

Post-Harvest Biotechnology or genetic engineering Solutions: Extending Shelf Life and Reducing Food Waste

Abstract

Post-harvest losses and food waste have become critical challenges in the global food supply chain, contributing to economic losses, environmental degradation, and food insecurity. This article explores the innovative applications of post-harvest biotechnology and genetic engineering as promising solutions to address these issues by extending the shelf life of perishable products and minimizing food waste. Advancements in genetic engineering techniques have paved the way for the development of crops with enhanced resistance to pests, diseases, and environmental stresses. Additionally, the manipulation of genes associated with ripening and senescence has allowed scientists to engineer fruits and vegetables with extended shelf life. These genetically modified organisms (GMOs) exhibit improved post-harvest characteristics, providing a longer window for transportation, storage, and consumption. Biotechnological interventions also include the use of biocontrol agents and beneficial microorganisms to suppress post-harvest pathogens, thereby reducing spoilage and decay. The development of bio-preservatives, such as antimicrobial peptides and natural compounds, offers an eco-friendly alternative to traditional chemical preservatives, contributing to both food safety and sustainability. Furthermore, the integration of smart packaging technologies with genetic modifications enhances the monitoring and control of environmental conditions during storage and transportation. Intelligent packaging materials equipped with sensors can detect changes in temperature, humidity, and gas composition, enabling real-time adjustments to prolong the freshness of perishable goods. This highlights the dual role of post-harvest biotechnology and genetic engineering in not only extending the shelf life of agricultural products but also in minimizing food waste throughout the supply chain. As the world faces the challenges of a growing population and environmental concerns, these innovative solutions present a promising avenue for ensuring food security and sustainable agriculture. However, it is essential to address socio-economic and regulatory aspects, fostering public acceptance and responsible deployment of these technologies to realize their full potential in shaping a more resilient and efficient global food system.

Keywords: environmental, eco-friendly, storage, bio-preservatives

Introduction

An essential part of the physiological processes that occur in fruits, vegetables, and decorative crops is played by ethylene, which is a gaseous plant growth regulator that contains two carbon atoms. At micromolar quantities, it has the ability to hasten the ripening process of ethylene-sensitive fruits, leafy greens, and vegetables, which ultimately results in the deterioration of the fruit and its waste during the postharvest stage [1]. With the ultimate goal of extending the shelf life of produce and enhancing its quality after harvest, a number of different approaches have been undertaken in order to gain a better understanding of the pathways that regulate ethylene as well as the biochemical and physiological processes that are dependent on ethylene [2].

The purpose of this review paper is to provide an overview of contemporary breeding approaches that are focused on ethylene and metabolism related to ethylene, as well as possible postharvest technological applications for the postharvest control of ethylene-sensitive crops. Postharvest physiology (ethylene dependent) for mature and immature fruits

and vegetables, postharvest quality management of vegetables: fresh and fresh cut products, and the evolution of breeding technologies for facing old and new challenges in postharvest quality of vegetable crops are some of the topics that are discussed in this paper [3]. The paper also highlights the implications of new breeding and management strategies for maintaining the quality and marketability of various crops during the postharvest period. Ethylene was the first phytohormone to be discovered, and it plays a crucial role in a variety of physiological and developmental processes. These activities include seed dormancy, germination, fruit ripening, and defence against biotic and abiotic stimuli [4]. It is possible for ET to cause the deterioration of vegetables, fruits, and ornamental crops because of the role it plays in the ripening process. In breeding efforts, biotechnological research, and transgenic studies, the production of ET and ET-dependent pathways has been addressed with the goal of reducing waste and extending the shelf life of products [5].

ET receptors, protein kinases, transcription factors, and other transcription factors are all components of the conventional ET signaling pathway. An alternative mechanism that involves AHP and ARR regulatory proteins has also been suggested as a possible explanation. According to the findings of recent studies, the production of food ought to be increased by a factor of two in the next fifty years in order to prevent malnutrition, starvation, and other related repercussions. In order to minimize food waste and guarantee quality throughout the whole food production and distribution chain, it is essential to preserve the quality of the food once it has been harvested [6].

The extensive network of biological processes that involve this phytohormone is highlighted in this review, which covers the function that ET plays in postharvest quality as well as the advancement of breeding technology. There are a number of roles that ET plays in the postharvest management of fruits, vegetables, and ornamental crops [7]. Some of these roles include influencing the marketability of immature and non-climacteric fruits, acting as a signaling molecule in the development of certain postharvest physiological disorders, and influencing the storability and quality of a number of vegetables, including leafy greens, which are frequently sold as fresh cuts [8].

Editing plant genes has been regarded as the most significant advancement in plant breeding since the Green Revolution. This technological advancement has the potential to produce new crops that possess desirable features. The majority of authorities, on the other hand, believe that in order to keep up with the rise of the population, food production will need to double during the next fifty years [9]. Despite the fact that horticultural crops are frequently rich in nutrients and contain bioactive phytochemicals, they are frequently disregarded or undervalued in the context of global food security. A further 25–40% of all fruits and vegetables that are produced around the world are never consumed once they have been harvested, with an average of 33 percent of them being never consumed [9].

According to the authors, technology-assisted breeding for new and enhanced fruit, vegetables, and ornamentals that are compatible with supply chain constraints but supplied to the consumer at peak quality could be an important part of the solution both in the short term and in the long term. This paper investigates the possibility that gene editing could have a significant and quantitative impact on postharvest waste and loss [10]. This is accomplished by establishing a connection between the bio physiology of postharvest produce, the requirements of the produce sector, and the vast amount of molecular research that is already available.

It is the consideration of genes that could potentially affect the quality of produce as well as its shelf life that is the primary emphasis of this review [11]. The authors investigate the measures that are used to extend the shelf-life of produce across the supply chain, as well as the influence that supply chain management has on the qualities that consumers like to see in products. In addition to this, they provide a concise overview of the CRISPR–Cas9 approach

in order to highlight the adaptability, simplicity, and power with which characteristics can be altered [12].

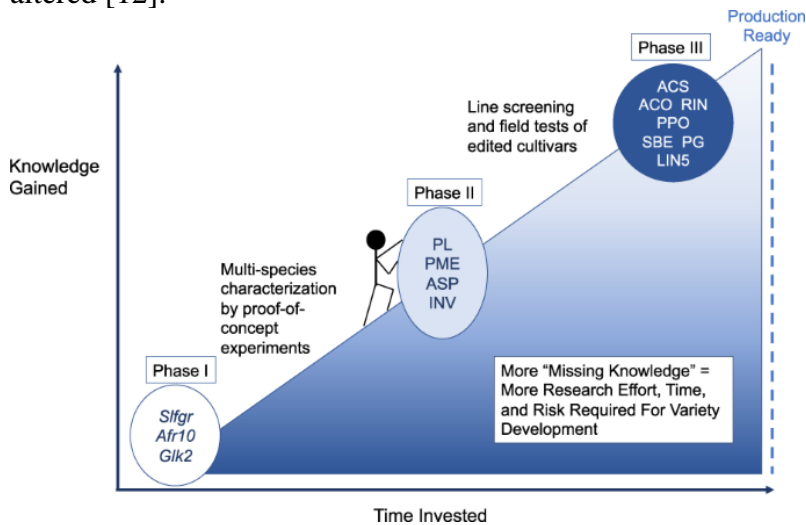


Fig. 1. shelf-life of produce across the supply chain

Climacteric and non-climacteric principles

There has been a significant amount of research conducted in recent years regarding the impact of ET on the climacteric fruit ripening process, with a particular emphasis placed on tomato as a model fruit for these fruits. There have been new ways established to limit losses in order to prevent the deterioration of products while they are being stored and transported [13].

Through the use of various therapies on ET-receptors, molecular studies have demonstrated that it is possible to gain a better understanding of how ET influences the storability and marketability of climacteric fruits. Using 1-methylcyclopropene (1-MCP), a recent study explored various ET receptors at a transcriptome level in tomato during ripening and off vine [14]. The purpose of the study was to gain a better understanding of the regulation of ripening time and the function that ET activity plays. The metabolism of methyl jasmonate (MJ) and ethylene tetrahydrocannabinol (ET) has been shown to interact in a way that promotes ripening in climacteric fruits, according to recent studies [15].

In addition, it has been demonstrated that other plant hormones interact with ET metabolism, which in turn regulates the ripening of fruit. Exogenous injection of salicylic acid (SA) has been demonstrated to slow the ripening process in a variety of species. On the other hand, the ripening of avocados is not only dependent on the action of ethylene tetrahydrocannabinol (ET), but it is also regulated by a complex interaction between a number of other hormones, including as auxin, jasmonates, gamma-aminobutyric acid (GA), and ABA [16]. The application of melatonin has been discovered to boost the production of ethylene and to up-regulate the expression of the gene that is responsible for the production of 1-aminocyclopropane-1-carboxylic acid (ACC) [17].

There has also been research conducted on tomato to study the relationship between the activity of ET and membrane catabolic enzymes such phospholipases and lipoxygenase. In order to get the conclusion that lipoxygenase could be a regulator of ET synthesis through the activity of the enzyme ACO, the primary isoform of the lipoxygenase producing gene, TomloxB, was silenced. Therefore, the conclusion was reached. The possibility exists that this discovery could be beneficial for extending the shelf life of tomatoes and enhancing postharvest management [18].

During the postharvest treatment of a variety of climacteric fruits, technological approaches have the potential to mitigate and mitigate the adverse effects of ET exposure. Through the induction of DNA methylation as a response of ET signaling systems, heat treatments have been proposed as an effective method for extending the amount of time tomatoes can be stored. Packaging, controlled and changed environment, and one-microgram per cubic centimetre are some more options [19].

Extending the shelf life of a product has been accomplished by the utilization of ET absorbers and scavengers, ventilation systems, ozone, active oxygen, and 1-MPC. Vacuum ultraviolet photolysis is a unique technology that has been developed for the purpose of reducing the harmful impact of ethylene tetrahydrofuran (ET). This technique is based on the creation of extremely reactive radicals that have the ability to eliminate pathogenic bacteria while simultaneously oxidizing ethylene tetrahydrofuran (ET) to carbon dioxide and water [20].

In another study, it was shown that treatments with ethanol were able to prevent tomato fruit from ripening while simultaneously promoting the production of ET. This finding suggests that additional research should be conducted on this subject [21].

Non-climacteric fruits, such as zucchini, have a shorter shelf life compared to fruits that are consumed commercially when they have reached their full ripening stage. There are possible dangers and problems associated with the postharvest management of these fruits, one of which is the development of chilling injury (CI) in certain fruits when they are being stored in cold conditions [22]. In zucchinis that were treated to cold storage and held at a temperature of 20 degrees Celsius, there was an increase in the production of ET, which indicates that ET and genes related to ET are involved in the development of CI. There is a possibility that the rise in ET production is connected to the rise in oxidative stress, which is a significant contributor to the development of CI in immature fruits [23].

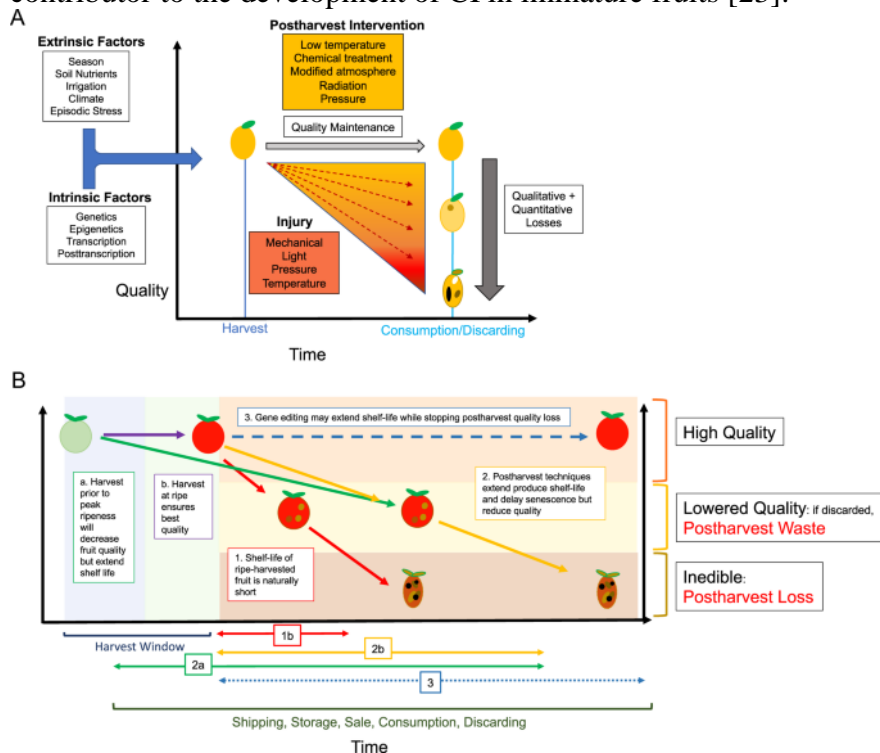


Fig. 2. Climacteric and non-climacteric principles

In order to effectively alleviate the adverse effects of ET-mediated CI, it has been suggested that the utilization of the ET-inhibitor 1-MCP could be as an effective method. CI symptoms were reduced in zucchini cultivars that were sensitive to CI, and genes involved in ET

production and perception were downregulated as a result of the administration of 1-MCP into the plant. It has also been suggested that shrink-wrapping packaging could be a useful technique for mitigating the adverse consequences of ET-mediated CI [24].

In a recent study, ET receptors and related proteins were compared in climacteric and non-climacteric fruits. The researchers discovered that both types of fruits share a significant number of the pathways that contribute to the perception and signaling of ET. In contrast, the amount of ethylene-responsive components found in non-climacteric fruits, such as grapes and citrus, was significantly smaller. During the process of strawberry ripening, it has been discovered that ET receptors, such as FaEtr1 and FaErs1, play a significant role in the development of non-climacteric fruits [25].

It is possible to further our understanding of the physiological and metabolic processes that occur in non-climacteric fruits by inhibiting ET using 1-MCP. This can also help to extend the commercial life of crops, including non-climacteric fruits. Additionally, it has the ability to postpone the onset of senescence and disorders associated with senescence throughout the postharvest period, as well as restrict the development of certain physiological illnesses, such as chilling injury. It is possible for the effect of 1-MCP on respiration and the development of decay in non-climacteric fruit to vary and change depending on the crop [26].

Causes of losses in post-harvest

The postharvest losses of agricultural commodities can be ascribed to a variety of variables that have an effect on the quality of the output. The most common reasons are poor harvesting, which can lead to significant losses as a consequence of a variety of circumstances, including assaults by birds and rodents, microorganisms, and natural disasters for example [35]. When it comes to separating grains from panicles, delayed threshing and cleaning are very necessary. However, poor drying might result in the growth of microbes in the grains, which is not something that is ideal for storage and grinding operations [36]. Among the biological explanations is respiration, which is responsible for the catabolization of stored organic molecules into simple chemicals in order to release energy that is necessary for metabolic activities. The rate of respiration is directly proportional to the pace of degradation of horticulture products, which may be measured in terms of the amount of carbon dioxide or oxygen that is consumed [37]. The physiological process known as transpiration is the process by which water is lost from the living tissues of a plant in the form of vapour. The significant amount of water that is lost from harvested food puts at risk its quality, nutritional value, palatability, and the demand that it receives from customers. The storage conditions should be lowered in order to reduce the amount of transpiration loss [38]. This may be accomplished by limiting the amount of air movement, lowering the temperature of the air, increasing the relative humidity, utilizing protective coverings such as waxing, and utilizing modified atmospheric packing and polyethylene films [39].

Postharvest infections are frequently caused by microbes in agricultural product that has been kept. Some examples of these microbes are *Penicillium* sp., *Botrytis* sp., *Fusarium* sp., and *Phytophthora infestans*. It is typical for pathogenic microorganisms to enter through mechanical damages and bruises that occur during harvesting and other agricultural activities [40]. These bacteria have the potential to negatively impact both the quality and quantity of product, as well as limit its marketability. Ethylene is a gaseous hormone that plays an active part in the postharvest processing of agricultural products. It is responsible for managing the ripening process in fruits and vegetables, but it also has unfavourable effects on crops, such as early ripening and skin damage [41].

Temperature, relative humidity, atmospheric condition, and exposure to light are all examples of environmental factors that can induce conditions. There is a correlation between

temperature and the postharvest life of items that have been kept. When temperatures are high, the rate of transpiration and water loss increases, whereas when temperatures are low, microbial proliferation is encouraged. The relative humidity, which is determined by the difference in vapour pressure between the air in the surrounding area, is an essential factor in determining the quality of product that has been previously harvested [42]. It is essential to regulate the gaseous composition around product in order to lessen the amount of respiration and increase the shelf life. It is also possible for light exposure to create physiological changes in produce, such as the production of green tubers as a result of the formation of solanin and chlorophyll, both of which are hazardous for human ingestion [43].

Technologies intervention

With the use of modern technology, scientists are now able to make regulated changes to the structure of genes inside plant genomes. These changes, which may be categorized as either gene editing or gene targeting, are extremely beneficial. The approach known as genome targeting is capable of bringing about permanent modifications at a particular location, which enables targeted mutagenesis to take place [44]. The cellular DNA repair mechanisms are activated whenever a Double-Stranded Break (DSB) takes place. The repair of the double-stranded breaks can be accomplished using either the homology-directed repair (HDR) mechanism or the non-homologous end joining (NHEJ) method mechanism [45].

Since the NHEJ repair mechanism is dependent on both the structure of donor DNA and the repair machinery of the target cell, it is more widespread in plant species than the homology-directed repair (HDR) mechanism. This is due to the fact that the NHEJ process occurs more frequently. For the development of DSB-dependent genome editing technologies in plant cells, it is vital to find unique restriction enzymes that are selective and rare-cutting [46].

These enzymes must be able to generate double-stranded breaks at specified genomic locations. Nucleases are enzymes that are able to detect lengthy nucleotide sequences and cause double-stranded breaks at the places that they are targeting. Meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the recently introduced CRISPR-Cas endonuclease are some of the designed nucleases that are utilized in gene editing methods [47].

Zinc Finger Protein (ZFP) is coupled with the FokI restriction enzyme to form ZFNs, which are endonuclease systems that have been chemically produced. In order to facilitate the integration of the targeted transgene into donor genome sequences, stimulate the repair of defective transgenes, replace donor DNA sequences with foreign DNA molecules, and make the integration of the targeted transgene into donor genome sequences easier, ZFNs have been used as site-specific mutagens in model plant species as well as crop plant species [48]. The fusing of the Transcription Activator like Effector with the catalytic domain of the FokI endonuclease resulted in the development of TALENs. Plant infection is caused by TALE proteins, which are naturally present in the proteobacterium *Xanthomonas* and play a role in the process [49]. They do this by transporting the TALE proteins to the nucleus of the plant cell, where they then attach to the promoter region of the target gene and initiate transcription. There are three distinct domains that make up the structure of TALENs. The N terminal is packed with a type III secretion system (T3SS) and non-canonical repeats (NCR), while the C terminal is comprised of a transcription factor binding site, Nuclear Localization Signal (NLS), and an activation domain. It was discovered that the CRISPR/Cas system is a naturally occurring RNA-guided DNA endonuclease system that is prevalent in prokaryotic species, namely in archaea and eubacteria [50].

The technique known as CRISPR has gained widespread acceptance for its potential applications in genome editing and gene therapy across a wide range of creatures and living

systems. In plant systems, it is the approach of genetic engineering that is the most exact, accurate, easy, and site-specific [51]. It has the capability of producing plants that have unwanted features knocked out. Adaptation, expression, and interference are the three phases that make up the CRISPR/Cas system. During the process of adaptation, bacteria take on the short and unique protospacer sequences of invaders and integrate them across neighbouring CRISPR loci in their genome. This allows the bacteria to continue to adapt to their environment. The CRISPR array is expanded with these sequences, which are known as new CRISPR spacer sequences. The purpose of this expansion is to construct a memory of the creature that invaded the cell [52].

The subsequent step is an expression, which involves the translation of the CRISPR locus into pre-crRNA. This is followed by the further processing of the pre-crRNA into mature crRNAs. The crRNA creates a complex with the Cas protein once the protospacer from the invasive organism has been incorporated into the CRISPR locus. Thereafter, the crRNA-Cas complex makes a complementary base pairing with the protospacer of the invader that is invading for the second time. In the last step, the Cas endonuclease protein is utilized to facilitate the cleavage of the DNA in a manner that is dictated by the crRNA [53].

The Cas9 endonuclease and a guide RNA are the two essential components that are utilized by the system in order to target a certain gene respectively. Spacer DNA is formed when small segments of foreign DNA are incorporated into the CRISPR repeat-spacer array that is present inside the chromosomes of the host organism as a result of exposure to an invading virus or plasmid. They serve as a marker for species that have invaded the area. Enzymatic cleavage and transcription of this spacer DNA both result in the production of short CRISPR RNA, also known as crRNA [54].

It is possible to transport the CRISPR/Cas9 complex to the target cell in a variety of ways, including plasmid DNA, messenger RNA, or ribonucleoproteins. It is vital that the complex be delivered to the target cell in an effective manner. Another method that is currently being developed for editing the genome is called ribonucleoprotein (RNP), and it consists of a Cas9 protein and a gRNA [55]. One of the most important aspects of RNP-based genome editing is that it does not include the use of DNA or transgenes in the editing process. This method also guarantees that there will be little off-target effects and is DNA-free, which results in lower toxicity [56].

The use of CRISPR/Cas9 technology to modify the genome of plants presents substantial prospects for the regulation of genomic and epigenetic processes. It is possible for the Cas9 and sgRNA complex, which are both components of the CRISPR/Cas9 system, to act as a scaffold in order to direct any number of effectors or markers to specific DNA locations [57]. Double-stranded breaks, on the other hand, leave the door open for off-target gene editing; hence, various methods have been devised to circumvent these drawbacks. An example of such a method is the utilization of the dead Cas9 variation, also known as dCas9. In this version, the two catalytic domains are rendered inactive by the process of point mutation [58].

The editing of plant genomes has been accomplished through the use of a technique known as base editing, which does not necessitate the creation of double-strand breaks. The utilization of base editors, also known as CBEs, is required for this process. These editors cause cytosine to be delaminated into uracil, which is subsequently converted into thymidine by means of DNA replication or repair processes [59]. For the purpose of targeted base editing, CBEs have been applied for crops such as Arabidopsis, rice, and tomato plantations. Adenine Deaminase (ABEs) that have been coupled with dCas9 are responsible for the base mutation that occurs when adenine is converted into inosine. Inosine may then be base paired with cytosine, which results in a change in the DNA that is being changed [60].

It is possible to change gene expression by epigenetic alterations such as DNA acetylation,

methylation, or Histone modification. These modifications do not involve any changes to the DNA sequence of the parents [61]. Through the utilization of epigenetic modifiers that have been fused with the dCAS9 protein, these alterations can be accomplished. As an illustration, Kang and his team effectively targeted the Oct4 gene by demonstrating the use of CRISPR/Cas9 to produce methylation at particular CpG sites. Through modifications made by Gallego-Bartolome and his team, the CRISPR/dCas9 SunTag system was modified to target DNA demethylation in plants, which ultimately led to late blooming [62].

In order to facilitate CRISPR-mediated genome editing, a novel toolkit called prime editing has been created. This toolkit does not need donor templates or double-strand breaks (DSBs). The Cas9 nickase, a prime-editing guide RNA (pegRNA), and a primer binding site (PBS) are the three primary components that are involved in this process [63]. There are a number of characteristics that set PegRNA apart from other small guide RNAs. These characteristics include a sequence that is complementary to the target site of the template DNA at the 5' end, a Primer Binding Site (PBS) at the 3' end, and the sequence that carries the desired alterations subsequent to the PBS [64].

It has been demonstrated that genome editing can increase the quality of post-harvest products in ornamental plants, fruits, and vegetables. The focus of attention that was formerly placed on extrinsic visual-quality features has shifted to intrinsic attributes, such as the nutritional, physiological, and physiochemical elements that are associated with fruits and vegetables [65]. Post-harvest loss has been a significant bottleneck, and it is imperative that the potential of novel editing tools be utilized in conjunction with the use of traditional editing techniques. Meganucleases, ZFNs, TALENs, and CRISPR/Cas are all examples of specific types of site-directed nucleases (SDNs) that are often utilized in plant editing technologies for the purpose of modifying genomes [66].

Sterility, self-incompatibility, high heterozygosity, low frequency of recovering desirable alleles and traits, and extended life cycles are some of the challenges that conventional breeding operations face. CRISPR has the ability to circumvent these challenges, which make it difficult or even impossible to perform these operations [67]. Due to the ease with which undesired characteristics may be removed, there has been an increase in the amount of research conducted on the non-interference of constitutive genome editing on other cellular operational capabilities. Through the use of CRISPR-TSKO (tissue-specific knockouts) or TSGE (tissue-specific gene editing), it is possible to induce somatic mutations in particular cells, tissues, and organs of the plant genome. This results in the generation of more accurate KO (knock-out) variations in plants [68].

Through the targeting of genes in fruits, such as cassava promoters, chimeric transcription factors (XVE), and estradiol-induced XVE, genome editing has brought about a revolution in the food business. These techniques have been utilized for a wide range of crops, including fruits such as carrots, Arabidopsis, apples, tobacco, and soybeans, among others. Site-directed nuclease-1 (SDN1) is one of the repair methods that are utilized in genome editing [69]. This enzyme generates non-homologous end products following the breakage of host DNA by CRISPR/Cas9. Within the plant, the Homology-Directed Repair (HDR) pathway is utilized by SDN2 in order to regulate gene activity through the modification of the sequence. Specifically, SDN3 makes advantage of a DNA insertion or substitution at a particular location within the DNA [70].

Through the use of targeted mutations, Oligo-directed Mutagenesis (ODM) is able to modify the genomic locus. The use of ODM has been demonstrated to be effective in the cultivation of maize, rice, and oilseed rape. PEG-fusion, electroporation, and biolistics are the components that make up the delivery system for SDNs. This method does not require any expression system to function. The Agrobacterium-mediated delivery in plants, on the other

hand, is the most reliable transformation for gene editing [71].

Tools for editing the genome have been studied on tomato and have developed to the point that they can be achieved in various other fleshy fruits, resulting in the discovery of a large number of prospective gene targets. The food sector has also been experiencing losses as a result of browning reactions that occur in strawberries after harvest. Through genome editing, it has been possible to conduct research on natural bioactive substances that include anti-browning extracts. This research has the potential to replace artificial additions. Through the utilization of the activity control of the Polyphenol Oxidase (PPO) enzyme, an industrial strategy for the regulation of the problem has been developed [72].

The rate at which innovations that target modification in the genome of plants have been developed has been astounding. Extensive research has been conducted on these innovations. ZFNs, TALENs, EMNs, mi-RNA, and CRISPR have all contributed to the revolution that has taken place in ONM (Oligonucleotide directed mutagenesis) and ENs (Engineered nucleus respectively). Through the use of miRNA and CRISPR knock-out mutations, browning activity in eggplant berries was decreased by 52%. This was accomplished by modulating the PPO and POD genes [73].

The post-harvest storage of newly picked fruits is essential to ensuring their freshness and extending their shelf life. It has been determined that chemical-free storage may be achieved by the utilization of optimized storage conditions that include controlled atmosphere, DCA (dynamic controlled atmosphere), or ULO (ultra-low environment). In order to combat the activity of microorganisms, chemical treatments of pesticides have been utilized; nevertheless, the demand for their utilization has decreased due to the presence of harmful by-products and residues involved. Comprehensive study has resulted in the development of alternatives to chemical treatments that make use of the physical-natural blend. These alternatives include the application of essential oil, the application of led light, and edible coating (EC). It is necessary to do research on enzymatic inhibitors, as well as Nano and microencapsulation of bioactive chemicals, in order to develop and execute methods that will enhance and make use of natural extracts [74].

An impending network of signaling and biological pathways has been deconstructed by the use of genome editing on fruits. This network is expected to be of assistance in the investigation of genes that have not been found up to this point that induce beneficial post-harvest phenotypes [75].

Gene editing in post-harvest management

The area of agriculture has been changed by gene editing, notably in terms of boosting crop quality and strengthening postharvest features in horticultural crops. CRISPR–Cas9 is the gene-editing method of choice at the moment because it enables precise modification of plant genomes and has been effectively utilized to induce beneficial features in a wide variety of crop species. Through the use of CRISPR, it is possible to circumvent additional obstacles like as sterility, self-incompatibility, and high heterozygosity, low frequency of recovering desirable alleles and features, and extended life cycles, all of which can either prolong or halt the process of conventional breeding [76].

A prokaryotic system that protects organisms from viral infection, CRISPR has been co-opted by scientists to delete undesired nucleotides or insert new or changed ones in order to promote desirable features in an organism of interest. CRISPR is a system that has been used to defend organisms from viral infection. In the process of CRISPR editing, a synthetic guide RNA (gRNA) is tailored to a protospacer adjacent motif (PAM) that has been located in the sequence of interest. This gRNA, together with the Cas protein sequence, is then introduced into a cell, where it is processed by the gene expression apparatus of the cell. A double-stranded break (DSB) is produced by the Cas protein that is generated in the plant. This break

occurs at the bases that are designated by the gRNA. As a result of the fact that the repair of the double-strand break (DSB) in DNA is typically not faithful to the original sequence, non-synonymous mutations may be introduced into the genome [77].

For both fundamental and practical plant research, it is frequently desired to have the capability of precisely expressing the Cas protein in a regulated spatial-temporal way, as well as in cooperation with other enzymes. The single-base substitution of a gene or genes of interest can be accomplished by the use of precise site-directed editing. This has been accomplished in cereals as well as in horticulture crops like tomato and potato. Through the use of certain promoters, tissue-specific knockouts conducted with the CRISPR technology, which is referred to as CRISPR-TSKO, have the capability to produce somatic mutations in cells, tissues, and organs. An additional gene-editing approach calls for the utilization of an inducible chimeric transcription factor (XVE) in order to regulate the production of the Cas protein in planta [78].

Targeting gene regulatory regions using CRISPR can result in fine-tuned expression and boost post-transcriptional modification of gene expression, which can have an effect on phenotype. Through the use of CRISPR targeting, this novel approach to crop improvement, known as de novo domestication, has been proven in a number of different plants belonging to the wild *Solanum* genus [79].

In conclusion, a variety of CRISPR procedures and methodologies may be utilized to bring about subtle alterations in the expression of a single gene or several genes. Furthermore, CRISPR possesses significant worth as a tool for dissecting the network of biological pathways that are accountable for ripening, senescence, and quality [80].

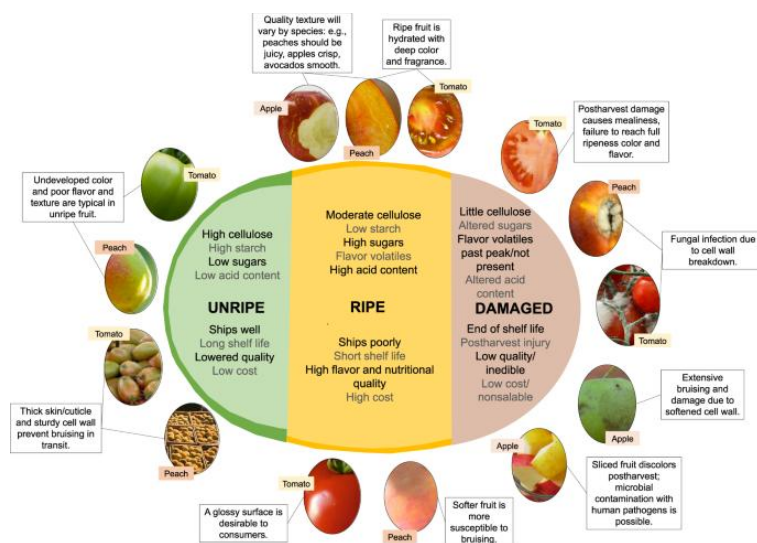


Fig. 3. Gene editing in post-harvest management

Method adopted

Emerging non-thermal physical technologies have experienced a spike in popularity as postharvest techniques to increase the shelf life of fruits and vegetables have become increasingly popular. Traditional thermal processing procedures, which need a significant amount of water and can have a detrimental effect on the quality of fresh commodities, are the target of these technologies, which aim to replace them. In order to lessen the amount of microorganisms that are present in fruits and vegetables and to maintain their quality and freshness, more recent methods have been implemented. These methods include microwave heating, high hydrostatic pressure, pulsed electric fields, high hydrostatic pressure, and cold

plasma [81].

A microwave heating method is an alternative to traditional heating procedures, which can result in a decrease in the amount of flavour-related chemicals and vital nutrients in plant tissue owing to the application of heat and the delayed diffusion of heat throughout plant tissue. The use of microwaves has been implemented to control the development of microorganisms during minimum processing, so limiting the loss of quality while simultaneously guaranteeing that the impact on the environment is limited [82]. Despite this, there is a paucity of published material concerning the use of this cutting-edge physical method for decreasing the amount of quality loss that occurs during the postharvest period. Pulsed electric field (PEF) technology has garnered significant interest because to its capacity to produce safe food with minimum heat generation. This is achieved by employing pulses ranging from microseconds to milliseconds, which are characterized by a high electric field intensity. The fresh flavour, texture, and functional characteristics of items that have been treated with PEF are better preserved, and these products also have a longer shelf life and a higher level of microbiological safety [83].

The majority of applications for high hydrostatic pressure (HHP) technologies include the reduction or inactivation of microorganisms as well as the denaturation of enzymes. On the other hand, due to the large diversity of product types, HHP can also have an effect on the functionality of proteins, such as enzymes and tissue structure, in a particular and differentiated manner. The effects of HHP treatment have been documented for a variety of minimally processed horticulture commodities, whole produce, and juice. These applications have demonstrated a high level of effectiveness in enhancing food safety features and preserving quality [84].

Cold plasma is a new technique that is used to manage the development of microorganisms, and it is widely employed in the industry that deals with processed and slightly processed fruits and vegetables. There have been a number of scientific studies that have revealed the usefulness of non-thermal plasma on various horticulture goods. Specifically, some fruit-based fresh-cut products have been treated to plasma treatment, which has resulted in favourable results in terms of quality metrics and the suppression of microbial development. During the fresh-cut processing of numerous goods, the use of plasma-activated water (PAW) has also been subjected to a greater amount of research. This has enabled producers to avoid the cell damage that is caused by direct exposure to cold plasma. PAW is a viable alternative to the standard approach of washing [85].

Emerging non-thermal physical technologies have demonstrated promising results in prolonging the shelf life of fruits and vegetables, as stated in the conclusion. However, further study is required in order to attain a sufficient quality level through the use of procedures that are cost-effective [86].

Physiological changes, such as enzymatic browning brought on by tissue damage and high respiration rates, as well as physical variables, such as mechanical injuries and the loss of exterior protective coatings, have a substantial impact on the quality of fresh-cut fruit and vegetables during refrigeration. Sanitizing, reducing enzymatic browning, improving texture, and using nutrients to fortify these goods are some of the innovative food processing technologies that are now being explored and applied. Some examples of these technologies are dipping and vacuum impregnation procedures [87].

Dipping treatments include immersing the product, either with or without the use of mechanical agitation, and then removing any surplus solution that has been absorbed by the product. This procedure is frequently utilized on commodities that are whole, peeled, shredded, and sliced, as well as on items that are more susceptible to spoilage. This is because it promotes the dispersion of the solution, which covers the largest surface area of the product without causing any harm or stress. One of the most significant benefits of these

dipping treatments is the elimination of cellular exudates, which are known to have a negative impact on the quality of commodities after they have been harvested [88]. The factors of the dipping process that need to be adjusted include the amount of time spent soaking, the frequency of dipping, the solute composition, the temperature, and the concentration of the solution. These variables vary depending on the food product of interest. To determine whether or whether dipping treatments with calcium (Ca) salts can increase the shelf life of items, a number of experiments have been conducted. Ca enrichment provides a number of benefits, some of which include the reduction of microbial growth as a result of a decrease in activity water, the enhancement of texture, acceptability, and storability, and the avoidance of browning as a result of oxidation events and the formation of off-flavours in fresh-cut foods [89].

The utilization of natural extracts for the purpose of anti-browning agents (polyphenols, carotenoids, organic acids, and bioactive peptides) is a relatively new method that has been implemented in order to enhance the quality of fresh-cut fruit and vegetables and to extend their shelf life. Several natural compounds derived from tomato skin, pineapple juice, pomegranate peel, mango peel, aloe vera gel, and extracts from pumpkin, artichoke, grape, and broccoli have been shown to be effective in preventing browning in fresh-cut fruits [90]. Food vacuum impregnation, also known as VI, is a technique that gives manufacturers the ability to directly introduce, dissolve, or suspend items in the void fraction (also known as the pores) of a food matrix in a regulated manner. VI is comprised of two primary steps: (1) the pressure is reduced in the system (under vacuum), the native gases and liquids are removed, and the product pores are expanded under the action of pressure gradients until mechanical equilibrium is achieved; (2) the atmospheric pressure is restored (relaxation period) and, with the opposite pressure gradient, the external solution fills the pores while the tissues relax, until a new equilibrium has been reached [91].

Before the application of the VI treatment, it is necessary to take into consideration the porosity, the tissue structure, the size and geometry of the food, the impregnation solution (concentration and type of solute), and the process parameters (vacuum pressure, exposure time, relaxation time at atmospheric pressure, temperature, product/solution relationship, and agitation among other things). The fact that fruit and vegetables have a significant amount of intercellular space that is filled by gas makes VI an appropriate instrument for the incorporation of substances that enable food manufacturers to increase the shelf life of their products without altering the cellular structure of the food [92].

Primary packaging materials that are successful include edible active packaging, which is based on natural components. This type of packaging slows the ripening process, maintains nutritional characteristics, and avoids quality loss by reducing natural processes like as gaseous exchange, respiration, and transpiration rates. Recent research has demonstrated that the effectiveness of edible coatings can be considerably enhanced by the use of active natural components that possess antioxidant and/or antibacterial characteristics. These active packaging materials are intended to interact with food by releasing components that possess biological qualities, therefore improving the oxidative stability of the food product and limiting the growth of diseases that are transmitted through food [93].

Examples of edible active packaging include coatings made of alginate and chitosan that have been enhanced with an extract from olive leaves. These coatings extend the shelf life of delicious cherries, keep the phenolic and antioxidant components intact, and delay the ripening process. Chitosan and ascorbic acid inhibit browning, retain flesh firmness, delay microbial growth, and maintain phenolic compounds throughout storage. Alginate coatings with citric and acetic acid allow fresh-cut mangoes to retain quality attributes such as antioxidant activity, phenolic content, and colour. Chitosan and ascorbic acid also prevent browning [94].

It has been demonstrated that gallic acid-grafted chitosan film is beneficial for the preservation of mushrooms. This film has been shown to have a reduced respiration rate, browning degree, malondialdehyde content, electrolyte leakage rate, superoxide anion generation rate, and hydrogen peroxide content when compared to commercial polyethylene film. Pectin-based coatings that are enhanced with lemon by-product extract ensure that fresh-cut carrots keep their structural integrity for a period of fourteen days as long as they are stored at a temperature of four degrees Celsius throughout storage [95].

There have been reports that the incorporation of lemon essential oil into chitosan edible coatings has the ability to decrease the respiration rate of strawberries and boost the antifungal activity of chitosan against *Botrytis cinerea*. In addition, the effects of chitosan edible coatings that include natural functional compounds derived from olive cake and orange peel (OPE and OCE, respectively) on fresh Barhi date fruit have been investigated [96]. In order to preserve raspberry fruits, active film pads produced from chitosan have been utilized. These pads have been enhanced with ethanolic extracts of green tea and rosemary, which are natural antifungal agents. These pads have been used to reduce the incidence of fungal growth, maintain overall quality while being stored, and increase the shelf life of the raspberry fruits by up to 14 days [97].

In the field of agri-food research, the study of microbiota is becoming an increasingly popular topic of interest, particularly in relation to the microbiota of fruits and vegetables. The naturally occurring bacteria, yeasts, and filamentous fungus (molds) that make up these intricate microbial ecosystems are responsible for their formation. At the time of harvest, the variety of microorganisms can have a major impact on the quality and safety of goods that are produced after harvest. It is possible for these undesirable microorganisms to be linked to undesirable microorganism pathogens, manufacturers of toxins, bacteria that contain antibiotic resistance genes, and potential spoiling activities. Controlling these undesirable microorganisms may be accomplished by the utilization of a variety of techniques, which are frequently coupled within the framework of barrier technology applications. These microbes can originate from postharvest processes, operators, and surroundings [98].

When it comes to the topic of biological therapies, biocontrol is regarded to be one of the most important solutions since it involves the utilization of certain microbes that have the ability to restrict the growth of microorganisms that are not wanted. The idea of having the microbiome of the fruit or vegetable as a target offers a privileged perspective in understanding the variables involved in biocontrol solutions, including the currently emerging interest in products developed for preharvest applications but also demonstrate interest in terms of postharvest biological control [99].

The use of yeast and bacteria as control agents is included into each and every one of the solutions that are now available on the market. The successful application of yeast in postharvest biocontrol is due to their general inclination for physically colonizing the surfaces, effectively competing for resources to overcome widely used pesticides, releasing lytic enzymes, and inducing host resistance. Yeasts possess these characteristics. In addition, there is a wide variety of particular killer toxins that may be bio-produced by these eukaryotic microorganisms. These killer toxins have specific biological targets within the cell of the microscopic creature that is vulnerable to them [100].

Even among prokaryotic microorganisms, the biological armament against postharvest pathogens includes the release of lytic enzymes, the creation of biofilms, and the process of resistance induction. Steric competition and antagonism in terms of nutritional variables are also included in this arsenal. In terms of postharvest management, the genera *Bacillus* and *Pseudomonas* are widely regarded as being among the most effective antagonists. In the area of agriculture, *Bacillus* is widely acknowledged as a biological alternative to conventional

chemical fungicides and bactericides. On the other hand, *Pseudomonas* has a broad diffusion in the field, which is evidence of the genus's resistance in the agricultural environment [101]. Specifically with regard to postharvest applications, the diverse group of lactic acid bacteria (LAB) offers a promising reservoir of possible bio-based solutions for sustainable agriculture. This is especially true in the realm of prokaryotes themselves. It is generally accepted that LAB have a long history of being used in food fermentations without any adverse effects, and a number of LAB species are recognized as having eased pathways in determining their safe usage in food. The biological processes that are utilized in the management of undesirable microorganisms on fruits and vegetables after harvesting are diverse and belong to classes that have been extensively researched in other food applications [102]. Within the framework of hurdle technology, future perspectives in the field of biocontrol in the postharvest of fresh plant products demonstrate the necessity of a clear and shared global regulatory environment. This is necessary in order to expedite the implementation of innovative actions and the evaluation of biocontrol, in conjunction with other physical and chemical solutions [103].

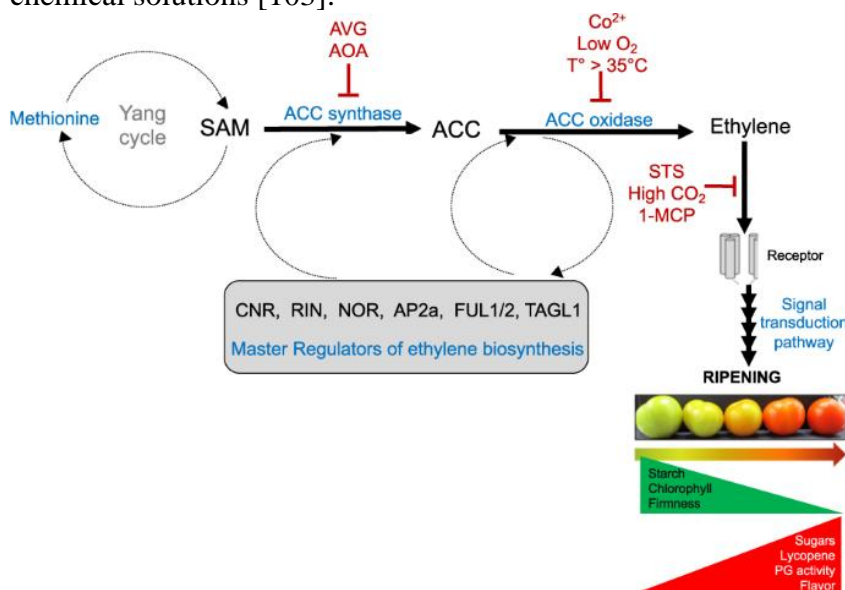


Fig. 4. Implementation of innovative actions and the evaluation of biocontrol, in conjunction with other physical and chemical solutions

Computer based technologies

Computer vision systems, also known as CVSs, are a technology that is utilized for the in-line grading of fruits and vegetables. This technology is contactless and non-destructive. These systems are able to extract visual information from photographs, which allows them to classify and grade items, evaluate quality, identify faults, and estimate internal attributes. Consumer value stores (CVSs) offer food management that is objective, consistent, and widespread across the supply chain [104]. This helps to reduce loss and waste while simultaneously enhancing customer satisfaction. In most cases, they make use of a digital camera, an illumination system, and a personal computer in order to extract classification characteristics and construct models by employing statistical methods or machine learning approaches. In order to evaluate the quality and marketability of fresh goods, CVSs have been created [105]. This is because colour is an essential factor in determining market acceptability. In comparison to conventional colorimeters, CVS technology is able to provide an evaluation that is more objective and consistent. This is because it can estimate colour characteristics at the pixel level. An innovative CVS was created not too long ago with the

purpose of determining the quality level of rocket leaves and distinguishing between different growing methods by utilizing colour information derived from digital photographs. Through the utilization of a random forest model for the purpose of automatically selecting relevant variables for classification, the CVS that was presented was able to attain an accuracy of around 95% in quality-level evaluation and between 65-70% in cultivation strategy differentiation [106].

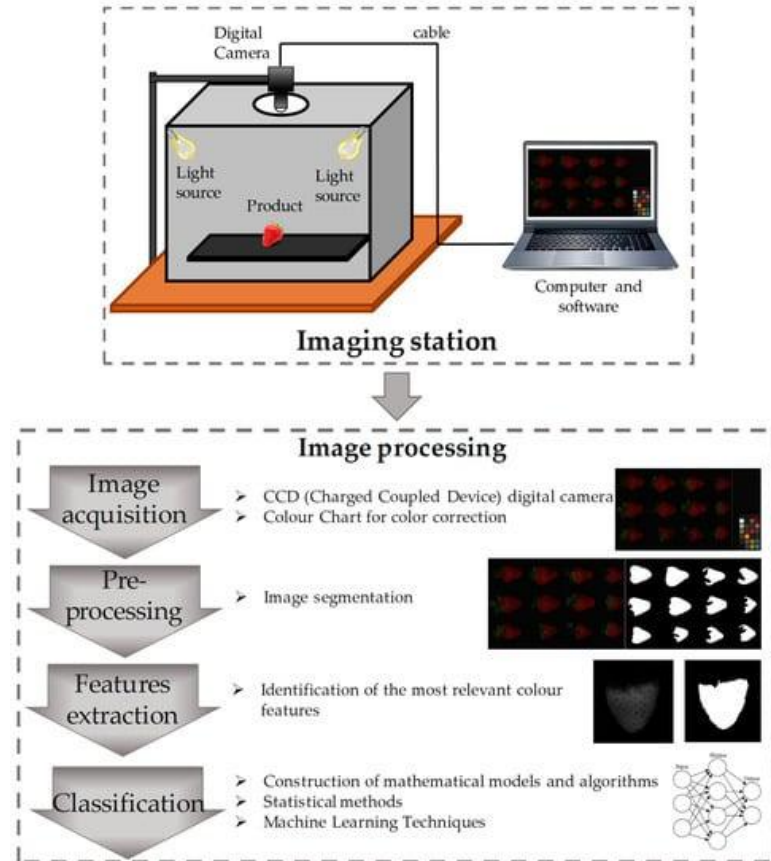


Fig.5. Computer based technologies

The CVS is an efficient instrument that may be used to check the quality of fresh-cut fruit throughout the supply chain, therefore minimizing food loss and guaranteeing that freshness is maintained at the market level. Artichokes, nectarines, iceberg lettuce, radicchio, apples, and potatoes are just some of the foods that have benefited from its utilization in past applications [107]. The identification of regions that are impacted by shadows or highlights created by light interaction with a plastic bag is a significant problem that has to be solved in order to conduct quality-level evaluation through the packaging material. This topic pertains to packaged items. A strong and powerful segmentation strategy that picks the regions where colors may be measured adequately is required in order to attain performances that are comparable to the results achieved on unpackaged samples [108].

In addition, CVS may be utilized for the purpose of assessing the internal quality of agricultural goods. It is possible for ripening or senescence processes to induce changes in the nutritional content of a product while it is being stored after harvest. These changes can also lead to changes in the food's visual characteristics, such as its colour and/or texture. The yellowing of green leafy vegetables is only associated with the loss of chlorophyll, but the browning processes that take place on the surfaces of freshly cut items are brought about by the activity of polyphenol oxidase and peroxidase on phenolic substances. When the colour of

a certain fruit is improved, the total soluble solids content increases, and the acidity of the fruit lowers. This is because the various degrees of the ripening stages have a statistical impact on the total soluble solids and pH values [109].

It has been determined via the development of CVS regression models that the antioxidant activity (AA) and total phenols (TP) levels of carrots may be estimated. The method of image processing has been utilized in order to forecast the enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD) on banana samples, with the objective of assessing the browning of the peel after nine days of storage at a temperature of 25 degrees Celsius.

Nevertheless, the correlation coefficients did not reveal any significant differences between the values of PPO and POD that were predicted and those that were taken into account [110]. Recently, a CVS was used in order to differentiate between the ripening phases of strawberries by harvesting them at three distinct times. These stages include half-ripe and red. It was shown that titratable acidity was statistically associated to the image data, making it a useful indication for the non-destructive evaluation of the ripening stage of strawberries. Titratable acidity is one of the chemical markers of ripening [111].

In order to anticipate the concentrations of chlorophyll and ammonia, which are objective markers of senescence, for both unpackaged and packed rocket leaves, a combination of image analysis and the random forest model was developed. When it came to predicting total soluble solids and pH, the findings showed that an SVM-R model that worked on the features in the HSV colour space performed significantly better than an MLR model compared to its performance [112].

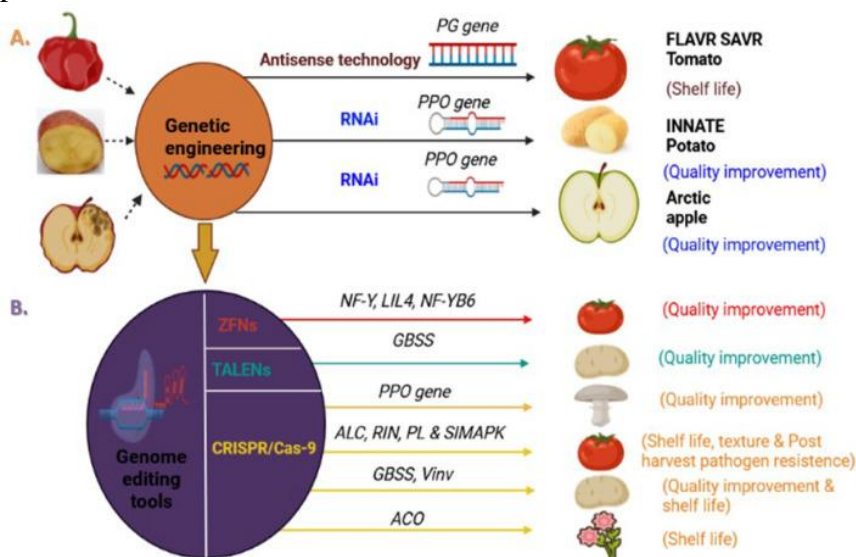


Fig. 6. Concept of genetic engineering

Utilizing the RGB value retrieved from photographs taken with a smartphone camera, Li et al. developed a cutting-edge and intelligent system that can accurately estimate the shelf-life and quality of kiwifruit while it is being stored in a cold environment. There was a negative correlation between the R to B ratio values and titratable acidity, vitamin C content, and hardness. On the other hand, there was a positive correlation between the R to B ratio values and soluble solids content, total soluble sugar and total plate counts. The approach that these authors have presented, which is based on the analysis of smartphone images, is more straightforward and quicker than previous prediction methods that have been published. This makes it possible to evaluate the postharvest quality of kiwifruit in a way that is more

expedient [113].

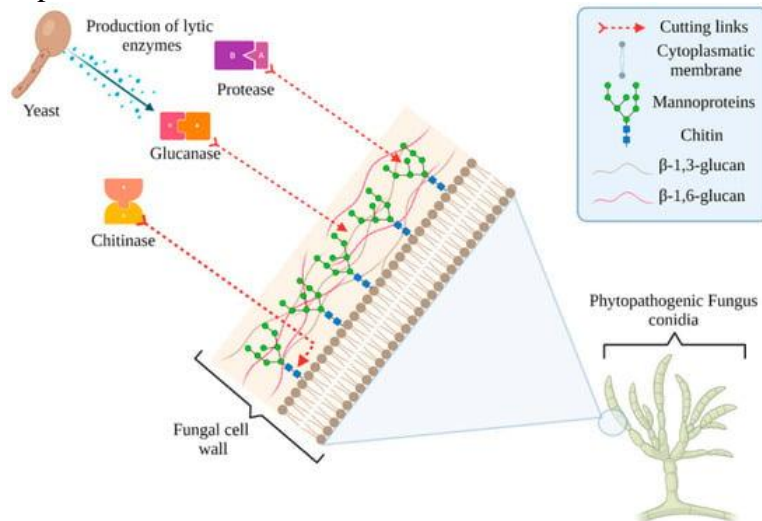


Fig. 7. Production of lytic enzymes

A potential sensing technique that has arisen as an alternative to conventional approaches such as headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC-MS) is the electronic nose, also known as the E-nose. E-nose is a sensing device that differentiates and categorizes food matrices with diverse fragrance signatures by utilizing electronic chemical sensors that are both partly selective and broad-spectrum. The apparatus for handling samples, the detector, and the data collection system are the components that make up these devices [114].

In order to evaluate the volatile organic compound (VOC) profiles of horticultural food products, the olfactometric methodology is utilized. This methodology offers a number of benefits, such as low cost, ease of use, rapidity, non-destructiveness, elimination of the need for preliminary sample preparation steps, use that is friendly to the environment, and automatic data handling. Metal oxide semiconductor (MOS) sensors are the ones that are utilized in E-nose applications the most commonly because of their quick reaction time, great sensitivity, and extremely inexpensive cost [115].

There have been a great number of publications that have been published on the utilization of E-noses in the study of various foods that are extracted from plants. More specifically, new research suggests that E-nose is a mechanism that may be utilized for the purpose of determining the stage of ripening that fruit is in. For instance, Palumbo et al. utilized E-nose in conjunction with attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy and image analysis (IA) in order to differentiate between two phases of ripening in the strawberry variety known as "Sabrosa." The results of the correlation study between the data from the E-nose and the VOC profiles that were acquired by HS-SPME/GC-MS in the past indicated that the responses from the E-nose were consistent with the findings of the HS-SPME/GC-MS analysis [116].

In order to determine the stage of maturation of both white berry and blackberry, Aghilinategh et al. investigated the possibility of combining the responses of a MOS gas sensor E-nose with appropriate pattern recognition techniques. These techniques included artificial neural networks (ANN), principal component analysis (PCA), and linear discriminant analysis (LDA). In terms of classification performance, the findings demonstrated that the ANN demonstrated the highest level of accuracy, with a score of over 88 percent [117]. Through the use of an E-nose, evaluated the fruit quality indices of naturally and artificially ripe crab apples. These indexes included titratable acidity, soluble sugar content, sugar–acid ratio, soluble solids quantity, soluble protein level, and taste

profile. Based on the data that was collected, it was determined that the RF algorithm, which has an average recognition accuracy of around 98%, is the most effective algorithm for discriminating between crab apples that are naturally ripe and those that are artificially ripe [118].

There have been a number of studies that have demonstrated the potential of the E-nose, when paired with chemometric techniques, can be utilized in the classification of fruits and vegetables according to their geographical origin. Li et al. conducted an investigation into the origin of 303 maca samples that were gathered from over 100 different locations within the major growing area in China. They used GC-MS and a MOS-based E-nose to identify the volatile and olfactory fingerprints of the maca samples, respectively. The results of correlation and multi-regression studies demonstrated that each and every sensor had a statistically significant link with certain maca variables [119].

Cozzolino et al. investigated the possibility of using the E-nose as a quick method for distinguishing between samples of the sweet cherry variety known as "Ferrovia." These samples were packed in either an atmosphere with a high concentration of carbon dioxide (CO₂) (16% O₂ + 20% CO₂ + 64% N₂) or air (20% O₂ + 0.03% CO₂ + 80% N₂) for a period of up to 21 days. Based on the storage circumstances as well as the amount of time that the fruit was stored, the projection to latent structures (PLS) methods that were used to the E-nose data suggested that the fresh sample, as well as the packed or unpackaged fruit, could be categorized [120]. Through the use of one or more E-nose sensors, researchers were able to correlate samples with certain taste profiles by conducting a correlation analysis between the responses of the E-nose and the overall volatile organic compounds (VOCs) that were found in a prior study using HS-SPME/GC-MS on the same cherry samples [121].

An electronic nose that was fitted with eight MOS sensors was utilized by Ghasemi-Varnamkhasti et al. [193] in order to assess the level of freshness of strawberries that were kept in three distinct forms of polymer packaging. These types of packaging included polypropylene (PPP), ethylene vinyl alcohol (EVOH), and polyvinyl chloride (PVC). Using pattern recognition techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), and support vector machine (SVM), the researchers were able to accurately categorize unpackaged and packed samples, as well as investigate the impact of polymer packaging on the freshness of strawberries [122]. The response surface technique, also known as RSM, was taken into consideration while selecting the optimal sensor array. This was done with reference to the contribution that each sensor made to the categorization of the samples. On the first, eighth, and sixteenth days, sample headspace profiles were investigated. While the principal component analysis (PCA) was able to explain 84% of the variation in the data, the linear discriminant analysis (LDA) was able to classify all of the sensor responses with an accuracy of 86.4%. In addition, the support vector machine (SVM) technique was able to effectively differentiate between the samples by 86.4% and by 50.6% in training and validation, respectively, when utilizing a polynomial basis function (C-SVM). Additionally, when utilizing a radial basis function (Nu-SVM), the SVM technique provided

an accuracy of 85.2% and 55.6% in training and validation, respectively [123].

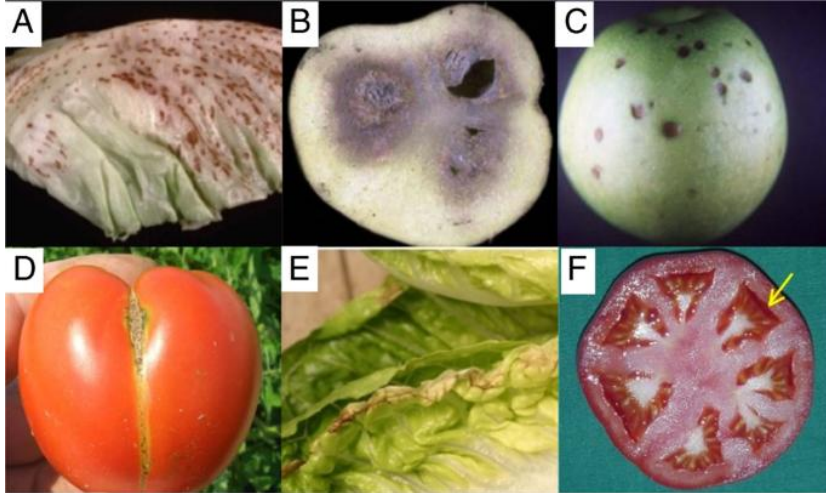


Fig. 8. Diseases in fruits

An investigation on the changes in freshness quality of postharvest spinach from one to twelve days of cold storage. This investigation was carried out utilizing machine vision and an E-nose, in conjunction with chemometrics. The procedure was both quick and non-destructive. During the period of cold storage, the freshness of the spinach was categorized into four different classes by ten expert panelists. For the purpose of determining the freshness of spinach, a backpropagation artificial neural network (BPNN), a support vector machine (SVM), and a K-nearest neighbors (KNN) were utilized [124]. When it came to the prediction of spinach freshness, the findings that were produced by applying the BPNN model linked to machine vision revealed the same outcome as the KNN technique, with a classification accuracy of 85.4%. The BPNN model, on the other hand, which was derived using E-nose data, made it possible to get a superior outcome in comparison to the SVM technique, with classification accuracies of 81.2% and 75.0%, respectively. Additionally, the BPNN model, which was constructed on the basis of multisensory data fusion through the utilization of machine vision and E-nose data, achieved a classification accuracy of 93.7%, which resulted in a significant improvement in the accuracy of the freshness evaluation of postharvest spinach [125].

It is possible to distinguish distinct functional groups in a molecule and, consequently, the chemical composition of a product through the utilization of a technology known as near-infrared (NIR) spectroscopy. This approach works by establishing a connection between the vibrational properties of matter and specific internal characteristics. Near-infrared (NIR) spectroscopy is one of the infrared (IR) methods that is utilized extensively and successfully in a variety of industries. These industries include the food industry, agriculture, chemicals, pharmaceuticals, textiles, polymers, cosmetics, and medical applications. In NIR spectroscopy, there are three different modes of setup that can be utilized, depending on the particular application [126].

Post-harvest management through gene editing in vegetables

There is a wide variety of foods that are derived from vegetables, and each one has a unique sensitivity to the action of ethylene (ET) during postharvest and control throughout shipping, storage, and sale. ET, which can induce yellowing and quick produce deterioration after harvest and storage, is especially sensitive to the Brassicaceae family of vegetables, which includes the Brassicaceae family of edible plants. It is necessary to develop innovative ways

in order to control the detrimental impacts of ET activity since broccoli florets are especially vulnerable to its action. The utilization of ET absorbers and packaging, which has been demonstrated to be successful in increasing the shelf life of this perishable vegetable product, is an efficient solution [127].

Gene expression studies have demonstrated that the expression of ET signaling genes is increased when broccoli and kale are stored at room temperature, but the expression of these genes is decreased when the temperature is lowered. The expression of senescence association genes (SAGs) and ET receptors is specifically reduced when the temperature is low, which suggests that temperature plays an important role in the regulation of ET throughout storage and shelf life. When applied to cabbage, abscisic acid treatments cut the amount of ET biosynthesis and accumulation that occurs within the storage package by fifty percent, while having no impact on the amount of water or chlorophyll present [128].

Both ET and cytokinin treatments have an effect on the postharvest quality of broccoli heads in Brassica. This is because they modify the activity of lipoxygenase, which ultimately results in degreening. Using the ET antagonist (1-MCP) resulted in a decrease in the breakdown of chlorophyll as well as the expression of genes that are linked with chlorophyll catabolism.

This was accomplished by a variety of activities on a number of chlorophyllases [129].

During the postharvest process, ethylene has a significant role in determining the quality of fresh and fresh cut goods. During the postharvest period, the economic success of a crop is impacted by all of the environmental and physiological manifestations that were discussed earlier. The extension of storage duration and marketability are both critical considerations; nevertheless, quality must be maintained at the highest feasible level from the field to the ultimate customer [130].

As a possible therapy for delaying the onset of senescence and extending the shelf life of products by influencing the metabolism and effect of ET, melatonin has been the subject of research. The use of melatonin treatments was successful in delaying the onset of senescence, reducing the impact of ET, and enhancing the qualitative characteristics of broccoli florets. In addition to having a greater chlorophyll content, broccoli that had been treated with melatonin had improved colour preservation after storage [131].

In a recent research, it was shown that the presence of hydrogen sulphide, selenium, and 1-MCP can delay the ripening and senescence of tomato fruits while they are being stored. This allows for the fruits to retain higher amounts of chlorophyll, starch, soluble proteins, and ascorbic acid in comparison to the control fruits that were not treated. During the postharvest period, these treatments could function as signal molecules that are responsible for the suppression of ET. In tomato, treatments with selenium were efficient in inhibiting the expression of ET biosynthesis genes and boosting the enzymatic antioxidant systems, all while extending the shelf life of the tomato [132].

In the case of broccoli, amino acids were successful in reducing the generation of ET, delaying the discoloration process, and identifying the maximum antioxidant capacity.

Through the use of modified environment storage, an efficient method was implemented in order to preserve the qualitative characteristics of both whole and fresh cut romaine lettuce while it was being stored. By taking this technique, it was possible to modify the internal gas composition of the package, which had an impact on the generation of ET, the sensory quality, and the marketability of the product [133].

The postharvest commercial management of crops, from the field to the fork, entails various problems during transit, and in the case of ripe fruits, there is a considerable potential danger of negatively influencing the quality of the product. There is a high probability that tomatoes will suffer from mechanical damage, which will ultimately result in degraded quality. An investigation that was conducted not too long ago demonstrated that even the slightest compression of tomato fruits can have a substantial impact on the product's quality and

marketability [134].

The transition from traditional breeding to transgenesis for ET has been a significant step in the development of breeding methods for postharvest quality products. The enzyme ET is responsible for regulating a wide variety of biochemical, physiological, and molecular activities that have an impact on postharvest processing. An alteration in the processes of ET biosynthesis and perception might have beneficial impacts by extending the shelf life of the product; nevertheless, additional efforts can assist emphasize the postharvest quality of crops [135].

The tomato is frequently regarded as the crop that serves as a reference for the development of fleshy fruits, and the ripening of tomatoes is controlled by both ET-dependent and ET-independent mechanisms. In traditional breeding, the majority of research that are connected to molecular aided selection (MAS) are based on crossings that were carried out between cultivated tomato or elite lines and related species, or most typically, mutants. An additional optimization of breeding efforts may be achieved by the availability of short life cycle genotypes, also known as Microtom. These genotypes serve as a model transformation system for functional genomics investigations [136].

Complex regulatory networks, which are subject to polygenic regulation and quantitative inheritance, are responsible for determining a number of qualitative characteristics that are associated with ET-dependent ripening. The tomato has been subjected to transgenic transformation in order to shed light on ET as well as the biochemical and metabolomics pathways that it possesses. An illustration of a transgenic transformation that was utilized in order to explore the ET route [137]. This investigation was followed by a metabolomics method. The introduction of commercial tomato cultivars that were created via the use of recombinant DNA technology was yet another approach applied. A great shelf-life was demonstrated by Flavr Savr, which was licensed by the Food and Drug Administration in 1994. This was made possible by the incorporation of the antisense polygalacturonase (PG) gene, which is an enzyme that is dependent on ET and is involved in the process of fruit softening [138].

Over the course of the past half-century, researchers have been examining the connection between ET biosynthesis and the induction of the climacteric peak of respiration during the process of fruit ripening in tomato plants. Nevertheless, the molecular and metabolomics pathways that are connected to this phenomenon are still being researched. Gene transfer by genome editing techniques can also result in the integration of the transgene and the cisgene, which allows for more predictable expression. This is in addition to the technology of transgenic transformation. The type II clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 (CRISPR-associated) technology is the most widely used site-specific genome editing technique. This technology was initially discovered in *Streptococcus*

pyogenes and was used for the first time in 2013 in five distinct plant species [139].

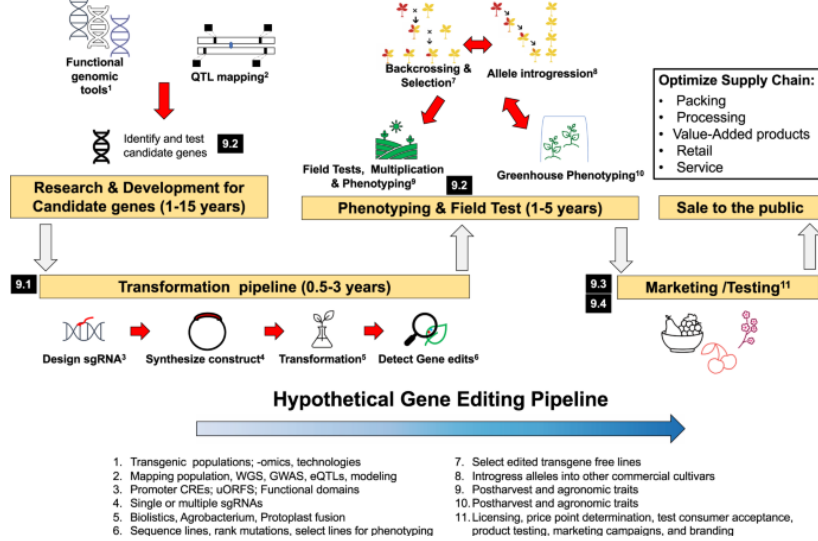


Fig.9. Post-harvest management through gene editing in vegetables

The transcription of ripening-regulated genes, which are responsible for determining the quality of climacteric fruits, is a result of the synthesis, control, and perception of ethylene (ET). There are several different components that are involved in ET signaling. These components include ET receptors that are located in the membrane of the endoplasmic reticulum, constitutive triple response 1 (CTR1), ET-insensitive 2 (EIN2), TFs including EIN3 and EIN3like, and ET response factors (ERFs). Multiple studies have demonstrated that ET and other phytohormones, like as auxins and ABA, engage in a reciprocal relationship [140].

During the process of ripening, substantial alterations take place in terms of texture, colour, and aroma. These alterations are controlled by ET in conjunction with transcription factors (TFs) and the genes that are downstream of them. TFs have been the subject of extensive studies and in-depth research, with the ET signal pathway being a complicated transduction network that ultimately results in the activation of ERFs, which are transcriptional regulators that are located farther downstream. The APETALA2/ethylene response factor, also known as AP2/ERF, is a crucial component in the regulation of ET-responsive genes and plays a significant role throughout the ripening process. In addition to the RIN-MADS, CLEAR NON-RIPENING, TAGL1, and LeHB-1 families of transcription factors, there are other families of TFs that are involved in the ET response and the ripening of fruit. These genes encode positive regulators of ripening [141].

Through the study of the ripening phenotype and the mapping of the genes that are responsible for the mutation, spontaneous tomato mutants have been utilized in order to examine the activities of ET-dependent genes. Monogenic tomato mutants that are considered to be of the utmost significance include ripening-inhibitor (rin), nonripening (nor), colorless nonripening (Cnr), green-ripe (Gr), green flesh (gf), high pigment1 (hp1), high pigment2 (hp2), and never ripe (Nr). New varieties that have a longer shelf life and a lower rate of produce degradation or losses have been developed with the use of these mutations, which have been employed for commercial purposes and for producing parental lines that show a delay in the ripening and textural deterioration of the fruit [142].

A number of significant crops, including broccoli, cauliflowers, Brussels sprouts, cabbage, kale, mustard (greens), and collards, are members of the Brassicaceae family, which is derived from the genus Brassica. Research on ET has been carried out in order to shed light

on the most important biochemical and physiological processes in these crops; nevertheless, in comparison to other crops such as tomatoes, only a limited number of research have been carried out for the goals of breeding and molecular analysis. In recent times, a number of gene families known as senescence-associated genes (SAGs) and two families of transcription factors known as NAC (NAM, ATAF, CUC) and WRKY have been identified in *Arabidopsis thaliana*. These gene families are involved in the process of senescence, and they have also been investigated in Brassicaceae plants like cabbage, kale, and broccoli [143].

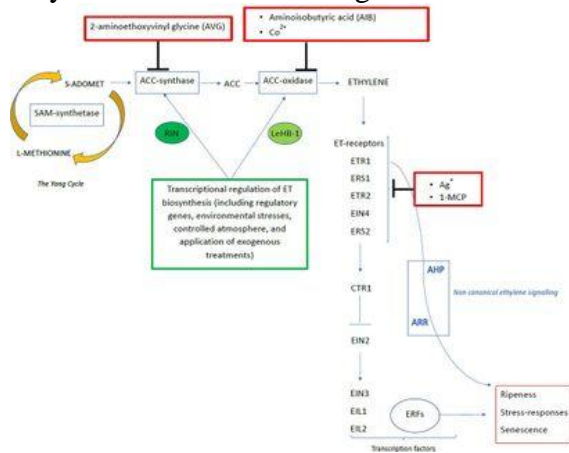


Fig. 10. transcription of ripening-regulated genes

The administration of both ET and propylene, which is a functional counterpart of ET, had an effect on the yellowing of the florets in broccoli. It was possible to generate a mutant broccoli ET-response sensor (*ers*) gene by the process of directed mutagenesis. Additionally, two plasmids were created, which resulted in the creation of two transgenic lines. The transgenic broccoli exhibited ET insensitivity, including a delay in the senescence of the leaves and a delay of one to two days in the yellowing of the head, which was evaluated by the drop in chlorophyll and colour angle on the broccoli heads [144].

Using antisense transformation designed broccoli to express the ACO gene. They demonstrated that this resulted in a block into ET production, which led to an increase in the transformation rate. This was due to the fact that the inhibition of ET biosynthesis led to an increase in the manufacture of shoot regeneration boosting chemicals, which were polyamines [145].

Due to the fact that it is grown all over the world and has a high economic value, the zucchini, also known as *Cucurbita pepo*, is considered to be one of the most significant crops in the Cucurbitaceae family. For breeding goals connected to parthenocarpy, ET has been thoroughly researched in zucchini; however, less attention has been paid to the quality of the fruit after harvest. Using MUC-16 as the progenitor genetic background (WT) and ethyl methane sulfonate (EMS) as the mutant agent, created a mutant collection consisting of around 3800 M2 families. Seedling germination, sex determination, sex expression parthenocarpy, and fruit set were all altered as a result of mutations that conferred ET insensitivity or altered ET sensitivity, according to a consistent form of mutant phenotyping [146].

In the case of zucchini, it has been discovered that ET plays a role in the susceptibility to chilling damage (CI), with types that are more resistant exhibiting a lower production of ET when exposed to low temperatures. An ET-insensitive mutant *etr2b* and its wild-type counterpart were examined in a recent study with regard to the synthesis of ET, the pace of respiration, and the amounts of oxidative stress. A demonstration of the role that ET plays in the development of cold-induced postharvest oxidative damage was made possible by the utilization of an ET-insensitive cultivar of zucchini known as *etr2b* [147].

Distinct expressions of the ACS and ACO genes have been studied in Spanish genotypes under cold storage at three distinct temperatures over a period of seven days: four degrees Celsius, twelve degrees Celsius, and twenty degrees Celsius. CpACS and CpACO have been shown to have lower expression levels in genotypes that are more resistant to CI. On the other hand, the genotypes that are the most vulnerable to CI have shown a chilling-induced ET peak at 4 degrees Celsius following the manifestation of apparent symptoms for CI [148]. For the purpose of genotyping, sequencing analysis was performed on an interspecific population that was developed from a hybrid between zucchini (*C. pepo* ssp. *pepo*) and Scallop (*C. pepo* ssp. *ovifera*). This cross resulted in the generation of over 7,000 thousand SNP markers and a high density linkage map that covered 2,817.6 cM of the whole genome. There are 48 QTLs that have been discovered for vine, blooming, and fruit quality based on a three environmental study. Additionally, various TF that are involved in the ET metabolism have been identified in the DFeF_9 area, which is near to other genes that affect the flowering process [149].

A reassessment of the ethylene signal (ET) signal and TFs in tomato plants has been brought about as a result of the use of new breeding techniques (NBT) that make use of CRISPR/Cas9. Increasing knowledge and understanding of ethylene-dependent pathways, which play a critical role during postharvest and may be used to create innovative techniques for enhancing crop quality and shelf life extension, is a promising strategy that has the potential to be utilized [150].

Targeted deletion or substitution of regulatory proteins CNR and NOR, as well as transcription factors AP2a, FUL1, and FUL2, has been accomplished by the application of CRISPR technology in tomato. By removing the genes that code for the TFs through the application of gene-editing technologies, further information on the roles of the TFs was obtained. When compared to the normal line and the mutant line, edited lines exhibited a greater level of ethylene production. Additionally, these lines ripened more quickly, which is further evidence that AP2a plays a detrimental function in the generation of ET [151]. Recent years have seen CRISPR/Cas9 deletion and RNAi silencing of *rin* in wild-type tomatoes only partially restore the non-ripening phenotype. This is due to the fact that it did not suppress the onset of ripening, and the mutant fruits displayed a modest red hue. When the *rin* mutant allele was inactivated, the induction of ripening was partially recovered. On the other hand, deletion or RNAi silencing of the chimeric RIN-MC mutant protein in a *rin* background partially restored ripening [152].

CRISPR/Cas9 mutagenesis was utilized in order to suppress the expression of the genes that code for AP2a, NOR, FUL1, and FUL2. Because different knockout alleles for ET biosynthesis have the potential to increase fruit shelf life without compromising organoleptic or nutritional quality, this NBT may have a practical application in tomato breeding for postharvest purposes [153].

Additionally, this NBT has been utilized to develop mutants that have increases in postharvest quality measures associated to ET. This is in addition to the CRISPR/Cas9 techniques that have been utilized to explore the function of ET in null mutants for ripening. In 2016, Uluisik and colleagues created tomato mutants for pectate lyase, an enzyme that is dependent on ET and plays a role in the process of fruit softening. These mutants demonstrated an increase in fruit texture and postharvest quality, without affecting the colour or the amount of soluble solids present. The following pectin-degrading enzymes, namely β -galactanase, polygalacturonase, and pectate lyase, have been subjected to the generation of additional CRISPR/Cas9-based mutants for texture-related genes [154].

There is no study that has been published on the application of CRISPR/Cas9-induced mutations for ET in breeding objectives linked to postharvest quality in Brassica and zucchini, as far as we are aware. The results of these research offered additional evidence that

mutations in the tomato ripening transcription factor that were produced by CRISPR/Cas9 did not completely eliminate the process of ripening. This finding suggests that the ripening transcriptional regulatory network had partial "back-up" qualities with numerous crucial sites of control [155].

Benefits of biotechnologies inventions in post-harvest

Increasing demand for extrinsic quality characteristics in fruits and vegetables has led to the commercialization of genetically engineered crops. Some examples of these crops include a papaya that is resistant to viruses, a variety of squash, an apple that does not brown, a pineapple that is fleshy, an eggplant that is resistant to insects, Brassica oleracea, tomato, potato, and *Lactuca sativa*. In order to generate cultivars of superior quality, approaches for altering the genome have been utilized. These techniques include included mutations that boost nutritional advantages. Increased flower and fruit size, fruit ripening, inflorescence branching, enhanced ascorbic acid synthesis, fortification of beta-carotene, transition of perennial to annual (kiwi fruit), decreased tuber-browning, and improved berry quality (strawberry) are some of the adaptations that contribute to these changes [156].

It has been demonstrated via research that the flavour of fruit directly controls specific organic acids that influence organoleptic qualities, which in turn directly affect the overall personality of a fruit. The acidity of the fruit harvest, which is a key characteristic, has a direct influence on the quality of the fruit, particularly for processing in the future. Early-ripening apples often contain high levels of acidity and low levels of sugar, which results in a reduced market demand for fresh consumption and a low proportion of total organic acids to total carbs. Therefore, the market demand for fresh apples generally decreases. In order to combat post-harvest impacts on early-ripened fruits that were produced at room temperature, the utilization of ultraviolet C has proven to be effective. These impacts include a delay in the ripening process, senescence, the preservation of the significant ratio of fruit firmness, biosynthesis of flavonoids, phenolic content, enhanced antioxidant and defence responsive molecules, and more [157].

Both post-harvest loss and waste are becoming increasingly unsustainable as a result of the fact that the global crop production from horticulture is not sufficient to fulfil the nutritional requirements of humans. Injury to the body, internal bleeding, early spoiling, and damage caused by insects are all examples of postharvest loss, which occurs unintentionally. In 2015, the average value of ornamental items was \$16 billion, indicating that their appeal has skyrocketed in recent years. There is a significant amount of moisture present in ornamental crops, and the cold-chain process can result in a loss of up to fifty percent of the farm's value. The value of ornaments drops by fifteen percent for every extra day that they are in transit, and the vase-life of decorations typically only lasts for ten to twelve days after the client makes the purchase [158].

It is essential to move items along a cold chain as rapidly as possible in order to guarantee the quality and shelf life of the output. In order to guarantee the shelf life and quality of the crop, it is necessary to regulate the temperature, the moisture content, the amount of ethylene hormone, and the storage proportion of oxygen to carbon dioxide. When infectious organisms seek to invade harvested items, it is probable that the fruit components will get caramelized. This will result in fruits that are infected or coated in spores, as well as the metabolic by-products that are produced by the fruits [159].

On the other hand, fruits that are either overripe or under ripe are more likely to develop physiological abnormalities, which can result in the goods being thrown away and possibly even food waste. Incorrect cultural practices, such as cold snaps, weather, water stress, heavy rainfalls, pathogens, physiological disorders, plant health, safeguarding, water management, fertilization, and cutting, can cause harm to fruits and vegetables during the pre-harvest

period. This damage can be caused by a variety of factors. The loss of harvesting time can be caused by a number of factors, including an erroneous calculation of the harvesting time, harvesting at the incorrect time, harvesting practices that are not appropriate, and failing to apply pre-cooling to fruits [160].

According to the International Refrigeration Institute (IIR), the absence of cooling systems in developing nations is responsible for the generation of 23 percent of the food waste that occurs in these countries. Constant cold storage ensures that items reach consumers in immaculate form; nevertheless, in impoverished countries, the absence of suitable storage facilities adds to the degradation of both qualitative and quantitative aspects of food production, beginning with the harvest of food and ending with the consumption level. Fresh food may be preserved for longer by taking preventative steps, such as maintaining a cool temperature, maintaining a relative humidity, transporting and packing it appropriately, and so on [161].

There is a wide range of ethylene sensitivity and bloom duration across ornamental plants, including roses, liliiums, lisianthus, chrysanthemums, and carnations, among others. Some degree of success has been achieved by the utilization of breeding techniques in combination with ethylene screening. Molecular approaches, on the other hand, have the potential to extend the lifespan of ornamental plants through the process of gene editing. This may be accomplished by using the CRISPR/Cas9 system to alter the 1-aminocyclopropane-1-carboxylate oxidase1 (PhACO1) gene with the ethylene generating enzyme [162].

A multitude of abiotic stresses, including but not limited to dehydration, soil salinity, and extreme temperatures, pose significant threats to the growth, production, and quality of crops. It is more likely in tropical locations than in temperate regions to experience abiotic stress, which is characterized by high temperatures and drought stress, which restricts the development and output of crops. Through the use of CRISPR/Cas9 technology, researchers are working toward the goal of increasing food productivity by developing transgenic crops that are able to produce more and readily survive harsh and varied circumstances [163].

An innovative method of genetic engineering that has been created for the purpose of crop enhancement is known as precision genome editing. The targeting of certain genes in plants has been accomplished by the utilization of methods such as TALENs, ZFNs, RGENs, and CRISPR/Cas9. The genome editing tool known as CRISPR/Cas9 has the ability to change virtually any sequence in order to reveal its role inside the genome of an organism.

Dehydration resistance, high-temperature resistance, auxin resistance, and drought resistance in tomatoes are just few of the tolerant plants that have been developed with the use of this technique [164].

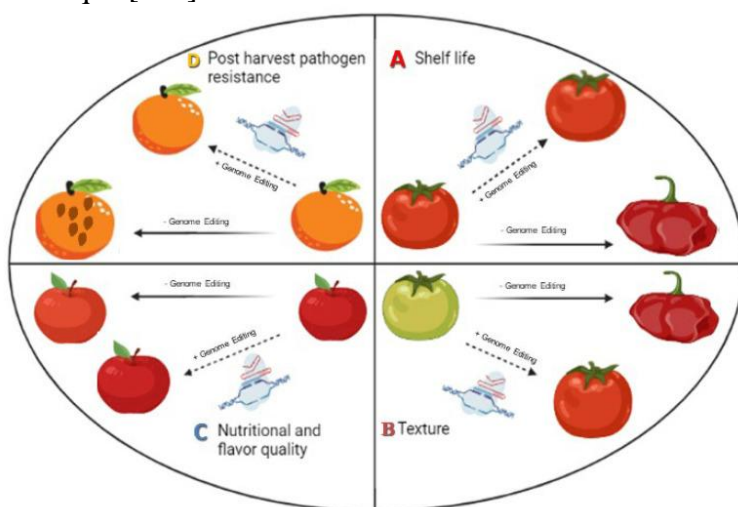


Fig. 11. Benefits of biotechnologies inventions in post-harvest

In order to gain a better understanding of the mechanism behind NCED4 and the silencing of SIMAPK3 in tomatoes when they are subjected to heat stress, CRISPR/Cas9 techniques have also been utilized to induce mutations in lettuce plants. The ARGOS8 gene promoter was modified into GOS2 in order to raise the expression level of the ARGOS8 gene, which was then utilized to generate drought-resistant maize crops. This technology has also been applied. Overexpression of the melatonin gene, which is responsible for the production of melatonin, was found to be a resistance mechanism in plants to abiotic stress [165].

The CRISPR/Cas9 genome editing technology is now being used to further broaden the use of these techniques to genome-wide screening for the purpose of improving desirable features. Mutants that exhibit either gain-of-function or loss-of-function can be produced using the CRISPR/Cas9 system by the precise modification of certain bases at certain gene sites. It is beyond a shadow of a doubt that the CRISPR/Cas9 technology has the potential to replace traditional techniques of crop breeding [166].

Through the use of the gRNA sequencing approach, it is possible to find unique allelic variants that correspond to a particular desirable characteristic that might be introduced into plant populations through the application of this genetic editing technology.

Insects, viruses, fungus, and bacteria are all examples of biotic stressors that have the potential to assault plants and inflict significant harm. Tools based on CRISPR/Cas9 have been created in order to obtain plants that are resistant to disease. Despite this, there has not been a significant amount of progress achieved in the application of the CRISPR/Cas9 genome editing technology for the purpose of enhancing biotic stress tolerance in ornamental crops, fruits, and vegetables. The response of plants to biotic stress is more intricate than that of other environmental stressors, since it involves the overexpression and downexpression of a number of essential genes in response to biotic stress [167].

For crops like apples (*Malus domestica*) and grapes (*Vitis vinifera*), which have extensive life cycles and generation periods, the CRISPR/Cas9 technology is very necessary. The application of CRISPR/Cas9 in a variety of fruits, including citrus reticulate, grapes, apples, *Solanum tuberosum*, bananas, cucumbers, papayas, and citrus, has been detailed in a number of studies. Producing crops that are resistant to biotic stress is vital for the production and growth of ornamental plants, fruits, and vegetables since these types of plants are vulnerable to the effects of biotic stress [168].

Conclusion

The application of genetic engineering techniques can enhance the natural resilience of crops, making them less susceptible to spoilage and decay. This not only extends the shelf life of the produce but also reduces the amount of food waste generated. For instance, genetically modified fruits like the Flavr Savr tomato have been engineered to ripen without softening, a key factor contributing to its longer shelf life. Moreover, biotechnological interventions in post-harvest processes can help maintain the nutritional quality of the food products. This is particularly important in developing countries where post-harvest losses are high and nutritional security is a major concern. By ensuring that a greater proportion of harvested crops reach the consumer in a fresh and nutritious state, these technologies can play a significant role in enhancing food security. However, it's important to note that while these technologies offer promising solutions, they are not without their challenges. Ethical considerations around genetic modifications, potential ecological impacts, and the need for regulatory frameworks are some of the issues that need to be addressed. It's crucial that these technologies are developed and used in a responsible and sustainable manner. Furthermore, while these technologies can significantly reduce food waste, they are only part of the solution. Addressing food waste requires a multi-pronged approach that includes improving

infrastructure, enhancing supply chain management, and changing consumer behaviour. In the future, as we continue to innovate and refine these technologies, we can look forward to a more sustainable and efficient food system. The potential of post-harvest biotechnology and genetic engineering solutions is immense and harnessing this potential could be key to solving some of the most pressing challenges of our time. In the final analysis, post-harvest biotechnology and genetic engineering solutions represent a powerful tool in our arsenal to extend shelf life and reduce food waste. As we move forward, it's imperative that we continue to explore, invest in, and refine these technologies. With careful management and responsible use, they hold the promise of a more sustainable and secure food future.

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