry in India, by and large, takes the following precautions to prevent disease in hatcheries and grow-out farms.

Disease Prevention Measures in Hatcheries:

1. Proper site selection. In choosing the location for a hatchery, due care should be taken to locate it in a place where good quality water is ensured for maintenance of broodstock, spawning, and larval rearing.

2. Water treatment. Disinfect sea water with calcium hypochlorite (20-30 ppm) or sodium hypochlorite (150 ppm) for 1-2 d. Remove excess chlorine from sea water by neutralizing with sodium thiosulfate. Filter or sterilize by UV treatment or other means, sea water used for all hatchery operations.

3. Maintenance of cleanliness of hatchery facilities. The tanks used for broodstock, spawning, and larval rearing should be kept thoroughly clean.

4. Disinfection of broodstock. For this purpose, 20 ppm formalin or other appropriate drugs can be used as short bath or dip treatment.

5. Observe appropriate care at the time of spawning by thoroughly removing the scum formed after spawning.

6. Stock only healthy nauplii at an optimal stocking density. The nauplii can be disinfected by dip treatment in 200-300 ppm formalin.

7. During larval rearing, siphon out unused feed, sediments, debris, and wastes accumulated at the bottom and sides of the tanks.

8. Feed the larvae with optimal amounts of good quality well-balanced feed.

9. Use antibiotics carefully and at the correct doses, preferably after ascertaining the *in vitro* sensitivity of the pathogens. Low doses of antibiotics lead to the development of antibiotic resistant mutants of bacteria and higher doses may be toxic to the shrimp larvae or the other fauna and flora of the culture system.

10. Examine larvae microscopically every morning before changing water for any signs of abnormality, fouling protozoa, filamentous bacteria, fungal infections, and presence

of swarming bacteria within the hemocoel. Maintain good water quality parameters at all times.

11. Disease outbreaks due to viral infection can be avoided by quarantine measures, and by destroying carriers and clinically diseased animals.

Disease Prevention Measures in Grow-out Farms:

1. Select farm sites properly, ensuring that they are far from industrial, agricultural and domestic sources of pollution.

2. Before stocking, drain and sundry the ponds thoroughly. The black layer of soil formed during the previous crops should be removed and the pond tilled. Lime can be applied at the rate of 200-600 kg/ha depending on the pH of the soil.

3. Stock only healthy postlarvae after achieving an optimal algal bloom in the ponds. Maintain optimal density of shrimp larvae.

4. Good water quality in the pond should be maintained.

5. Feed the shrimp with a balanced diet at optimal quantity. Care should be taken to avoid overfeeding and accumulation of uneaten feed.

6. Routinely examine the health status of the shrimp. Frequent microscopic examination of gills, hepatopancreas and haemolymph for microbial infections or any disease signs should be done. Clinically diseased and infected shrimp should be destroyed by burning or burying with lime in soil away from the shrimp farm.

7. Practice adequate quarantine measures before transporting shrimp post larvae or broodstock to different geographical locations. They may harbour pathogenic microorganisms, particularly viruses, without showing external clinical signs.

8. If available, use disease-resistant stocks for culture purposes. The resistance of shrimp to various infectious agents appear to be species specific and is probably a genetically acquired trait.

9. Vaccines may also be used for controlling specific diseases.

Therapeutic Measures

Disease control programs in aquaculture must consider various factors such as stocking density, environmental parameters, rate of water exchange, the type of feed used, and phytoplankton blooms. However, in spite of the best management practices adopted in hatcheries and grow-out systems, disease outbreaks will still occur, necessitating use of drugs for their control. Although some drugs have been advocated for treatment of diseases (see Tables 2 and 4), these should be employed only as a last option. Many aspects of the dynamics of drug use in aquaculture are yet to be studied. Various aspects of using drugs for disease control such as their dosages, intervals of administration, duration of exposure of fish, their effect and efficacy in controlling the disease, withdrawal period from the tissues, effects on non-target species, etc. remain to be clearly understood. As drugs are useful only if they are applied during the early phase of disease, correct diagnosis at an early stage is a very important aspect that will help to control the disease.

Criteria for Selection of Drugs for Disease Control:

The following criteria are used to select appropriate drugs for disease control:

1. The sensitivity of the pathogen to the drug or antibiotic based on *in-vitro* tests must be known.

2. The antibiotic or chemical should reach the pathogen and kill it without adversely affecting the host.

3. The antibiotic or drug should not adversely affect the user and the natural flora and fauna.

4. The drug should rapidly be broken down to avoid problems with tissue residues.

5. The metabolites of the drug should be harmless to the cultured animal and the consumer.

6. The drug should be stable under normal storage conditions.

Methods of Application of Drugs:

The treatment methods currently being followed include applying the therapeutic agent to the pond water or administering it along with feed. The various methods that are commonly followed are given below:

1. Oral route

The chemotherapeutant may be incorporated in the feed at the correct dose and fed to the shrimp. However, application of medicated feeds needs to be clearly understood, otherwise, the drug may diffuse into the water and create problems (Choo 1994). Drug therapy by this method should preferably start during the initial stages of the disease, since fish in the advanced stages of disease feed poorly. Compounds like antibiotics, sulfa drugs and nitrofurans are widely used along with feed to treat bacterial diseases, both in shrimp and freshwater carp culture systems in India (Tables 7 and 9).

In carp culture systems in the State of Andhra Pradesh, the required quantity of poultry feed supplement (poultry feed supplements with various proportions of antibacterial activity are commonly available to the fish farmers) is added to the normal fish feed by mixing 4:1 mixture of de-oiled rice bran and oil cake into a dough (Rao *et al*. 1992). Unlike the common practice of broadcasting the feed over the pond surface, the feed is kept suspended in perforated bags tied to bamboo poles. Normally, 10-20 bags of size 20 x 30 inches are used per ha, the bags being tied individually to bamboo poles fixed at regular intervals in the pond. Each bag has two rows of perforations and can contain up to 12 kg of feed. Typically, the feed within a perforated bag is eaten by the fish within 2 h. This method results in minimal feed wastage and reduced antibiotic leaching as compared to broadcasting the medicated feed over the pond (Rao *et al*. 1992).

2. Immersion treatment method

This method is followed to treat ectoparasitic diseases, bacterial surface ulcerative lesions and external fungal problems. The therapeutic agent is added directly to the pond water or sprayed over the surface. Agricultural grade pesticides sold under different brands have been regularly used to treat against helminth and crustacean parasites. The current method of pesticide application in carp farms in Andhra Pradesh involves dissolving the required quantity of pesticide in 10-20 L of water and spraying the solution over the pond surface with hand-held agricultural sprayers. In large fishponds, hand-held sprayers are operated from boats. Use of this method is very popular for the application of Nuvan and Malathion to combat infection by *Argulus* spp. (Rao *et al*. 1992).

3. Dip treatment

In this method, the fish or shrimp are held in containers with a strong solution of the chemotherapeutant for short durations. This method may be useful when only a small portion of the stock is affected with non-systemic infections such as fouling, shell disease, necrosis of appendages, etc.

4. Bath treatment

This method is applicable only when a small portion of the stock is affected with disease. The fish are given bath treatment in containers for 30-60 min in a solution containing the drug.

5. One-time application

In one-time application, a low concentration of the chemical is applied to the culture tanks or ponds for an indefinite period. However, this method poses pollution problems.

6. Injection

This method is practical to use when only a small number of large and valuable fish are to be treated.

7. Topical application

This method is also applicable only for a small number of valuable broodstock suffering from nonsystemic diseases such as shell disease.

Hazards and Adverse Impacts on Culture Organisms and Farm Productivity

The adverse impacts of chemical inputs on farmed species and farm productivity will be discussed separately for carp culture and shrimp culture because of the inherent differences between the two industries. The interaction between the pond environment and the external environment, and the qualitative and quantitative differences in the use of chemical inputs in the two fundamentally different aquaculture practices in the country are obvious.

Impacts on Farm Workers and Consumers

Aside from reports of workers in shrimp hatcheries and farms suffering from over-exposure to bleaching powder or chlorine, incidences where toxic substances have affected farm workers or consumers, where residues have affected product quality or where pathogens resistant to chemotherapeutants have been encountered have not been reported in India.

Environmental Impacts

There are few environmental problems encountered in freshwater carp culture, as in these inland fish farms the pond-culture units are typically closed systems. However, release of large volumes of water containing nutrients, organic matter, and wastes at the time of fish harvest was reported to have resulted in eutrophication of receiving waters and algal blooms (Pathak and Palanisamy 1995). The concentration of ponds in areas with limited water resources aggravated the situation. Development of semi-intensive pond culture for Indian major carps around Kolleru Lake in Andhra Pradesh has resulted in heavy discharge of pond effluents during harvest. This has contributed to the deterioration of lake water quality, leading to increased disease outbreaks.

There has been considerable debate on the environmental impacts that shrimp pond effluents may have on the receiving waters and the ecosystem. A comparison of shrimp farm effluents with other discharges has shown that their pollution potential is considerably less than that of domestic or industrial waste water. However, effluents produced during pond cleaning have greater pollution potential, although for a much shorter period. Although shrimp farm effluents are less noxious than many other coastal effluents, water pollution problems arise because of the large volumes discharged. Pollution also results when shrimp farms are concentrated in areas with limited water supply or with poor flushing capacity. In such situations, the shrimp farms themselves are most severely affected, resulting in “self-pollution.”

Conclusions

Although information on the quantity of chemicals and therapeutants used in aquaculture is not available at present, the tremendous upsurge in their use in the last five years is primarily due to the phenomenal growth in shrimp grow-out culture and hatchery operations and to the expansion of the carp industry. Considering the stagnation in fish harvest from nature, the future fish requirements of the country can only be met from expansion of freshwater and coastal aquaculture, and the utilization of the seaward side of the country’s coastline, which is presently untapped for mariculture. This definitely would call for higher usage of chemicals in aquaculture and would require careful environmental planning and prudent usage of chemicals and therapeutants. The country at present lacks a suitable policy for the manufacture, sale and use of such chemicals. It is, therefore, absolutely necessary at this stage to frame suitable policies and legislation and to create the infrastructure for their implementation and monitoring. The indiscriminate use of such chemicals can pose serious hazards to public health and aquatic life. The most serious problem at the moment is a lack of information on the use of chemicals by the aquaculturists in India and their appropriateness. Most veterinary grade chemicals and biocides are freely available to the aquaculture sector. Often, farmers are unaware of the nature of a disease and its etiology, and easily fall prey to errant advisers. This leads to indiscriminate use of hazardous chemicals, which may prove dangerous. Hence, it is necessary to create an awareness among aquaculturists of the potential hazards of indiscriminate drug use, and to provide them necessary assistance through timely diagnosis and prescription of appropriate drugs. In order to achieve this, it is incumbent on the development agencies to provide mobile diagnostic laboratories at the district level and man them with adequately trained personnel. The diagnostic laboratories, together with pollution control agencies in the states, may be empowered to monitor the use of chemicals by the aquaculture industry, as well as to maintain information databases on the subject.

The need for further research to standardize prophylactic and control measures in aquaculture is real, since the industry is of recent origin. Research needs to be strengthened to develop safer and biodegradable chemicals for use in aquaculture. Extension services need to be strengthened to educate aquaculturists on the safe use of therapeutants and chemicals.

In order to provide adequate services and to carry out research in these areas, it may be necessary to evaluate our expertise in the field before steps are taken to strengthen our capabilities. It may be also be useful to share information with other countries in the region that have acquired expertise in the field. These steps may enable us to grow without endangering our aquatic habitat and human health.

References

Anon. 1988. Norwegian aquaculture: controlling the antibiotic explosion. Animal Pharmacy, P.J.B. Publication Ltd., Surrey.

Choo PS. 1994. Degradation of oxytetracycline hydrochloride in fresh and seawater. Asian Fish. Sci. 7:195-200.

CIBA. 1995. Shrimp diseases, their prevention and control. CIBA Bull. No. 3. Central Institute of Brackish Water Aquaculture, Madras. 17 p.

CIFRI. 1985. Carp culture - package of practices for increasing production. Aquaculture Extension Manual, New Series No. 2. Central Inland Fisheries Research Institute, Barrackpore, West Bengal. 30 p.

FAO/NACA. 1995. Regional Study and Workshop on the Environmental Assessment and Management of Aquaculture Developement (TCP/RAS/2253). NACA

Environment and Aquaculture Development Series No. 1. Network of Aquaculture Centres in AsiaPacific, Bangkok.

Konar SK, Ghosh TK, Dey M. 1990. Hazards of aquatic pollution, its complexity and abatement measures. In: Proceedings of the Second Indian Fisheries Forum held on May 27-31, 1990 in Mangalore, India. p. 195-198.

Government of India (GOI). 1993. Handbook on Fisheries Statistics. Ministry of Agriculture, Fisheries Division, Government of India, New Delhi.

Government of India (GOI). 1995. Guidelines for Sustainable Development of Brackishwater Aquaculture. Ministry of Agriculture, Fisheries Division, Government of India, New Delhi.14 p.

NEERI. 1995. Impacts of aquaculture farming, and remedial measures in ecologically fragile coastal areas in the states of Andhra Pradesh and Tamil Nadu. Investigation Report for the Supreme Court of India, National Environmental Engineering Research Institute, Nagpur, India. 84 p.

Pathak SC, Palanisamy K. 1995. Shrimp and carp aquaculture and the environment in India. Report on a Regional Study and Workshop on Aquaculture Sustainability and the Environment. ADB RETA 5534, Network of Aquaculture Centres in Asia-Pacific, Bangkok.

Rao KG, Mohan CV, Seenappa D. 1992. The use of chemotherapeutic agents in fish culture in India. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 505-513. Fish Health Section, Asian Fisheries Society, Manila.

Sarkar SK. 1983. Influence of cow dung and mustard oil cake on the effectiveness of fertilizers in fish production. Environ. Ecol. 1:30-40.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/4/2024

VALUE CHAIN EXTENSION - A REVIEW

Dr. T. Kungumaselvan
Assistant Professor (Agricultural Extension)
Department of Agricultural Extension Education
SRM College of Agricultural Sciences, Baburayanpettai, Chengalpattu-603 201

Abstract

India is an agrarian country with more than 70 % of the people depends on agriculture and allied sector for the livelihood. Among them most of the farmers belonged to marginal and small farmers with the land holding of less than 2.5 acres and 5 acres respectively. Due to the reason of fragmented land holding and lack of awareness and knowledge on marketing aspects, they could not able to reap higher income from agriculture. This article explores the ways related to value chain extension through which farmers can get high income for their produce through the help of extension agents and organizations.

Introduction

Agriculture along with the allied sectors contributed significantly to the economy of India and more than 70.00 per cent of the people had agriculture as major occupation. Further, 82.00 per cent of the farmers were belonged to marginal to small farmers category. Even though we have lots of marginal and small land holdings, we are happy to be self-sufficient in cereal production. But the resources available for the production is not same for all the states of India. And also farmers are facing many constraints viz., poor market infrastructures, poor market linkage, improper post-harvest management of produce and weak value chain process.

Agriculture and Value Chain

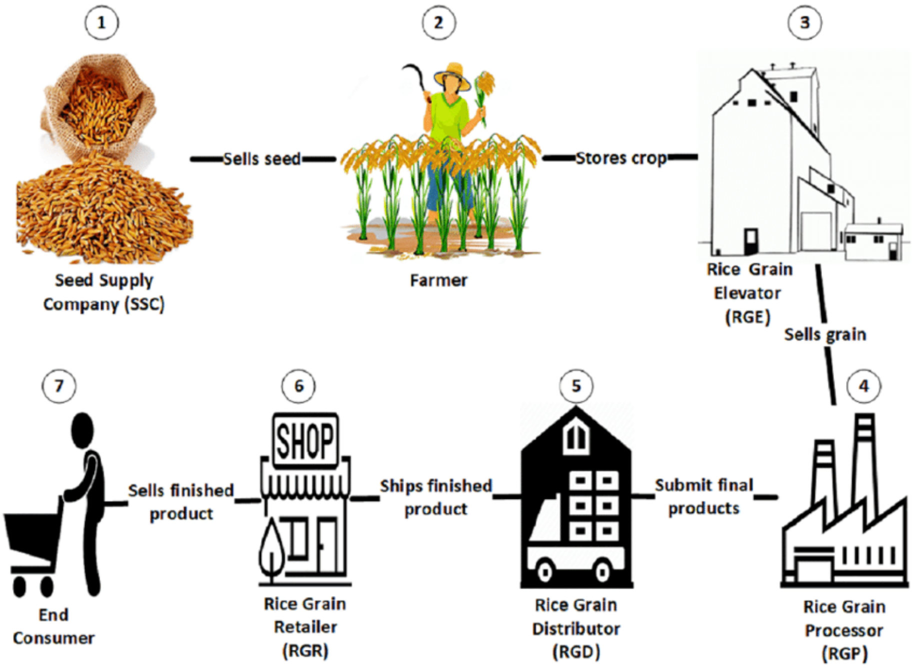
The agricultural value chain concept is discussed since the beginning of the millennium, primarily by those who working in agricultural development in developing countries. Value chain can be referred to the whole range of goods and services necessary for an agricultural product to move from the farm to the final customer or consumer. The term "agricultural value chain" refers to the interconnected series of activities and processes involved in the production, processing, distribution, and consumption of agricultural products (Oyelami & Ladele, 2018). At the heart of the agricultural value chain concept is the idea of actors connected along a chain producing and delivering goods to consumers through a sequence of activities. A major subset of value chain development work is concerned with ways of linking producers to companies, and hence into the value chains.

The Indian food basket has been increasingly diversifying towards high value, protein and nutrition rich foods such as fruits and vegetables, milk, eggs, meat, and fish.

Changing consumption preferences as a result of increasing income, increasing population, and urbanization together with higher global demand have lead in a demand-driven structural change in Indian agriculture. The fast developing, high value agricultural markets provide immense chance to India's smallholders who constitute 86.10 per cent of total farm holdings (Gualti *et al.*, 2022. Extension and Advisory Services (EAS) play a crucial role in providing support to farmers (Davis & Sulaiman, 2014) by facilitating their access to appropriate markets, enhancing their understanding of and adherence to quality standards required for their products in markets, and assisting them in obtaining a fair portion of the consumer price (Arulmanikandan *et al.*, 2023).

Linking farmers to market

The goal in linking farmers to market is to invest in ways that enable farmers or group of farmers to access market that match their capacities, production, investment and risk profiles (Yadav, 2018). While there are examples of fully integrated value chains that do not involve smallholders (e.g. Unilever operates tea estates and tea processing facilities in Kenya and then blends and packs the tea in Europe before selling it as Lipton, Brooke Bond or PG Tips brands), the great bulk of agricultural value chains involve sales to companies from independent farmers. Such arrangements frequently involve contract farming in which the farmer undertakes to supply agreed quantities of a crop or livestock product, based on the quality standards and delivery requirements of the purchaser, often at a price that is established in advance. Companies often also agree to support the farmer through input supply, land preparation, extension advice and transporting produce to their premises.

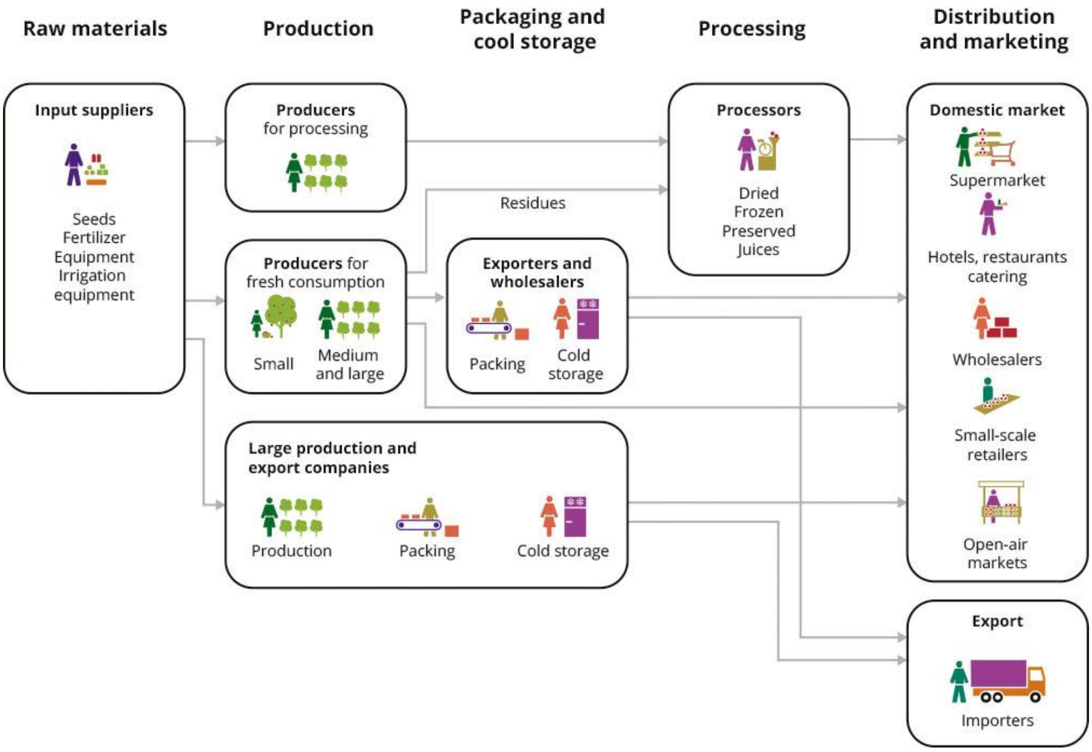


(Source: Yakubu *et al.*, 2022)

Fig: Rice Value Chain

Inclusive Value Chain

Work to promote market linkages in developing countries is often based on the concept of "inclusive value chains", which usually places emphasis on identifying possible ways in which small-scale farmers can be incorporated into existing or new value chains or can extract greater value from the chain, either by increasing efficiency or by also carrying out activities further along the chain. In the various publications on the topic the definition of "inclusion" is often imprecise as it is often unclear whether the development aim is to include all farmers or only those best able to take advantage of the opportunities.



(Source: FAO, 2021)

Fig: value chain of vegetables

Agricultural Value Chain Finance

Agricultural value chain finance is concerned with the flows of funds to and within a value chain to meet the needs of chain actors for finance, to secure sales, to buy inputs or produce, or to improve efficiency. Examining the potential for value chain finance involves a holistic approach to analyze the chain, those working in it, and their inter-linkages. These linkages allow financing to flow through the chain.

For example, inputs can be provided to farmers and the cost can be repaid directly when the product is delivered, without need for farmers taking a loan from a bank or similar institution. This is common under contract farming arrangements. Types of value chain finance include product financing through trader and input 6 supplier credit or credit supplied by a marketing company or a lead firm. Other trade finance instruments include receivables financing where the bank advances funds against an assignment of future receivables from the buyer, and factoring in which a business sells its accounts receivable at a discount. Also falling under value chain finance are asset collateralization, such as on the basis of warehouse receipts, and risk mitigation, such as forward contracting, futures and insurance.

Use of Information Communication Technology in Value Chain Management

ICTs have grown in importance as a tool for increasing the efficiency of the agricultural value chain. In particular, there has been a sharp increase in the use of mobile technologies. The goal of agricultural value chains is to increase market performance and economic productivity through diverse alliances of primary producers and other economic actors. ICT services are getting cheaper, and more people in underdeveloped nations can now afford the technologies. Through SMS, applications can provide farmers with direct support. Their technology setup, business scale, degree of education, and information-gathering methods set them apart.

ICTs definitely have a significant impact on reducing information and communication asymmetries amongst agricultural value chain participants and on breaking the cycle of rural poverty. Examples include the Kenyan-developed app iCow, which offers details on the length of the gestation period, cow artificial insemination, and cow maintenance. A significant portion of the population without bank accounts can obtain mobile payment services thanks to apps like M-Pesa, which will facilitate value chain transactions.

Conclusion

In rural areas, agriculture is the main source of income. However, for a variety of reasons, small and marginal farmers faced more challenges in being successful in the agricultural sector. When planning the next support services, the extension staff must, nevertheless, take into consideration a number of limits and view these limitations through the lens of value chain extension. Value Chain Extension fills the knowledge void left by implementation. Value chain interventions connect farmers with agribusinesses, processors, and distributors, resulting in a more equitable and transparent supply chain. In summary, it transforms agriculture by encouraging cooperation, creativity, and long-term growth. With this method, farms become networked centers of productivity, knowledge, and economic wealth.

Reference

Arulmanikandan, B., Vaishnavi, P. and Jayan, R. (2023). The Role of Agricultural Value Chain Extension in Facilitating Agricultural Transformation. Emerging Trends in Agricultural Extension Education. P: 528-544.

Davis, K. and Sulaiman, R. (2014). The new extensionist: Roles and capacities to strengthen extension and advisory services. Journal of International Agricultural and Extension Education, 21(3), 6-18.

Food and Agriculture Organization. (2021). Market and value chains: Responding to health conscious consumer.

Gualti, A., Ganguly, K. and Wardhan, H. (2022). Agricultural Value Chains in India. India Studies in Business and Economics. ISBN 978-981-33-4267-5.

Oyelami, B. O. and Ladele, A. A. (2018). Agricultural Value Chain Extension: A panacea for Agricultural Transformation in Nigeria. Journal of Agricultural Extension, 22(1): 173-184.

Yadav, H. (2018). Linking Farmers to Market: Opportunities and Challenges. Proceedings of the Feed The Future India Triangular Training Program.

Yakubu, B.M., Latif, R., Yakubu, A., Khan, M.I. and Magashi, A.I. (2022). RiceChain: secure and traceable rice supply chain framework using blockchain technology. *Peer.J Comput. Sci.*, 5.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/5/2024

ADVANCED BREEDING STRATEGIES FOR RICE CROP IMPROVEMENT

M. R. Prajapati[1], V. P. Patel[2], C. P. Chandramaniya[3] and S Bhattacharya[4]

[1]PhD Research Scholar, Navsari Agricultural University, Navsari, Gujarat
[2]Associate Research Scientist, Regional Rice Research Station, NAU, Vyara, Tapi, Gujarat
[3]PhD Research Scholar, Anand Agricultural University, Anand, Gujarat
[4]PhD Research Scholar, Junagadh Agricultural University, Junagadh, Gujarat

Introduction

Agriculture, the most important sector of the economy that is highly dependent on climate. Climate change affect directly or indirectly which is reflected by a lower crop productivity, that's why today the major challenges for plant biologists is global climate changes and to meet the demands of food and energy for the increased world population. Rice farming is a vital part of our agriculture sector, huge staple food in many country and provide minerals, vitamins and fiber it a healthy choice to eat. Rice grain makes up to 20% of the world's dietary energy supply and more than three billion people across the globe eat rice daily as major staple food. Current Rice productivity and production levels are not sufficient to feed the world's growing population. That's why Plant breeders need to innovative solution and give new cultivars which is high yielding with multiple stress resistances/Tolerances at faster rate in changing environment, utilizing advanced plant breeding strategies for sustainable growth of agriculture. In India, conventional breeding is still widely used for improving rice since it produces a huge number of recombinants with favorable traits. Robust phenotyping, which is based on breeders' experiences, is essentially the cornerstone of conventional breeding. But the major drawbacks of conventional breeding approaches, including high labor costs, times intensive, low efficiency, reliance on the environment, limited genetic gain, etc. Therefore, incorporation of modern tools along with the conventional breeding triggers new breeding strategies, like Marker assisted selection (MABC, MAGP and MARS), Genome editing, genetic enginnering, gene cloning, genomic selection, forward breeding, and haplotype-based precision breeding.

Rice – Modern crop for biological research

- Small genome size: 45 x 10^6 bp.
- Highly dense molecular map
- YAC and BAC libraries
- T- DNA insertion and deletion mutants
- Gene bank with 1,00,000 accessions

- Several wild species and
- Huge database

Modern breeding strategies

A. Marker assisted selection

Marker Assisted Selection [MAS] refers to indirect selection for a desired plant phenotype based on the banding pattern of linked molecular (DNA) markers. MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker which is tightly linked to the gene of interest. Its advantages include

- It may be simpler than phenotypic screening, which can save time, resources and effort.
- Selection can be carried out at the seedling, which may enable certain traits to be 'fast-tracked', resulting in quicker line development and variety release.
- Selection of traits with low heritability and distinguishing homozygotes from heterozygotes is possible.
- Markers can also be used as a replacement for phenotyping, which allows selection in off season nurseries making it more cost-effective to grow more generations per year
- Another benefit from using MAS is that the total number of lines that need to be tested can be reduced.

Various scheme under marker assisted selection

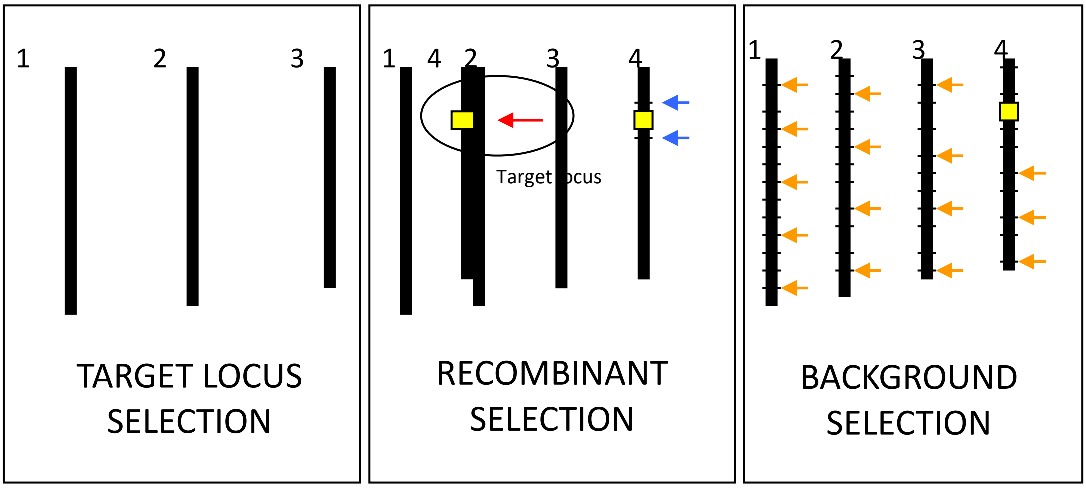
1. Marker assisted backcrossing

Backcrossing has been a widely used technique in plant breeding for almost a century. Backcrossing is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety. In most cases, the parent used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics, but major limitation of conventional backcrossing is selection of particular trait interfere with environmental effect, linkage drag, require more time, not accurate and not get enough recovery of recurrent parent which has desirable other traits. But using Marker in backcrossing will lead to solve all above mention problems.

There are three general levels of MAB. In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as **'foreground selection'** this may be particularly useful for traits that have laborious or time-consuming and difficult phenotypic screening procedures.

The second level involves selecting BC progeny with the target gene and recombination events between the target locus and linked flanking markers this is referred as '**recombinant selection**'. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e., size of the introgression).

The third level of MAB involves selecting BC progeny with the greatest proportion of recurrent parent (RP) genome, using markers that are unlinked to the target this is refer as '**background selection**'



2. Marker Assisted Recurrent Selection (MARS)

In order to accumulate favorable alleles in inbred lines from several genomic areas within a single population, marker assisted recurrent selection, or MARS, is another method for crop improvement. In contrast to MABC, it provides advantages in preserving the higher genetic variability resulting from repeated inter-mating among the chosen lines of heterozygous population, which raises the frequency of favorable alleles for a variety of traits of interest, especially those governed by polygenes.

However, India's efforts to breed rice have not shown much success. Using the MARS technique, IR58025B, a maintenance line of rice hybrid, has recently been enhanced for drought and salinity tolerance QTLs (Suryendra *et al.*, 2020).

3. Marker assisted gene pyramiding (MAGP)

Pyramiding is the process of concurrently combining many genes or QTLs into one genotype. Gene pyramiding is crucial for ensuring the persistence of rice resistance to BLB and for broad range resistance to the disease.

Because molecular or DNA-based marker are non-destructive and may assess markers encoding many target genes with a single DNA sample without phenotyping, molecular markers aid in selection. The most common use of pyramiding in plant breeding is the combination of numerous disease resistance genes, or QTLs, to give long-lasting disease resistance. Conventional breeding can still be used, but it is extremely difficult or even impossible in the early generations since every plant must be phenotypically screened for every characteristic under consideration. Because of this, it is exceedingly difficult to assess plants using damaging bioassays for attributes or from specific populations, such as the F_2.

In India, improved rice varieties/lines were introduced with different biotic and abiotic stressors through the use of marker-assisted selection.

Sr. No	Name of improved varieties	Gene combination	Stress	References
1	Improved Samba Mahsuri	*xa5 + xa13 + Xa21*	Bacterial blight	Sundaram *et al.*, 2008
2	Improved Tapaswini	*Xa4 + xa5 + xa13 + Xa21*	Bacterial blight	Das *et al.*, 2018
3	Swarna	*Xa4+xa5+xa13+Xa21+Sub1*	Bacterial blight & Submergence	Pradhan *et al.*, 2019
4	ADT 43	*Pi54+Pi33+Pi1*	Rice blast	Pandian *et al.*, 2019
5	MushkBudj	*Pi54+ Pi1+ Pita*	Rice blast	Khan *et al.*, 2018
6	Swarna-Sub1 (CR 2539-1)	*Sub1*	Submergence	Neeraja *et al.*, 2007
7	Aiswarya	*Saltol+ Sub1*	Salinity & submergence	Nair and Shylaraj, 2021
8	Pusa Basmati 1121	*Saltol*	Salinity	Babu *et al.*, 2017
9	MTU 1010	*qSALTOL + qSSISFH8.1*	Salinity	Bhandari *et al.*, 2019
10	CR Dhan 801	*qDTY1.1+qDTY2.1+qDTY3.1Sub1*	Drought and submergence	Kumar *et al.*, 2020
11	Samba Mahsuri-Sub1	*Sub1+DTY1.1+DTY2.1+DTY2.2 + DTY3.1*	Drought and submergence	Bhandari *et al.*, 2019
12	IR64-Sub1	*Sub1+qDTY1.1+qDTY2.2+qDTY3.1*	Drought and submergence	Bhandari et al., 2019

A. Gene cloning

Gene cloning refers to the production of a large population of a DNA fragment in pure form. It involves the formation of a recombinant DNA and its introduction into an appropriate host, such as Escherichia coli or Bacillus subtilis. The host used should be without plasmids. The walls of host bacteria are made permeable by treatment with calcium chloride or a lysozyme. The recombinant DNA is added to the culture in which such host bacteria are growing. The recombinant DNA is taken up by the bacteria along with the nutrients. It replicates whenever the bacterial cell divides.

The seven main steps involved in gene cloning are:

1. Isolation of DNA (gene of interest) fragments to be cloned
2. Insertion of Isolated DNA into the suitable vector to form the recombinant DNA
3. Introduction of the recombinant DNA into a suitable organism known as host and other steps too

This is done either for one or both of the following reasons:

(a) To replicate the recombinant DNA molecule in order to get the multiple copies of our GI.

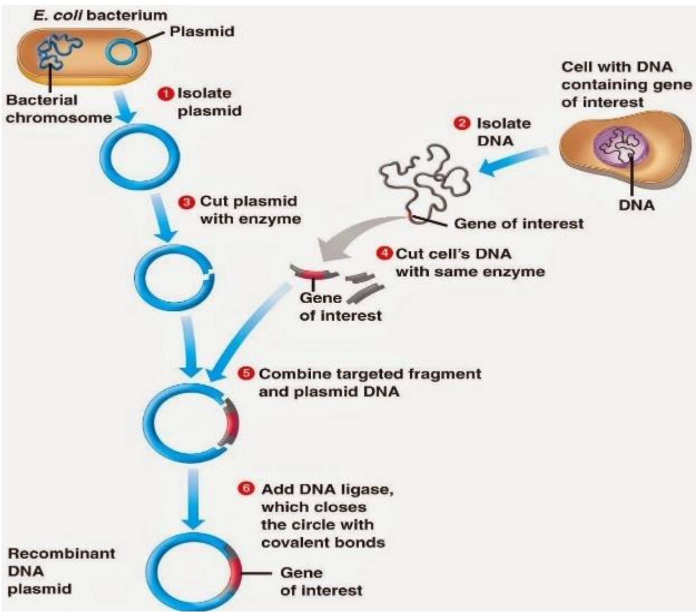
(b) To let our GI, get express and produce the protein which is needed by us. Introduction of the recombinant DNA into the host cell is done by various ways and strictly depends upon the size of the DNA molecule and the nature of GI. Some of the methods followed to carry out this step includes electroporation, micro-injection, lipofection, etc. When we carry out this pro-cess some of the host cells will take up the re-combinant DNA and some will not. The host cells which have taken up the recombinant DNA are called transformed cells and the process is called transformation.

4.Selection of the Transformed Host Cells and Identification of the Clone Containing the Gene of Interest

5.Multiplication/Expression of the Introduced Gene in the Hos

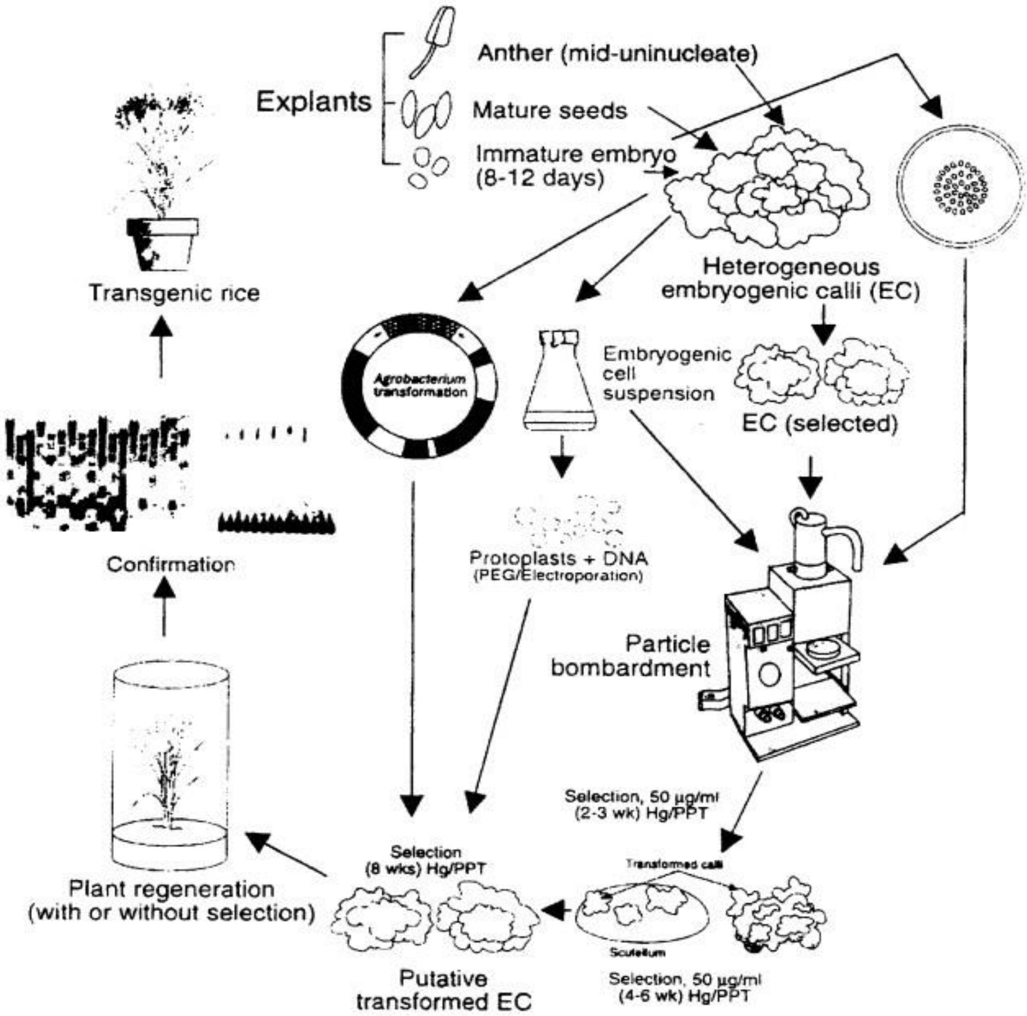
6.Isolation of the Multiplied Gene Copies/Protein Expressed by the Introduced Gene

7.Purification of the Isolated Gene Copy/Protein.



D. Genetic Engineering

Genetic engineering, the **artificial manipulation, modification, and recombination of DNA** or other nucleic acid molecules to modify an organism. The term is generally used to refer specifically to methods of recombinant DNA technology. Including various direct (gene gun) and indirect (agrobacterium) methods.



Datta, S. K. (2000)

Development and use of transgenic rice with agronomically important genes.

Rice	Method	Gene transferred	Traits
Japonica	Biolistic	*Xa21*	Resistances to BLB
Indica	Biolistic	PR genes	Resistances to sheath blight
Indica	Biolistic	*Bt* ML for hybrid Rice	Resistances to stem borer

Rice	Method	Gene transferred	Traits
Japonica	Agrobacterium	*Psy*, *lyc* and *crt* 1	Beta carotene synthesis
Indica/ Japonica	Biolistic and protoplast	Early nodulation	Biological N Fixation

Applications

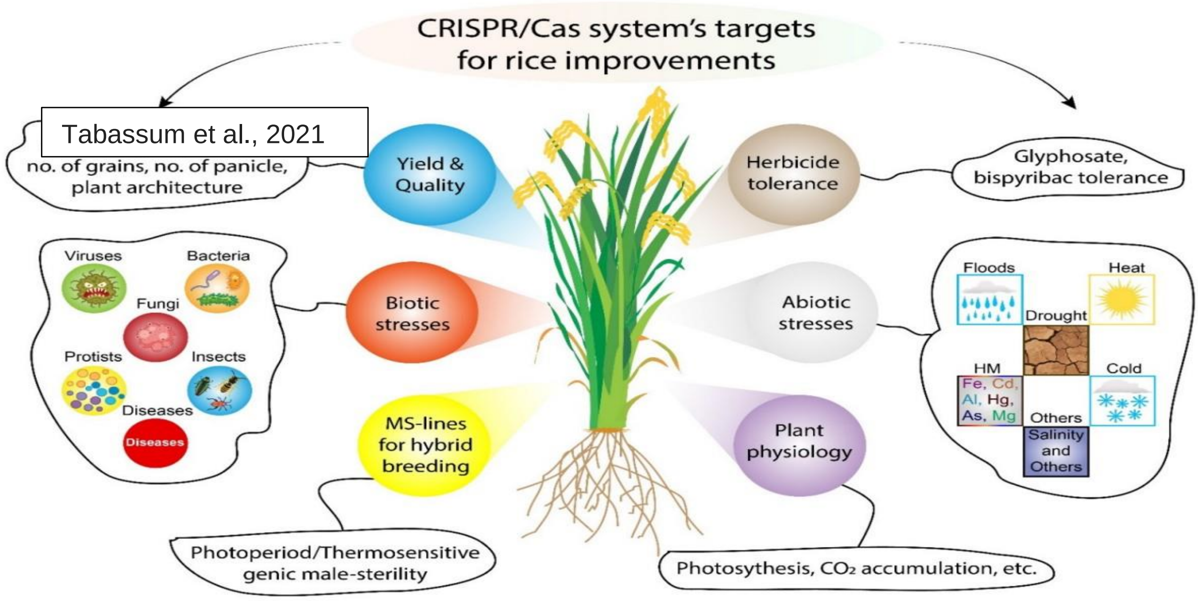
- Insect, pathogen and herbicide resistant plants
- Stress and senescence tolerant plants
- Genetic manipulation of flower pigmentation
- Modification of plant nutritional content
- Plant as bioreactors (therapeutic agents, antibodies)
- Edible vaccines
- Renewable energy crops
- Plant yield

E. Genome editing

Genome / Gene editing is a type of genetic engineering in which precise genome modifications are brought about using artificially engineered nucleases in organisms.

Genome editing encompasses a wide variety of tools using either a site-specific recombinase (SSR) or a site-specific nuclease (SSN) system. Both systems require recognition of a known sequence. The SSN system generates single or double strand DNA breaks and activates endogenous DNA repair pathways. SSR technology, such as Cre/loxP and Flp/FRT mediated systems, are able to knockdown or knock-in genes in the genome of eukaryotes, depending on the orientation of the specific sites (loxP, FLP, etc.) flanking the target site.

There are 4 main classes of SSN developed to cleave genomic sequences, mega-nucleases (homing endonuclease), zinc finger nucleases (ZFNs), transcriptional



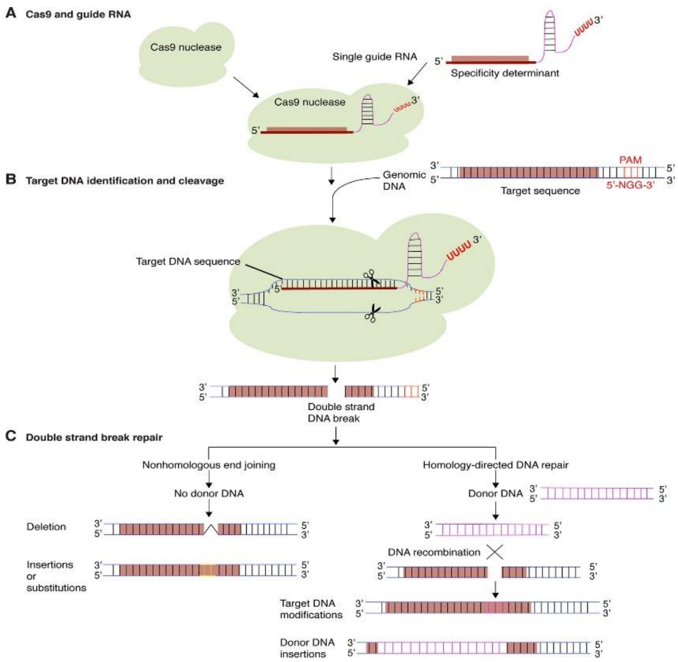
activator-like effector nucleases (TALENs), and the CRISPR/Cas nuclease system (clustered regularly interspaced short palindromic repeat/CRISPR-associated protein).

CRISPR/CAS Genome Editing System

A novel genome editing system, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has been developed in 2013. CRISPRs are genetic elements that gave immunity to bacteria to protect them against viruses. The protection mechanism includes 3 main stages: adaptation, transcription and interference. At the adaptation stage, when foreign DNA entered bacterial cells, a small fragment is inserted into the CRISPR locus of the host genome in specialized repeat structures separated from each other by short palindromic repeats and therefore they received the name CRISPR.

The Casgenes (CRISP associated), are located close to the CRISPR cassette express protein and have helicase and nuclease activity. At the transcription stage, the entire CRISPR locus is transcribed into a long pre-crRNA (poly-spacer precursor crRNA) and produce short crRNAs (CRISPR RNA) of 39–45 nucleotides containing one spacer sequence. The interference stage includes assembled ternary Cas9-crRNA-tracrRNA complex at the 3′ side of the crRNA-tracrRNA duplex structure and binds to Cas-proteins. The crRNA recognizes complement with the protospacer sequence, while tracrRNA is required for Cas-mediated DNA interference and Cas-proteins to cut matching foreign DNA sequences causing DNA degradation.

The CRISPR element consists a complex of non-coding RNAs and Cas proteins (CRISPR associated), which have nuclease activity. In contrast to the chimeric TALEN proteins, recognition by the CRISPR/Cas system is carried out via the complementary interaction between a non-coding RNA and the target site DNA.



Basic plasmid constructs used for this system contain the elements necessary for CRISPr/Cas9 activity: Cas9 nuclease/nickase, CRISPR mRNA, and tracrRNA. To change the target sequence, this construct only needs cutting off the original 30 nucleotide guide sequence flanked by unique restriction sites and replacing it with an artificially synthesized one. Both wild-type Cas9, which creates a double stranded break at the target site, and Cas9 Nickase, which creates a single stranded break, could be used in genome editing. Nickase is mutated Cas9 that cut only single stranded DNA, Nickase HNH cut the binding strand while Nickase RuvC cut the complementary strand. For genome editing using Cas9 Nicase 2 guide RNAs are required for each type and that will reduce off target events.

Steps:

- The first step is gRNA design and construct development.
- After confirmation of the construct, it is transferred either in *Agrobacterium* or coated on gold particles for biolistic bombardment.
- The construct is transformed to rice callus. The plants are regenerated from this callus.
- The regenerated plants are confirmed through PCR and restriction digestion, followed by sequencing.
- The plants are then screened for desired characteristics and moved to the greenhouse and then to field trials.

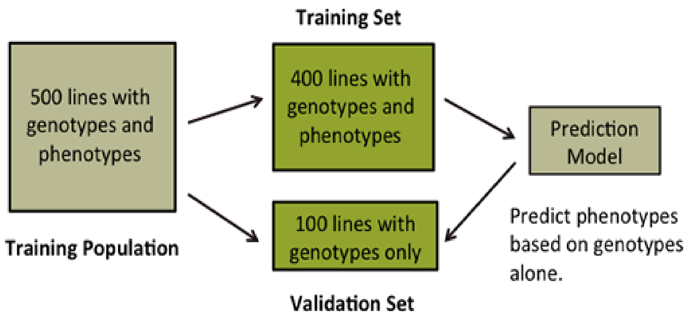
Examples of CRISPR/Cas9 system for enhancing abiotic stress tolerance of rice

Traits	Edited Gene	Improved Traits in Mutants	References
Drought	*OsSAPK2*	Reduced drought, salinity, and osmotic stress tolerance; role of gene in ROS scavenging, stomatal conductance and ABA signaling	Lou *et al.*, 2017
	OsPYL9	Drought tolerance; grain yield, antioxidant activities, chlorophyll content, ABA accumulation, leaf cuticle wax, survival rate, stomatal conductance, transpiration rate	Usman *et al.*, 2020
Salinity and Osmotic Stress	*OsRR22*	Salinity tolerance, shoot length, shoot fresh and dry weight	Zhang *et al.*, 2019
Rice blast	*OsALB1, OsRSY1,*	Resistances to blast	Foster *et al.*, 2018
Bacterial leaf blight	*OsSWEET14*	Resistances to BLB	Zeng *et al.*, 2020

F. Genomic selection

- Linkage mapping is limited by poor resolution, few alleles and need for mapping populations.
- QTL mapping cannot detect minor QTLs with low heritability.
- Association mapping is prone to false positives and unable to explain all the variance of a trait.

- MABC and MAGP improves only for introgressed QTL.
- MARS is based on significant major and minor QTLs.
- Genomic selection involves simultaneous estimation of all locus effects across the genome to calculate genomic estimation of breeding value (GEBV).
- Breeding value of the genotype is sum of average effect of the genes it carries.
- The breeding value (BV) of an individual/line represents the expected phenotype of its progeny.
- If an individual is crossed with a random selection of population, its BV relates to the deviation from the population mean.
- Conventionally, BV is estimated from half sib population and related to GCA and Additive gene action.



When use?

- Minor QTLs
- Low heritability
- When phenotypic selection is ineffective
- When cost of genotyping is less than phenotyping
- Does not deal with cause-and-effect relationship

G. Haplotype based breeding

A haplotype is an assortment of single nucleotide polymorphisms (SNPs) that come from a single parent and are inherited together. In addition to being defined as linkage between a set of SNPs, a haplotype can also refer to a collection of alleles or a series of SNPs on the same chromosome. After SNP markers are designed, superior haplotypes will be employed to transmit these better haplotype blocks for trait enhancement.

Additionally, specialized markers for selected better haplotypes might be utilized to test genotypes for desirable traits. Haplotyping may be used to determine the parental lines (maintainers and restorers) of rice hybrids prior to test crossing in order to produce new hybrids, using hybrid rice as an example.

Therefore, the constraints of linked, tightly connected, and gene-based markers to be used in trait improvement will be resolved by genotyping haplotypes for diverse characteristics and then identifying super haplotypes. Furthermore, the donors used in the bi-parental mapping population for QTL/gene discovery might not be accessible for need-based application. Nonetheless, by haplotyping the current rice germplasm, the found markers may be used to get the necessary donor genotype information for additional use.

H. Forward breeding for rice improvement

The creation of inbred lines with favorable alleles for the number of loci involved or the balance of positive alleles among the population's parents is referred to as forward breeding. This comprises the creation of a source population with several advantageous alleles, inbreeding, and trait-specific selection.

The forward breeding method has been effectively used to the betterment of rice. By combining MAS with forward breeding, it may be possible to create better rice lines with a variety of tolerant genes from different parents.

Using a crossing program involving recurrent parents (Swarna+drought) carrying drought tolerant QTLs (*qDTY1.1*, *qDTY3.1*) and four diverse donors with targeted genes like BB (*Xa4*, *xa5*, *xa13*, *Xa21*), Blast (Pi9), BPH (*Bph3 and Bph17*), and GM (*Gm4 and Gm8*), Dixit et al. (2020) adopted such a breeding approach to incorporate multiple genes for biotic-abiotic stress resistance/tolerance in rice. IC_3F_1s were created for the purpose of introducing desired multiple QTLs/gene combinations into a single rice genetic background after numerous rounds of inter-crossing.

Conclusion

With only one instance of the introduction of specific characteristics, marker-assisted back crossing (MABC) was able to transfer several traits linked to biotic and abiotic tolerance into high yielding rice varieties and parental lines of rice hybrids without sacrificing the background of recurring parents. However, given the effects of climate change, it is imperative to generate new rice varieties that are more genetically gain-tolerant and resistant to diverse stressors. This cannot be achieved with the current breeding program. Thus, cutting-edge breeding techniques including Genome editing, genetic enginnering, gene cloning, genomic selection, forward breeding, and haplotype-based precision breeding combined with a speed breeding strategy, and NGS technology might be used to upgrade conventional breeding technologies.

References

Babu NN, Krishnan SG, Vinod KK, Krishnamurthy SL, Singh VK, Singh MP, SinghR, Ellur RK, Rai V, Bollinedi H and Bhowmick PK (2017). Marker aided incorporation of Saltol, a major QTL associated with seedling stage salt tolerance, into Oryza sativa 'Pusa basmati 1121'. *Frontiers in Plant Science* 8: 41

Das G, Rao GJ, Varier M, Prakash A and Prasad D. (2018). Improved Tapaswini having four BB resistance genes pyramided with six genes/QTLs, resistance/ tolerance to biotic and abiotic stresses in rice. *Scientific Reports* 8(1):1-16

Datta, S. K. (2000). Potential benefit of genetic engineering in plant breeding: rice, a case study. *Journal of Applied Biological Chemistry*, 43(4), 197-206.

Foster, A.J.; Martin-Urdiroz, M.; Yan, X.; Wright, H.S.; Soanes, D.M.; Talbot, N.J. CRISPR-Cas9 ribonucleoprotein-mediated co-editing and counterselection in the rice blast fungus. *Sci. Rep.* 2018, 8, 14355.

Khan GH, Shikari AB, Vaishnavi R, Najeeb S, Padder BA, Bhat ZA, Parray GA, Bhat MA, Kumar R and Singh NK (2018). Marker-assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar MushkBudji. *Scientific Reports* 8(1): 1- 13

Lou, D.; Wang, H.; Liang, G.; Yu, D. OsSAPK2 Confers Abscisic Acid Sensitivity and Tolerance to Drought Stress in Rice. *Front. Plant Sci.* 2017, 8, 1–15.

Nair MM and Shylaraj KS (2021). Introgression of dual abiotic stress tolerance QTLs (Saltol QTL and Sub1 gene) into rice (Oryza sativa L.) variety Aiswarya through marker assisted backcross breeding. *Physiology and Molecular Biology of Plants pp*. 1-18

Neeraja CN, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard BC, Septiningsih EM, Vergara G, Sanchez D, Xu K, Ismail AM and Mackill DJ (2007). A marker assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics* 115(6): 767-776

Pandian BA, Joel J, Nachimuthu VV, Swaminathan M, Govintharaj P, Tannidi S, Sabariappan R (2018). Marker-aided selection and validation of various $${Pi} $$ Pi gene combinations for rice blast resistance in elite rice variety ADT 43. *Journal of Genetics* 97(4): 945-952

Pradhan SK, Pandit E, Pawar S, Baksh SY, Mukherjee AK, Mohanty SP(2019). Development of flash-flood tolerant and durable bacterial blight resistant versions of mega rice variety 'Swarna' through marker-assisted backcross breeding. *Scientific Reports* 9(1): 1-15

Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Reddy GA, Rani NS, Sarma NP, Sonti RV(2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica* 160(3): 411-422

Tabassum, J., Ahmad, S., Hussain, B., Mawia, A. M., Zeb, A., & Ju, L. (2021). Applications and potential of genome-editing systems in rice improvement: current and future perspectives. *Agronomy*, 11(7), 1359.

Usman, B.; Nawaz, G.; Zhao, N.; Liao, S.; Liu, Y.; Li, R. Precise Editing of the OsPYL9 Gene by RNA-Guided Cas9 Nuclease Confers Enhanced Drought Tolerance and Grain Yield in Rice (Oryza sativa L.) by Regulating Circadian Rhythm and Abiotic Stress Responsive Proteins. *Int. J. Mol. Sci*. 2020, 21, 7854.

Zeng, X.; Luo, Y.; Vu, N.T.Q.; Shen, S.; Xia, K.; Zhang, M. CRISPR/Cas9-mediated mutation of OsSWEET14 in rice cv. Zhonghua11 confers resistance to Xanthomonas oryzae pv. oryzae without yield penalty. *BMC Plant Biol.* 2020, 20, 313.

Zhang, A.; Liu, Y.; Wang, F.; Li, T.; Chen, Z.; Kong, D.; Bi, J.; Zhang, F.; Luo, X.; Wang, J.; et al. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol. Breed.* 2019, 39, 47.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/6/2024

BIOTECHNOLOGICAL APPROACHES TO MANIPULATE ETHYLENE BIOSYNTHESIS AND ITS ACTION IN FRUITS

Brijesh Kumar Yadav[1*], Prasoon Gunjan[1], Bhupendra Sagore[2]

[1]PhD Scholar, Division of Food Science and Postharvest Technology, ICAR-IARI, New Delhi-110012

[2]PhD Scholar, Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi-110012

*Corresponding author: bkybhu98@gmail.com

Abstract

Ethylene plays a pivotal role in various stages of plant development and postharvest quality, but excessive ethylene can lead to spoilage. Biotechnology has enabled the creation of transgenic plants with altered ethylene production or perception, providing control over ripening and stress responses. Different strategies, such as modifying SAM decarboxylase, SAM hydrolase, ACC synthase, ACC deaminase, and ACC oxidase, have been employed to reduce ethylene levels, with some techniques showing promise in delaying ripening and enhancing pathogen resistance. However, careful fine-tuning is required to balance ethylene reduction with the preservation of desirable postharvest qualities.

Introduction:

Ethylene is a key regulator of plant growth and development, as it is involved in most of the developmental processes of the plant, from seed germination, stem and root elongation, flowering, to leaves and flower senescence, fruit development and ripening. Moreover, ethylene is inducible also as a response to biotic and abiotic stress, such as pathogen attack, wounding, water logging and drought.

Ethylene derives from the "Yang" cycle; methionine is converted to ethylene with S-adenosylmethionine (SAM) and 1-aminocyclopropane-l-carboxylic acid (ACC) as intermediates. These two reactions are catalysed by ACC synthase (ACS) and ACC oxidase (ACO). Ethylene signal is perceived by receptor proteins and transduced trough a phosphorylation cascade that led to transcription factor activation and consequently ethylene-related gene expression. Due to its role in regulation flower senescence and fruit ripening, the control of ethylene production and perception has always been a major issue to prevent fruit/flower spoilage and prolong marketability.

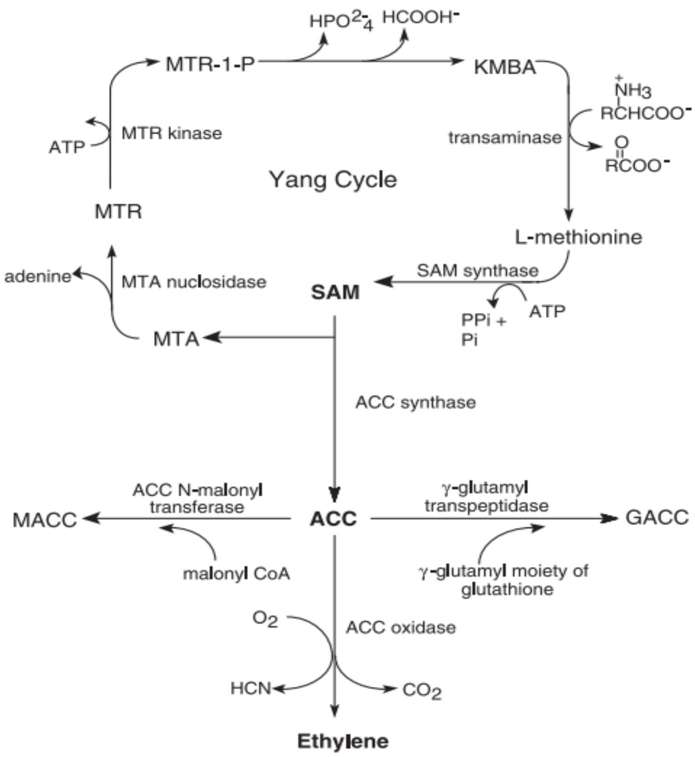


Fig. 1 Yang cycle and Ethylene Biosynthesis Pathway

Plant biotechnology enabled to control ethylene biosynthesis and perception in plants. Mutants and transgenic plants altered in ethylene perception or production have been generated either to elucidate ethylene function and the mode of action but also to improve fruit and flower shelf-life.

Ethylene Biosynthesis and Its Relation to Postharvest Quality of Fresh Produce:

Ethylene has a central role in the regulation of different biological processes associated with the postharvest life of many different crops including fruits and vegetables, leafy vegetables, flowers, tubers, and bulbs. Postharvest-related processes in which ethylene action is well documented include fruit ripening, senescence, abscission, softening and texture changes, development of physiological disorders, taste and aroma development, nutritional value, and fruit–pathogen interactions.

Manipulations of ethylene biosynthesis were achieved by inhibition of the two central biosynthesis-related genes, 1-aminocyclopropane-1-carboxylate oxidase (ACC) synthase (ACS) or ACC oxidase (ACO). Hormone perception was manipulated in most cases by introduction of a mutated form of ethylene receptor genes found to confer insensitivity when introduced into heterologous plant systems.

Although ethylene action in many postharvest-related contexts has negative implications and generally its inhibition has a postharvest advantage, in some cases ethylene action is required for maintaining or improving postharvest qualities. For example, downregulation of ethylene synthesis aimed at extending the shelf life of climacteric fruits often results in lower production of aroma compounds. Excessive inhibition of ethylene likely results in non-attractive fresh produce. One of the main challenges in biotechnological applications for modifying ethylene biosynthesis is the use of appropriate regulatory elements that will lead to the desired and appropriate effect in a given plant system for optimal postharvest benefit. Recently regulated ethylene insensitivity was obtained through the use of inducible expression of the Arabidopsis etr1-1 mutant ethylene receptor in tomato (Gallie, 2010).

Altering ethylene levels in plants

Modifying the amount of ethylene produced under ripening or stress conditions is the goal of a wide array of transgenic strategies. The first demonstrations of the transgenic approach came after the cloning of the ethylene biosynthetic genes encoding ACC synthase and ACC oxidase from tomato plants. Antisense technology was used to reduce expression of these genes in tomato. In both cases, it was possible to reduce ethylene production by more than 95% in the fruit. As a consequence, ripening of the transgenic fruits was suppressed.

An alternative approach to reduce ethylene biosynthesis has also been demonstrated. Rather than inhibit the native biosynthetic genes by antisense technology, the gene encoding a bacterial ACC deaminase was introduced into tomato, its expression serving to deplete the endogenous ACC pool. One potential advantage of reducing biosynthesis, rather than perception, of ethylene is that the process can be reversed by subsequent treatment with ethylene. The identification of genes involved in ethylene perception provides a new set of targets for transgenic modification. The ethylene receptor mutant etr1-1 should be of particular use owing to the dominant nature of this mutation. It is capable of rendering a plant ethylene insensitive in a background containing wild-type copies of itself as well as wild-type copies of other ethylene receptors. The etr1-1 mutation from Arabidopsis can also be used to generate ethylene insensitivity in other plant species. When transgenically expressed in tomato, the etr1-1 mutation delayed ripening and senescence of the fruit.

Table 1 Summarizes the types of transgenic plants that have been created by insertion of genes that alter the level of ethylene produced in the plant, fruit or flower.

Plant transformed	Gene and promoter	Consequences	References
Tomato	Tomato ACC oxidase in a sense orientation	87% reduction of ethylene in ripening fruit	Hamilton *et al.*, 1990

Plant transformed	Gene and promoter	Consequences	References
		68% reduction of ethylene in wounded leaves	
Tomato	ACC synthase in an antisense orientation with CaMV 35S	99.5% lower ethylene levels Fruit do not ripen	Ollere *et al.*, 1991
Carnation	ACC oxidase from carnation with MAC promoter	>90% reduction in ethylene in 5 days old plants	Savin *et al.*, 1995
Cantaloupe	MEL1(ACC oxidase) from cantaloupe in an antisense orientation with CaMV 35S	99% reduction of ethylene in ripening fruit 68% reduction of ethylene in wounded leaves	Ayub *et al.*, 1996
Broccoli	ACC oxidase from tomato in an antisense orientation	91% reduction in ethylene after 90 hour	Henzi *et al.*, 1999

Transgenic strategies to lower ethylene levels in order of their action within the ethylene pathway:

1) SAM decarboxylase:

The enzyme SAM decarboxylase, converts SAM to decarboxylated SAM that is then used in the polyamine biosynthetic pathway. SAM decarboxylase is an essential gene that is present in bacterial, plant and mammalian systems, and has been isolated from many sources. Expression of SAM decarboxylase following the insertion of the gene under control of either the cauliflower mosaic virus (CaMV) 35S constitutive promoter or the tet promoter, in both the sense and antisense orientation, has been studied in potato plants. It was thought that overexpression of SAM decarboxylase might enhance the flux of SAM through the polyamine pathway, thus reducing the amount available for ethylene biosynthesis. Unfortunately, ACC synthase shows a higher affinity for SAM than does SAM decarboxylase, and insertion of this gene, in both orientations, caused unusual phenotypes. No plants were produced from the sense insertion transformants, and plants raised from callus with the antisense insertion had higher levels of ethylene production (Kumar *et al*., 1996). This result notwithstanding, the external application of SAM decarboxylase on peaches has been studied and found to have an effect similar to the chemical ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG) on ripening fruit (Bregoli *et al*., 2002)

2) SAM hydrolase

Another SAM degrading enzyme is SAM hydrolase. It is found only in bacteriophage T3, and converts SAM to 5V-methylthioadenosine (MTA) and homoserine both of which re-enter the methionine cycle. In this way, SAM is diverted away from the ethylene pathway and no foreign metabolites accumulate. The insertion of this gene under control of the CaMV 35S promoter caused no change in ethylene levels or plant phenotype in tomato but caused extremely stunted phenotypes in tobacco (Good *et al*., 1994). The use of the ripening specific E8 promoter, from tomato, allowed for a greater level of SAM hydrolase mRNA, and subsequently reduced ethylene levels in transgenic fruit compared with controls (Good *et al*., 1994).

3) ACC synthase

ACC synthase is one of the rate-limiting enzymes in ethylene biosynthesis and many strategies aimed at reducing ethylene have targeted this step. However, the multiple forms of this gene within the plant genome have made the construction of an effective antisense strategy difficult. Theologis and Sato (1998) found that although the coding regions of the various ACC synthase genes in tomato are similar, the control regions are not, allowing for temporal and inducible expression under different conditions. In order to inhibit a tomato ACC synthase mRNA, the entire gene including the untranslated portions was inserted in an opposite orientation under the CaMV 35S promoter into the plant genome (Oeller *et al*., 1991). Transgenic tomatoes showed a 99.5% decrease in ethylene production and no ripening was observed, a phenotype that was reversed by the addition of exogenous ethylene (Oeller *et al*., 1991)

4) ACC deaminase

The enzyme ACC deaminase was first discovered in soil microorganisms (Honma and Shimomura, 1978) and shown to convert ACC to ammonia and a-ketobutyrate, both of which may be further metabolized by the microorganism. By inserting the gene for ACC deaminase into tomato plants under the control of the CaMV 35S promoter, fruit with delayed ripening were produced (Klee *et al*., 1991).

Both Lund *et al*. (1998) and Robison *et al*. (2001a) observed that transgenic tomato plants that expressed ACC deaminase and had a lowered level of stress ethylene, were significantly protected from pathogen damage.

5) ACC oxidase

One strategy for reducing ethylene involved insertion of an antisense version of ACC oxidase into tomato; the effect of this strategy was studied on ripening fruit and wounded leaves. Hamilton *et al*. (1990) isolated a gene whose mRNA was abundant in ripening tomato and attempted to discover its role in ethylene synthesis by overexpressing it, in an antisense orientation, in tomato plants. They found that there was not only a decrease in the amount of sense mRNA for the gene in ripening fruit and wounded tomato leaves, compared to controls, but that no antisense mRNA was detectable in either tissue of transgenic plants (Hamilton *et al*., 1990).

Cantaloupe melon produces a large amount of ethylene, during the climacteric phase of fruit ripening; even more than other melons or tomatoes. Auyb *et al*. (1996) produced a transgenic cantaloupe line with lower levels of ethylene synthesis due to the overexpression of an antisense ACC oxidase gene called Mel1. Ethylene production in ripening fruit and wounded leaves was reduced by 99% and 68%, respectively (Auyb *et al*., 1996).

Conclusion:

In conclusion, ethylene is a pivotal player in the regulation of various plant growth and development processes, as well as postharvest quality in fruits and vegetables. Its dual role as a growth regulator and stress response signal has made it a subject of intense study in the field of plant biotechnology. Through genetic manipulation, researchers have successfully altered ethylene biosynthesis and perception, leading to the development of transgenic plants with diverse ethylene-related characteristics. The study of ethylene insensitivity, exemplified by the inducible expression of the Arabidopsis etr1-1 mutant ethylene receptor in tomato, offers a promising avenue for further research and potential applications in the field of plant biotechnology. These advancements hold great potential for addressing agricultural challenges and ensuring the availability of high-quality produce in the global market.

References

Ayub, R., Guis, M., Amor, M. B., Gillot, L., Roustan, J. P., Latché, A., ... & Pech, J. C. (1996). Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. *Nature biotechnology*, *14*(7), 862-866.

Bregoli, A. M., Scaramagli, S., Costa, G., Sabatini, E., Ziosi, V., Biondi, S., & Torrigiani, P. (2002). Peach (Prunus persica) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiologia Plantarum*, *114*(3), 472-481.

Good, X., Kellogg, J. A., Wagoner, W., Langhoff, D., Matsumura, W., & Bestwick, R. K. (1994). Reduced ethylene synthesis by transgenic tomatoes expressing S-adenosylmethionine hydrolase. *Plant Molecular Biology*, *26*, 781-790.

Hamilton, A. J., Lycett, G. W., & Grierson, D. (1990). Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature*, *346*(6281), 284-287.

Henzi, M. X., McNeil, D. L., Christey, M. C., & Lill, R. E. (1999). A tomato antisense 1-aminocyclopropane-1-carboxylic acid oxidase gene causes reduced ethylene production in transgenic broccoli. *Functional Plant Biology*, *26*(2), 179-183.

Lund, S. T., Stall, R. E., & Klee, H. J. (1998). Ethylene regulates the susceptible response to pathogen infection in tomato. *The Plant Cell*, *10*(3), 371-382.

Oeller, P. W., Lu, M. W., Taylor, L. P., Pike, D. A., & Theologis, A. A. (1991). Reversible inhibition of tomato fruit senescence by antisense RNA. *Science*, *254*(5030), 437-439.

Honma, Mamoru, and Tokuji Shimomura. "Metabolism of 1-aminocyclopropane-1-carboxylic acid." *Agricultural and Biological Chemistry* 42.10 (1978): 1825-1831.

Klee, H. J., Hayford, M. B., Kretzmer, K. A., Barry, G. F., & Kishore, G. M. (1991). Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *The Plant Cell*, *3*(11), 1187-1193.

Kumar A, Taylor MA, Mad Arif SA, Davies HV. Potato plants expressing antisense and sense S-adenosylmethionine decarboxylase (SAMDC) transgenes show altered levels of polyamines and ethylene: antisense plants display abnormal phenotypes. Plant J 1996;9(2):147 – 58

Robison, M. M., Shah, S., Tamot, B., Pauls, K. P., Moffatt, B. A., & Glick, B. R. (2001). Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase. *Molecular Plant Pathology*, *2*(3), 135-145.

Savin, K. W., Baudinette, S. C., Graham, M. W., Michael, M. Z., Nugent, G. D., Lu, C. Y., ... & Cornish, E. C. (1995). Antisense ACC oxidase RNA delays carnation petal senescence. *HortScience*, *30*(5), 970-972.

Gallie, D. R. (2010). Regulated ethylene insensitivity through the inducible expression of the Arabidopsis etr1-1 mutant ethylene receptor in tomato. *Plant physiology*, *152*(4), 1928-1939.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/7/2024

THE GREEN SYMPHONY: EXPLORING THE EVOLUTION, SIGNIFICANCE, AND BENEFITS OF INTERIOR LANDSCAPING

Vallarasu S[1] and Ranjani M[2]*

[1]*Division of Floriculture and Landscaping, ICAR – Indian Agricultural Research Institute, New Delhi – 110012, India.*

[2]*Division of Food Science and Postharvest Technology, ICAR – Indian Agricultural Research Institute, New Delhi – 110012, India.*

*Corresponding Author: white183.vallu@gmail.com

Introduction

Interior landscaping, often referred to as landscaping, has evolved over the years from a trend of the 1970s into landscaping an enduring practice that remains integral to contemporary design. Indoor plants are no longer seen as non-essential luxuries but rather as essential elements that enhance the aesthetics of our buildings and contribute to the psychological well-being of people. They also play a vital role in purifying the air, and improving our general health. The interior landscaping profession, which has developed over the past 25 years, has gained legitimacy and offers excellent career opportunities. Today, the business has become more complex and competitive, requiring a high level of horticultural knowledge and business acumen.

Plants suitable for Interior Landscaping

Indoor plants offer a multitude of benefits beyond their aesthetic appeal. Commonly known indoor plants, along with their botanical names and specific uses, can contribute to a healthier and more pleasant living environment. The snake plant, scientifically known as *Dracaena trifasciata*, excels at absorbing various volatile organic compounds (VOCs), making it an excellent choice for improving indoor air quality. The spider plant (*Chlorophytum comosum*) is renowned for its ability to eliminate harmful substances like xylene and formaldehyde. Lucky bamboo (*Dracaena sanderiana*) serves as both a decorative element and a source of aesthetic pleasure.

Another beneficial plant, the weeping fig (*Ficus benjamina*), plays a role in clearing xylene from indoor air. Areca palm (*Dypsis lutescens*) serves as a natural absorber of formaldehyde, contributing to a healthier indoor atmosphere. The Boston Fern (*Nephrolepis exaltata*) is effective at purifying indoor air by removing toxic VOCs. For those seeking stress relief, the baby rubber plant (Pep*eromia obtusifolia*) boasts flowers that can help create a calming atmosphere. Pothos (*Epipremnum aureum*) is well-known for its ability to absorb xylene, toluene, and benzene, enhancing air quality. The flamingo lily (*Anthurium andraeanum*) reduces ammonia concentration, improving overall air quality.

Moreover, the grape ivy (*Cissus rhombifolia* Vahl) contributes to maintaining an optimal oxygen level indoors. Lastly, the vibrant Croton (*Codiaeum variegatum*) not only adds color to your living space but also has a soothing effect on the mind. These indoor plants offer a range of advantages, from purifying the air to enhancing the ambiance, making them valuable additions to any indoor environment.

Classification of interior landscape plants

1. Growth Types: Plants are categorized by their growth patterns, which include trees, shrubs, vine plants, and ground cover plants, each with distinct roles in ecosystems.

2. Intended Use Purpose: Plants serve various purposes, such as greening for aesthetics, acting as hedges and shielding for privacy and protection, windproofing, dust, sand, and smokeproofing for specific environments, aiding in tidal land reclamation, and contributing to fire prevention.

3. Appreciation and Aesthetic: Plants are appreciated for their flowers, fruits, leaves, and unique stem features, adding to the aesthetic appeal of our environment, and showcasing the multifaceted roles they play.

Table 1. Classification of Interior Landscape Technique

Technique	Style	
Planting Technique	Garden Style	
	Planter Style	Planter Style Tree Planting Type Hanging Type Hydro-Culture Type
	Container Style	Container Style Large Container Type
Form Technique		Island Style Cascade Style 3d Style
Structure Technique		Entrance Style Count Style Mall Style

Advantages of Interior Landscaping Compared to Others

1. Does not require a fertile piece of land to be grown: Indoor landscaping allows for plant growth without the need for a dedicated outdoor garden or fertile land. It's especially beneficial for those with limited outdoor space.

2. Efficient in removing air pollution as well as pocket-friendly: Indoor plants can help purify the air by removing toxins and pollutants. They are cost-effective compared to complex outdoor landscaping projects.

3. Accessible for persons with disabilities: Indoor landscaping can be designed to be accessible for individuals with disabilities, allowing them to enjoy and maintain greenery without the need to navigate outdoor spaces.

4. Easy and enjoyable care: Taking care of indoor plants is generally easier and more enjoyable since they are protected from harsh weather conditions and pests. This can make gardening a more pleasant experience.

5. Reduced risk of diseases: Indoor plants are less susceptible to diseases and pests compared to outdoor plants. The controlled indoor environment provides better protection.

6. Controlled environment for plant growth: Indoor environments can be precisely controlled for temperature, humidity, and lighting, allowing plants to flourish under optimal conditions.

7. Effective air purification: Indoor plants contribute significantly to improving air quality, making them a valuable addition to both homes and workplaces.

8. Relaxing hobby: Gardening indoors can be a relaxing and stress-relieving activity, promoting mental well-being.

9. Improved air quality enhances cognitive function: Cleaner indoor air with higher oxygen levels can improve memory and concentration, positively impacting cognitive function.

10. Stress reduction and enhanced productivity: The presence of indoor plants has been shown to reduce stress levels and increase productivity by up to 12%, making them beneficial for workplaces.

11. Aesthetic appeal: Indoor landscaping enhances the visual appeal of indoor spaces, creating a more pleasant and inviting atmosphere.

12. Access to fresh produce: Some indoor herbal plants can provide access to fresh herbs and produce for culinary purposes.

13. Improved indoor air quality: The presence of indoor plants contributes to better air quality by filtering out pollutants and adding oxygen.

14. Noise reduction: Indoor plants can help reduce background noise, making the environment more peaceful and conducive to relaxation and concentration.

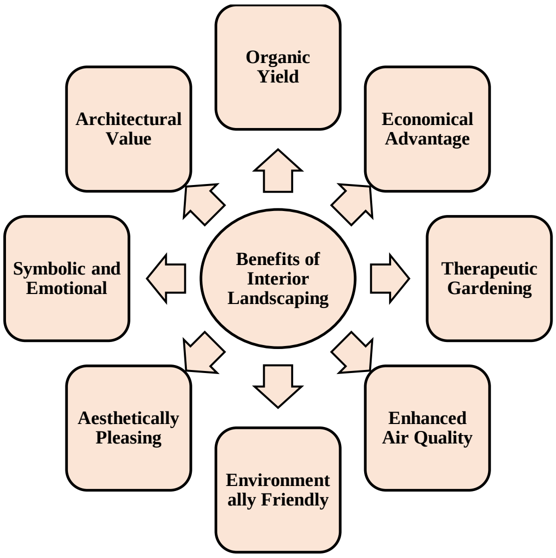


Fig 1. Benefits of Interior Landscaping

Conclusion

The history of interior landscaping is a testament to the enduring relationship between humans and plants. From the Chinese origins of indoor plant use thousands of years ago to the grandeur of the Hanging Gardens of Babylon and the innovation of the Romans in creating enclosed hothouses, the cultivation of plants indoors has evolved significantly. It has survived the Dark Ages, thrived during the Renaissance, and expanded with the discovery of new lands and species. Today, interior softscaping continues to thrive, bringing the beauty and benefits of the natural world into our homes and buildings. Whether it's a few pots on a windowsill or a lush garden room, house plants have become an essential part of our lives, offering a connection to the outdoors and a source of joy and beauty within our indoor spaces.

References

Won, J.-H. (1997). *Interior* landscape design (1st ed); Landscape Architecture Korea: Seoul. Korea.

Korean Horticultural Therapy & Wellbeing Association. *(For Horticultural Therapists) Horticulture*. (2019). *Bumin* cultural history (1st ed). Seoul, Korea.

Kim, W.-J., & Lee, T.-K. (2022). Greenness index and preferences for interior landscapes in residential spaces. *Sustainability*, *14*(9), 5183.

Mohamed, A. A., Elgizawi, L., & Abd Elhamid, N. (2022). Interior landscape techniques and its contribution to the interior places environmentally. Mansoura Engineering Journal, *(MEJ)*. October, *47*(5)

.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/8/2024

PACKAGING REVOLUTION: HOW NANOMATERIALS ARE TRANSFORMING THE FRESHNESS OF OUR FOOD

Vishwaradhya M Biradar[1], Sourabh Ajit Chougala[2], Pavan[3]*

[1&2]Ph.D. scholar, Department of Processing and Food Engineering, CAE, UAS, Raichur-584104
M.Tech. Student (Agril.Engg.) Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, GKVK, Bengaluru-560065
**Corresponding author email id: Vishwaradhyambiradar@gmail.com*

Introduction:

The prefix 'nano' is referred to a Greek prefix meaning 'dwarf' or something very small and depicts one thousand millionth of a meter (10^{-9} m). We should distinguish between nanoscience and nanotechnology. Nanoscience is the study of structures and molecules on the scales of nanometers ranging between 1 and 100 nm and the technology that utilizes it in practical applications such as devices *etc.*, is called nanotechnology. Nanoscience is a convergence of physics, materials science and biology, which deal with manipulation of materials at atomic and molecular scales; while nanotechnology is the ability to observe measure, manipulate, assemble, control and manufacture matter at the nanometer scale (Mansoori and Soelaiman, 2005).

The development of nanoscience can be traced to the time of the Greeks and Democritus in the 5th century B.C., when scientists considered the question of whether matter is continuous and thus infinitely divisible into smaller pieces, or composed of small, indivisible and indestructible particles, which scientists now call atoms.

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. The National Nanotechnology Initiative (NNI) in the United States define Nanotechnology as "a science, engineering and technology conducted at the nanoscale (1 to 100 nm), where unique phenomenon enables novel applications in a wide range of fields, from chemistry, physics and biology, to medicine, engineering and electronics" (www. nano. gov).

The basic and the key elements of nanotechnology are the "nanomaterials". The nanomaterials are the materials with less than 100 nm size ones at least in one dimension. That means they have very less size than that of microscale. The nanomaterials are usually 10^{-9} m in size that means it is one billionth of a meter. The nanomaterials show different physicochemical properties than the bulk material which inherently depends on their size and shape. Surprisingly the nanomaterials produce a unique character with new characteristics and capabilities by modifying the shape and size at the nanoscale level. The researchers are fascinated and working for the progress of knowledge in terms of their size, capability and expenditure. So special interest on the miniaturization of the device with economical is focusing mainly in the field of medicine and electronics. In upcoming days, the nanotechnology controls mankind in living, working and communicating fields (Lalitha *et al*., 2019).

Nano particles in food packaging:

In recent years, the application of active and intelligent packaging systems in the muscle-based food products, which are prone to contamination, has increased tremendously. The aim of packaging meat and muscle products is to suppress spoilage, bypass contamination, enhance the tenderness by allowing enzymatic activity, decrease weight loss, and retain the cherry red colour in red meats (Kerry *et al*., 2006). The advent of nanosensors provides food spoilage or contamination alarm to the consumers by detecting toxins, pesticides and microbial contamination in the food products, based on flavour production and colour formation (He *et al*., 2019). Most of the nanoparticles used for packaging in food industry have potential antimicrobial activity, acting as carriers for antimicrobial polypeptides and providing protection against microbial spoilage. Packaging material made of a coating of starch colloids filled with the antimicrobial agent acts as a barrier to microbes through the controlled release of antimicrobials from the packaged material (Sorrentino *et al*., 2007).

Nanoparticles are used as carriers to introduce enzymes, antioxidants, anti-browning agents, flavours and other bioactive materials to improve the shelf life even after the package is opened (Cha and Chinnan, 2004). Few metals and metal oxide nanoparticles (inorganic nanoparticles), namely iron, silver, zinc oxides, carbon, magnesium oxides, titanium oxides and silicon dioxide nanoparticles, are widely utilized as antimicrobials and in some conditions as food ingredients (He *et al*., 2019).

Nano material-based food packaging

- **Improved food packaging:** Improved food packaging is designed by adding nanoparticles to enhance mechanical and physical properties such as durability, strength, flexibility, biodegradability, thermal resistivity, UV absorptivity, water vapor and oxygen impermeability. The incorporation of metal oxides in polymers improves the mechanical, barrier, and light permeability properties (Garcia *et al*., 2018).
- **Active packaging:** Active packaging is a novel approach to enhance the shelf life of food products by preventing microbial invasion, moisture gain, oxidation, over ripening, *etc*., (Fig. 1).

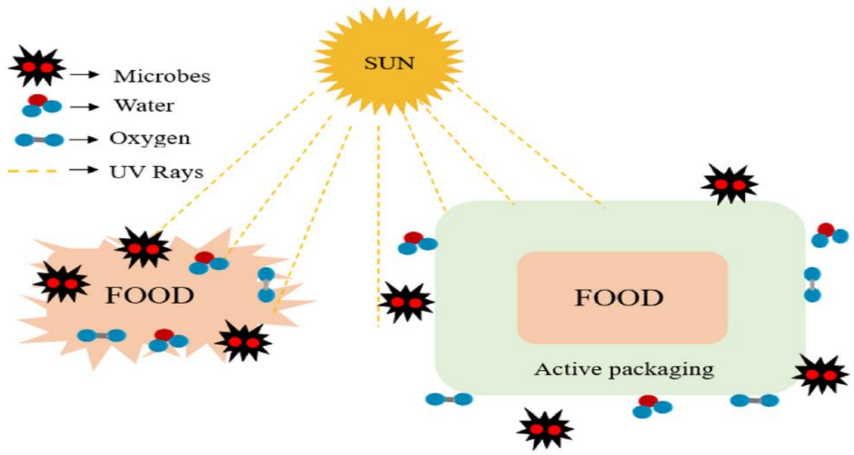


Fig. 1. Schematic representation of active packaging

In active packaging, some constituents such as oxygen scavengers, ethylene absorbers, antioxidants, carbon dioxide emitters, antimicrobial agents are intentionally added in the packaging material or in package headspace to amplify the properties of packaging polymer (Yousuf *et al*., 2018). Antimicrobial agents inhibit the growth of microbes either by destroying their cellular structure or inhibiting their metabolic pathway (Primozic *et al*., 2021). Nanoparticles contribute to the generation of reactive oxygen species that inhibits DNA replication and ATP formation resulting in cell damage or cell death shown in Fig.2 (Slavin *et al*., 2017).

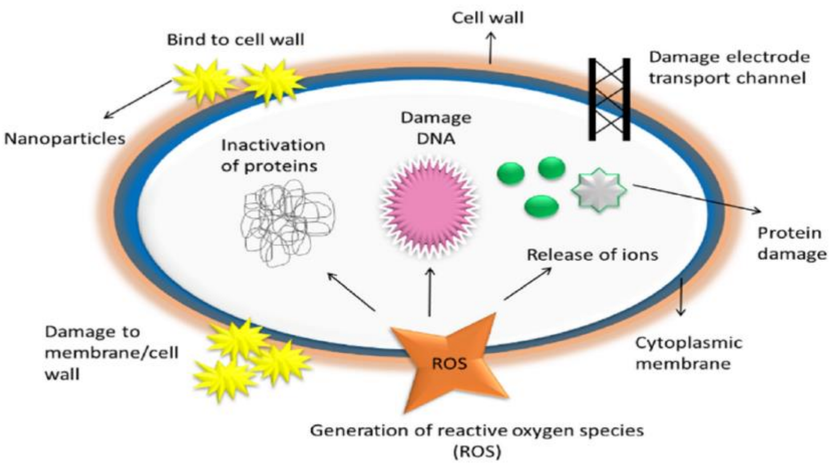


Fig. 2. Schematic representation of nanoparticle interaction on microbial cells.

Intelligent packaging: According to the European Commission, 2004 intelligent packaging materials are defined as “materials and articles that monitor the condition of packaged food or the environment surrounding the food” (Ghaani *et al*., 2016). Intelligent packaging is also termed as smart packaging (Fig. 3) as they consist of smart devices like barcodes, Radio Frequency Identification Tags (RFID), sensors, indicators to communicate, monitor, sense, record, track and indicate the information about food safety, quality, and history during the supply chain (Kalpana *et al*., 2019).

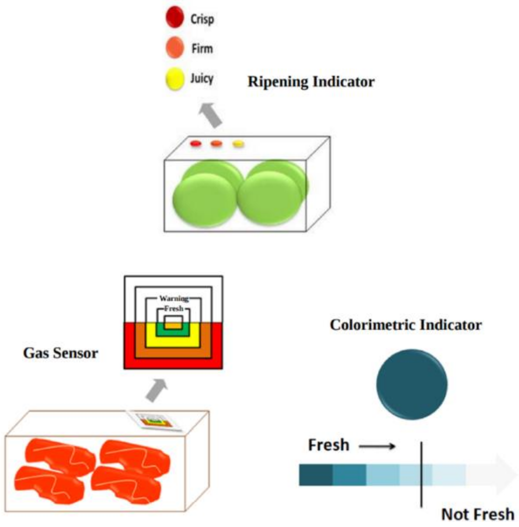


Fig. 3. Illustration of various smart packaging systems

Conclusion

The prefix 'nano', derived from Greek, signifies something very small or dwarfed, representing measurements at the nanoscale, specifically one billionth of a meter (10^{-9} m). Nanoscience, studying structures and molecules in the 1 to 100 nm range, converges physics, materials science, and biology, while nanotechnology is the practical application of nanoscience in fields like medicine, engineering, and electronics. The roots of nanoscience can be traced back to ancient Greece and Democritus, pondering the nature of matter. Nanotechnology, with its unique ability to manipulate matter at the nanoscale, holds great promise in the 21st century. Nanomaterials, with dimensions below 100 nm, exhibit distinct properties, making them crucial in nanotechnology. Nanotechnology's potential is particularly evident in fields like medicine and electronics, where miniaturization and cost-effectiveness are pivotal. In recent years, nanotechnology has found applications in the food industry, contributing to active and intelligent packaging systems. Nanoparticles in food packaging enhance mechanical properties, act as antimicrobials and enable active and intelligent packaging, providing consumers with information on food safety and quality throughout the supply chain. As nanotechnology continues to advance, its impact on various industries, including food packaging, is likely to grow significantly.

References

Cha, D. S. and Chinnan, M. S., (2004). Biopolymer-based antimicrobial packaging: a review. *Critical Reviews in Food Science and Nutrition*, 44(4): 223-237.

Garcia, C. V., Shin, G. H. and Kim, J. T., (2018). Metal oxide-based nanocomposites in food packaging: Applications, migration and regulations. *Trends In Food Science and Technology*, 82(1): 21-31.

Ghaani, M., Cozzolino, C. A., Castelli, G. and Farris, S., (2016). An overview of the intelligent packaging technologies in the food sector. *Trends in Food Science and Technology*, 51(2): 1-11.

He, X., Deng, H. and Hwang, H. M., (2019). The current application of nanotechnology in food and agriculture. *Journal of Food and Drug Analysis*, 27(1): 1-21.

https://www.nano.gov/

Kalpana, S., Priyadarshini, S. R., Leena, M. M., Moses, J. A. and Anandharamakrishnan, C., (2019). Intelligent packaging: Trends and applications in food systems. *Trends in Food Science and Technology*, 93(2): 145-157.

Kerry, J. P., O'grady, M. N. and Hogan, S. A., (2006). Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat science*, 74(1): 113-130.

Mansoori, G. A. and Soelaiman, T. F., (2005). Nanotechnology-An introduction for the standards community. Journal of ASTM International, 2(1): 1-22.

Primozic, M., Knez, Z. and Leitgeb, M., (2021). (Bio) Nanotechnology in food science and food packaging. *Nanomaterials*, 11(2): 292.

Slavin, Y. N., Asnis, J., Hnfeli, U. O. and Bach, H., (2017). Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology*, 15(2): 1-20.

Sorrentino, A., Gorrasi, G. and Vittoria, V., (2007). Potential perspectives of bio-nanocomposites for food packaging applications. *Trends in Food Science and Technology*, 18(2): 84-95.

Yousuf, B., Qadri, O. S. and Srivastava, A. K., (2018). Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: *A review. Lwt*, 89(1): 198-209.

Agri Mirror: Future India
ISSN: 2582-6980

AMFI/9/2024

BRASSINOSTEROIDS: A NOVEL GREEN MOLECULES FOR POSTHARVEST SHELF-LIFE EXTENSION OF FRUITS AND VEGETABLES

Sajeel Ahamad[1], Chhail Bihari[2], Maneesh Kumar[3], Amit Kumar[4] and Ajeet Kumar[2]

[1]Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi, 110 012, India

[2]Department of Horticulture Vegetable Science, Nagaland Central University, SASRD Medziphema, Nagaland, India

[3]Department of Horticulture, G.B. Pant University of Agriculture & Technology Pant Nagar, U.S. Nagar, Uttarakhand, India

[4]Department of Fruit Science, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

*Corresponding author: cbrathour500@gmail.com

Abstracts

Fruits and vegetable are highly perishable in nature having high moisture content around 80-90%. This high moisture leads to rapid deterioration leads to limits the shelf life so its need to use ecofriendly approaches that enhances the shelf life. Brassinosteroids (BRs), naturally occurring plant hormones, are found in all plants and affect a wide range of molecular, physiological, and biochemical processes. BRs regulate the ripening of climacteric and non-climacteric fruits, influence color development, and reduce chilling injury. By inhibiting enzymes like peroxidase and polyphenol oxidase, they delay enzymatic browning. Exogenous BR application prevents cell wall breakdown and slows fruit softening. These effects alleviate oxidative stress and extend the potential storage life of various fruits and vegetables. This article summarizes the diverse roles and mechanisms of BRs in prolonging postharvest shelf-life and preserving the quality of fruits and vegetables.

Keywords: Brassinosteroids, Browning, Delay softening, Chilling injury and Shelf life

Introduction

The demand for fresh fruits, vegetables, and flowers has increased significantly worldwide in recent years. Fresh fruits and vegetables are now recognized as essential components of a healthy human diet, while flowers play a vital role in various celebrations and events. However, ensuring a consistent supply of fresh produce to all consumers presents a significant challenge. Storing fruits and vegetables under low-temperature conditions generally extends their postharvest life, allowing them to remain fresh for a longer period. However, this practice can also lead to undesirable physiological disorders, primarily chilling injury and postharvest decay. These disorders can cause significant economic losses due to reduced marketability and consumer acceptance. Environmentally friendly and effective chemical treatments are needed to manage various postharvest problems in horticultural crops. BRs are natural plant hormones or phyto-hormones that were first discovered in rape pollen. BRs have been shown to have a significant growth-promoting effect on various crops. BRs play an important role in reducing various abiotic and biotic stresses at different levels. They have also been found to be effective in maintaining various quality attributes of fruits and vegetables. Applying Epibrassinosteroids (EBR) delayed the senescence of kiwifruit at room temperature. Postharvest EBR application also delayed chlorophyll degradation in lime fruits and leaf yellowing in broccoli florets (Cai et al., 2019).

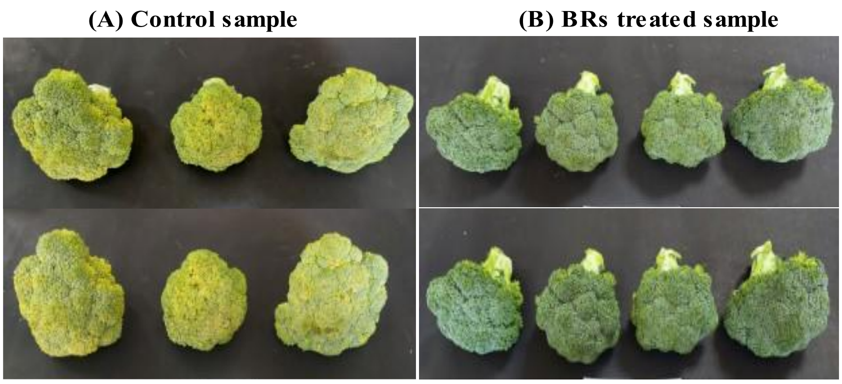
Biosynthesis and mechanism

BRs are made from a starting material called campesterol through a series of steps. Scientists have found that there are two main pathways for making BRs: early C-6 oxidation and late C-6 oxidation. In the plant Arabidopsis, BRs are made from campesterol, which is first turned into campestanol. The campestanol is then turned into castasterone, which is finally turned into BRs. The highest levels of BRs are found in pollen and immature seeds. BRs, naturally derived plant hormones, offer a promising approach to extend the shelf life of fruits and vegetables by delaying senescence, scavenging reactive oxygen species, modulating ethylene signaling, and enhancing stress tolerance. Their multifaceted effects reinforce cell wall integrity, preserve texture and firmness, and maintain nutritional content, making them an eco-friendly solution for reducing postharvest losses of fruits and vegetables.

Role of brassinosteroids in postharvest management of fruits and vegetables

Fruit ripening

BRs play crucial roles in the ripening process of various fruits. Applying EBR and Homo-Brassinolide (HBL) to tomatoes led to higher lycopene levels and faster chlorophyll breakdown. This enhanced ripening was linked to increased ethylene production, which sped up the ripening process. Applying BRs externally triggers their increased internal production due to the upregulation of the GhDWF4 gene, which ultimately accelerated ripening in tomato fruits. Another study found that external BR application



increased and upregulated FaBRI1 gene expression, accelerated red color development and ripening in strawberry fruits (Chai et al., 2013).

Fig 1. Effect of BRs on shelf life of broccoli after 10 days of storage

Chilling injury

Chilling injury (CI) is a major postharvest physiological disorder that affects a wide variety of horticultural crops during cold storage. CI generally depends on maturity stage and cultivar. CI leads to quality degradation and limits postharvest storage life. CI also negatively affects the integrity of cell membranes. BRs play an important role in the chilling tolerance of fruit and vegetable crops. It was observed that higher BRs concentration led to cold tolerance of cucumber. Application of 15 µmol L-1 BRs alleviated CI (indicated by reduced calyx discoloration and surface pitting) in green bell pepper fruits at 3 °C storage. Treatment of eggplant with 10 µmol L-1 EBR reduced electrolyte leakage that in turn reduced the symptoms of CI at 1 °C conditions (Wang et al., 2012).

Postharvest browning

Postharvest browning is a major undesirable disorder that occurs in various fruits and vegetables. Browning not only diminishes the visual appeal of the produce but also leads to decreased market potential and purchase decisions by consumers. BRs have been found to have anti-browning properties. In eggplant, treatment with 10 µmol L-1 EBR reduced the increase in PPO and POD activities and delayed pulp browning for 15 days at 1 °C. Application of 3 µL BL treatment significantly reduced browning of white button mushrooms for 16 days during storage at 4 °C. Similarly, treatment with 80 nmol L-1 EBR suppressed quinone production and reduced PPO and POD activities, along with reduced phenols oxidation, which effectively inhibited enzymatic browning in lotus root slices (Gao et al., 2017).

Table 1: Effects of brassinosteroids on postharvest quality and shelf life of fruits and vegetables.

Crop	Concentration	Results
Asparagus	10 µML^{-1}	Reduced weight loss and respiration rate and maintained fruit quality with higher vitamin C and total phenolic contents.
Broccoli	2 µML^{-1}	Reduced yellowing of florets and extended shelf life. Increased expression of BoACO3 and BoACS4 genes
Kiwifruit	5 µML^{-1}	Retarded firmness loss, decreased ion leakage and MDA content. Reduced sugars such as sucrose, glucose and fructose accumulation

Crop	Concentration	Results
Mango	10 µML-1	Alleviated chilling injury and up-regulated differential proteins. Increased ethylene production and accelerated ripening
Persimmon	10 µML-1	Increased cell-wall-degrading enzymes activities and accelerated ripening. Reduced expression of DkPL1, DkEGase1, DkPE2 and DkPG1 genes
Tomato	3 µML-1	Accelerated ripening due to increased respiration rate and ethylene production. Increased soluble solid content, lycopene and ascorbic acid content. Also increased ethylene biosynthesis related genes

Colour metabolism

Color in fruits and vegetables holds significant importance, influencing the purchasing decisions of consumers in the market. The color attribute plays a pivotal role in the market potential of specific crops, and any decline in color directly impacts the visual quality of the commodity. Employing a vacuum infiltration of 10 µmol L-1 BRs demonstrated a capacity to decrease chlorophyll degradation, preserving the green coloration of both 'Tahiti' and 'Persian lime' fruits by impeding the onset of yellowing. This treatment also exhibited an increased hue angle and decreased chroma values in both lime cultivars. Similarly, a 2 µmol L-1 EBR spray application had a comparable effect, reducing yellowing, elevating chlorophyll levels, and enhancing chlorophyll fluorescence in both lime cultivars. These interventions resulted in significantly diminished yellowing in broccoli florets by curbing chlorophyll breakdown and promoting higher chlorophyll fluorescence.

Ethylene biosynthesis and respiration rate

The regulation of ethylene and respiration is crucial for extending the storage and shelf life of produce, as heightened levels of these factors often lead to accelerated senescence. Implementing appropriate treatments to mitigate the climacteric peak of ethylene and respiration is essential. However, the impact of BRs on ethylene production and respiration rate appears to be complex and, at times, contradictory. In the case of 'Kensington Pride' mango fruits, an application of EBR treatment resulted in a significant increase in both respiration rate and ethylene production during ambient storage, suggesting a potential role in hastening the senescence process. Conversely, postharvest exposure of tomato fruits to 3 µmol L-1 BRs amplified the activities of ethylene biosynthesis-related genes, such as LeACS2, LeACS4, LeACO4, and LeACO1, consequently enhancing ethylene production and respiration rate. In stark contrast, the immersion of green asparagus spears in a 10 µmol L-1 BRs solution led to a substantial reduction in the respiration rate, pointing towards a potential positive impact on prolonging shelf life. These divergent effects underscore the need for a nuanced understanding of the interplay between BRs, ethylene, and respiration in different plant species.

Postharvest softening

The rapid softening of climacteric fruits poses a significant challenge in terms of their storage and shelf life potential. This softening process is closely associated with the disassembly of cell walls, and various enzymes, including pectin esterase (PE), polygalacturonase (PG), β-galactosidase (β-gal), endo-1,4-β-glucanase (EGase), and pectate lyase (PL), have been identified as key contributors to this phenomenon. Managing fruit softening necessitates a reduction in the activities of these enzymes. In the context of persimmon fruits, specific treatments were employed to achieve this goal, leading to notable outcomes. These treatments not only resulted in higher cellulose and pectin content but also increased acid-soluble pectin in persimmon fruits. Crucially, the reduced activities of EGase, PL, PG, and β-gal, coupled with the enhanced conservation of various pectin components, ultimately contributed to a significant suppression of softening in persimmon fruits during ambient storage. These findings highlight the potential of enzyme activity modulation as a targeted approach to mitigate the rapid softening that compromises the storage and shelf life of climacteric fruits (He et al., 2018).

Postharvest decay

Fresh vegetables and fruits, characterized by their perishable nature and living cells, undergo continuous respiration during ripening, which not only provides energy but also renders them more susceptible to decay in the later stages of postharvest storage. Postharvest decay poses a significant threat to the quality and quantity of produce, primarily attributed to bacterial and fungal pathogens. Among these, fungal diseases are particularly critical, resulting in substantial economic losses for commodities. The decay of fruits and vegetables after harvest can be attributed to latent infections originating in the field or through wounds incurred during harvesting and subsequent handling throughout the supply chain. Effectively controlling postharvest decay is imperative to minimize losses in the overall produce. In this regard, a postharvest treatment with 5 µmol L-1 BRs demonstrated promising results by increasing the activities of phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO). This treatment significantly suppressed *Penicillium expansum* induced blue mold rot in jujube fruits, suggesting the potential of BRs as a control measure against postharvest decay and its associated economic ramifications.

Conclusion

The exploration of BRs as novel green molecules for postharvest shelf-life extension of fruits and vegetables represents a promising avenue in agricultural research. The unique properties of BRs, derived from plant steroids, offer an environmentally friendly and sustainable solution to address the challenges associated with postharvest losses.

The positive impact of BRs on delaying senescence, preserving quality attributes, and enhancing stress tolerance in fruits and vegetables underscores their potential as a valuable tool in ensuring food security and reducing waste. Further research and practical applications will be essential to fully unlock the potential of BRs in transforming postharvest management, contributing to a more resilient and sustainable food system.

References

Cai, J.H., Luo, F., Zhao, Y.B., Zhou, Q., Wei, B.D., Zhou, X. and Ji, S.J. 2019. 24-Epibrassinolide treatment regulates broccoli yellowing during shelf life. Postharvest Biology and Technology, 154: 87-95.

Chai, Y.M., Zhang, Q., Tian, L., Li, C.L., Xing, Y., Qin, L. and Shen, Y.Y. 2013. Brassinosteroid is involved in strawberry fruit ripening. Plant Growth Regulation, 69(1): 63-69.

Gao, H., Chai, H.K., Cheng, N. and Cao, W. 2017. Effects of 24- epibrassinolide on enzymatic browning and antioxidant activity of fresh-cut lotus root slices. Food Chemistry, 217: 45-51

He, Y., Li, J., Ban, Q., Han, S. and Rao, J. 2018. Role of brassinosteroids in persimmon (*Diospyros kaki* L.) fruit ripening. Journal of Agricultural and Food Chemistry, 66: 2637-2644.

Wang, Q., Ding, T., Gao, L., Pang, J. and Yang, N. 2012. Effect of brassinolide on chilling injury of green bell pepper in storage. Scientia Horticulturae, 144: 195-200.

.

Particulars	Charges (in Rs.) AIASA Members	Charges (in Rs.) Non AIASA Members
One Single Article (If one author)	150	200
* If more than one author and other authors are not annual/life member (Maximum 3)	250	300
Annual Membership (Maximum 8 articles/Year)		
Masters/Ph.D. Scholars/ Project JRF/Young Professionals/Project SRF	450	500
Research Associates/ Assistant Professor or equivalent and Professionals including company representatives/Agricultural Officer and others	550	600
Life Membership Charges (Unlimited article for 10 years)		
Masters/Ph.D. Scholars/ Project JRF/Young Professionals/Project SRF	2800	3000
Research Associates	3600	4000
Assistant Professor /Teaching Assistant/any others professionals	4500	5000

Best two articles will be awarded on quarterly basis

Official Address:

Official Address: ALL INDIA AGRICULTURAL STUDENTS ASSOCIATION

(Registered Society under SR Act, 1860)

A/G-4, National Societies Block, National Agricultural Science Centre Complex, DPS Marg, PUSA, New Delhi - 110 012.

Website: https://aiasa.org.in/?page_id=2276

Email: aiasamagazine@gmail.com

Article Submission link: https://forms.gle/jqk5jukWwJfHSF6a7