Chapter

Role of Post-Harvest Physiology in Evolution of Transgenic Crops

Binny Sharma and Asha Kumari

Abstract

The increasing world population over few decades has led to increase in demand of food grains or agricultural commodities, thus possessing great impact on food security. Conventional farming approaches has been traditionally practiced but a lot of effort is required to make to enhance agricultural production. With changing climatic conditions plants are vulnerable to several stress factors. In order to combat such conditions, the agricultural systems are needed to be contemporary with advance and recent technologies. Crops after harvest are liable to a lot of changes which ultimately affect quality and quantity of produce, thus reducing economic value. Postharvest losses are decisive factors in reducing loss of produce and agricultural commodities. Thus in order to achieve maximum production, it is therefore essential to reduce post-harvest losses and ensures proper management of postharvest products. Postharvest physiology is the science which deals with quantitative and qualitative study of physiology of agricultural products after harvesting. Biotechnological and transgenic approaches are the recent and emerging technologies that possess great impact on agricultural production. Transgenic technology like genome editing, CRISPR/Cas9, TILLING are successfully used in various species to enhance production, possess resistance to abiotic and biotic stresses, enhance shelf life and improve nutritional quality. Transgenic crops or Genetically modified crops (GMO) like tomato, brinjal, soybean, cassava etc are cultivated globally. These techniques therefore are promising means in establishing food security, increasing crop production, reducing postharvest losses, production of secondary metabolites, hormones and plantibodies.

Keywords: postharvest physiology, transgenics, genome editing, crispr/cas9, plantibodies, genetically, modified crops

1. What is post harvest?

Post harvest physiology is the science which deals with quantitative and qualitative study of physiology of agricultural products (especially living plant tissues) after harvesting. The technologies used in postharvest physiology mainly consists of the techniques applied in agricultural produce after picking for the purpose of preservation, conservation, quality control/increment, processing, packaging, storage and many more. Postharvest technologies are concerned with enhancing nutritional value of food products as in order to meet consumer's needs. India is one of the largest producer of agricultural products and commodities in the world. According to FAO, the total foodgrain production in 2017-18 was estimated around 275 million tonnes and is the largest producer (25% global production), consumer (27% world's consumption) and importer (14%) of pulses in the world. Statistics presented by

National Horticultural Board also reveals that India stands second after China in the production of fruits and vegetables accounting average global production 13% and 21% respectively [1]. About one-third of agricultural produce available for human consumption in the world per year gets wasted. Food loss can be defined as condition where food is available for human consumption but not being consumed. The qualitative and quantitative losses of food commodities often occurs during postharvest operations commonly refers as postharvest losses. With the global increment in human population, the demand for food supply has been enhanced over few decades. Thus in order to increase food availability the losses of food commodities due to post harvest operations are needed to be minimize in order to provide substantial solution to food crisis, reduce pressure on natural resources, eradicate hunger and increase farmer's income [2]. The postharvest losses can be due to loss of weight, loss of quality and nutritional value, loss of viability and commercial loss. Postharvest losses of agricultural commodities may often contribute to the deterioration of quality and quantity as well. The deterioration of quality refers to various attributes including weight loss, change in color and visible quality, change in nutrient content and flavor whereas quantity refers to loss of amount of product respectively [3]. Therefore it can be concluded that postharvest losses are one of the major factor that not only affects agricultural production but also influences food supply chain and economic growth globally. Thus, postharvest loss can be summarized in **Figure 1**.

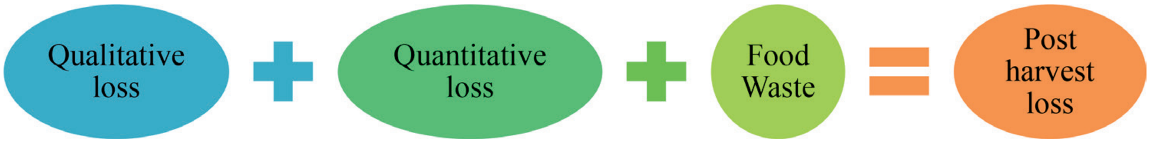

Figure 1.
Summary of postharvest losses.

2. Causes of postharvest losses

The postharvest losses of agricultural commodities may be due to various factors that affects the quality of produce.

2.1 Primary causes

Agricultural commodities like food grains are when grown and transported to consumers are subjected to various agricultural operations like harvesting, threshing, milling, packing, storage etc. The large number of losses may occur when harvesting is not done at adequate moisture content and time of harvesting. Delayed harvesting or too early harvesting may subject the crop severe losses by various factors like attack of birds, rodents, microbes, natural calamities etc [4, 5]. Threshing and cleaning are usually done to separate grains from panicles. However, threshing losses can occur in case of seed splitting, incomplete separation of seed from source, seed cracking due to excessive force [4, 6] . Delayed threshing result in loss of quality and quantity of commodities. In order to prolong the storage of food grains, it is necessary to store the commodities at safer moisture content. Improper drying result in microbe growth in grains and is not desirable for storage and grinding operations. Thus, drying is an important postharvest technology in order to improve quality, prevention from insects and rodents and for transportation [7, 8]. Lack of proper transportation facilities may result in loss of commodities to greater extent. However, the problem of loss due to transportation are relatively less in developed countries due to proper roads, infrastructures and processing equipment.

2.2 Biological cause

i. **Respiration**- Respiration is a physiochemical process in which stored organic materials like carbohydrate, lipids, fats are catabolized into simple compounds in order to liberate energy essential for metabolic processes. Respiration is an important phenomenon which influences the physiological and biochemical activities of horticultural produces like fruits and vegetables. In other words, the deterioration of horticultural products is directly related to respiration rate. The respiration rate of horticultural produce can be estimated in terms of O_2 consumed or CO_2 evolved in various processes like development, maturation, ripening etc.

ii. **Transpiration**- Transpiration is the physiological process which involves loss of water in the form of vapor from living tissues of the plant. The extreme loss of water from harvested produce is the major cause of deterioration which compromises its quality, nutrition, palatability and demand among consumers. Further, the transpiration loss can be ameliorated in storage conditions by:

- Reducing air movement
- Lowering air temperature
- Raising relative humidity
- Use of protective cover like waxing and protective methods like modified atmospheric packaging, polyethylene films.

iii. **Microbes**- Stored agricultural produce are often subjected to postharvest diseases caused by bacteria, mould, fungi and incidence of insect pest and rodents. The common pathogen that infects the postharvest produce includes *Penicillum sp.*, *Botrytis sp., Fusarium sp., Phytophthora infestans etc.* Mechanical damages and bruising during harvesting and other agricultural operations are common point of entry of pathogenic microorganisms which affect the quality and quantity of produce adversely and reduces marketability as well.

iv. **Ethylene**- Ethylene is a gaseous hormone which plays an active and important role in postharvest technology of agricultural produce. It is a Ripening hormone which controls ripening process in fruits and vegetables. However, it has also some undesirable effects on fruits like premature ripening, skin damage etc.

2.3 Environmental causes

a. **Temperature**- Temperature is one of the most crucial environment factor that affect the postharvest life of stored product. Generally, for every 10^0C increase in temperature, the rate of deterioration of produce increases to 2-3 folds [9]. High temperature increases transpiration rate, thus increasing water loss whereas low temperature favours microbe development. Undesirable temperature in storage conditions may cause chilling and freezing injury, heat injury which drastically affect the quality of postharvest produce.

b. **Relative humidity**- Freshly harvested fruits and vegetable possess 80-95% water by weight. The loss of humidity from horticultural produce is purely dependent on vapor pressure deficit between the surrounding air. The relative humidity is highly influenced by transpiration and respiration processes. In the meantime, where high relative humidity reduces the chances of water loss from produce it also harbors the pathogenic postharvest microorganisms as well.

c. **Atmospheric condition**- The composition of gaseous mixture mainly oxygen and carbondioxide plays an important role in controlling the quality of post harvested produce as it controls respiration, temperature, ethylene concentration etc. Therefore, it is necessary to regulate the gaseous composition around produce in order to reduce respiration and enhance shelf-life of produce [10]. Meanwhile the reduction of oxygen and increase of carbon dioxide in storage condition may reduce deterioration of postharvest produce. However, change in gaseous composition in storage chamber can also cause physiological disorder in produce. Eg-Hollow heart in potato can occur due to faulty oxygen balance and during transportation. The unbalanced gaseous composition may also cause other calamities likeirregular fruit ripening, soft texture, poor skin color development etc.

d. **Light**- Light exposure may also cause some physiological change in produce along with alteration in biological process. Eg: When potatoes are exposed to light, it forms green tubers due to formation of solanin and chlorophyll, which is toxic for human consumption (**Figure 2**).

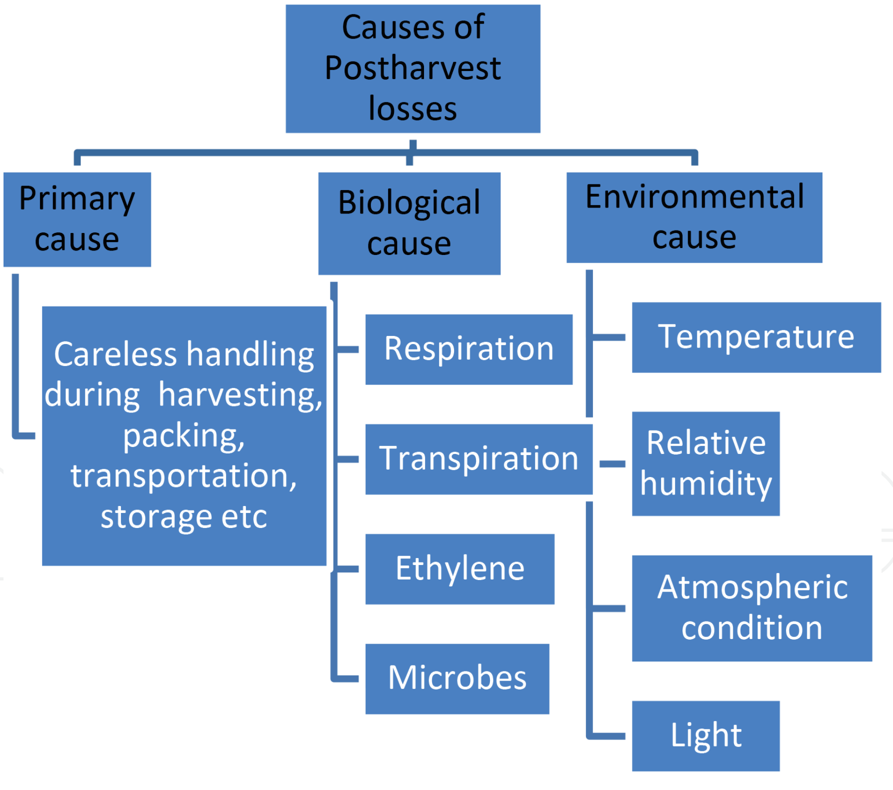

Figure 2.
Factors affecting postharvest losses.

3. Why post harvest technology?

The food processing industry in India encompasses wide variety of products including fruits and vegetables, milk and poultry, meat and its products, alcoholic beverages, fisheries, grain processing and confectionaries like chocolates, cocoa products, soya based products, high protein products and many more. The agricultural and food products when gets detached from plant or being harvested are prone to various physiological, biochemical and biological events that results in its degradation of loss of commodity. Thus, it is necessary to adopt and practice some measures in order to reduce the loss of food commodities. Several measures can be taken in order to minimize the loss which includes reduction in moisture content, control of microorganisms, denaturation of endogenous enzymes and ensuring proper packaging of food materials. Postharvest technology plays a vital role in flourishing the food industry by minimizing losses and spoilage and value addition of commodities. It ensures the use of optimum harvest factors, utilizes modern machinery to ensure reduced losses due to handling, packaging, storage and transportation as well. It mainly aims to meet the food requirement for growing population by minimizing losses and enhancing nutritive value of food products. Postharvest technology is well established in large as well as small scale industries. The storage of food products can be prolonged by adapting several technologies like use of thermal processing, drying, low temperature, chemical and biological reactions couples with several preservation technologies etc. Postharvest technology opens new opportunities for marketing of food products as processed food commodities are gaining popularity among consumers and it also generate new employment opportunities among individuals. The fundamental objective underlying in postharvest technology sector is to maintain quality of produce after harvest in terms of texture, appearance, flavor etc, to ensure proper safety of food and to reduce loss of food and agricultural commodities from harvest to consumption.

4. Role of ethylene in postharvest physiology

Ethylene is a gaseous hormone which can be produced by almost all parts of higher plants. It is a colourless, odourless gas with solubility in water 20 mg/lit at 20^0C and 250 mh/lit at 0^0C [11]. The meristematic and nodal regions of plants play active part in ethylene biosynthesis. However, the production of ethylene gets accelerated during leaf abscission, senescence and ripening processes. Wounding in plant and physiological stresses like chilling, drought, and diseases also enhances ethylene production in plants [11]. The biosynthesis of ethylene initially begins with amino acid methionine (Met) which gets converted to S-Adenosyl methionine (SAM) in presence of enzyme Adomet synthase. The enzyme ACC synthase converts SAM to 1-Aminocyclopropane-1-carboxylic acid (ACC) which further gets converted ethylene by enzyme ACC oxidase which can be described as below (**Figure 3**).

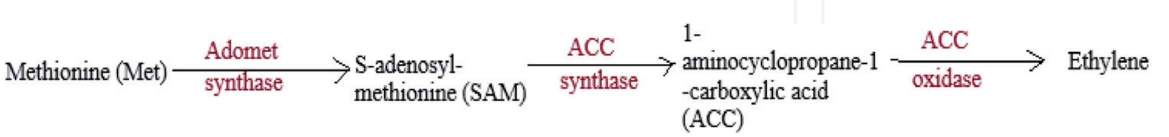

Figure 3.
Biosynthesis of ethylene.

Ethylene is considered as multifunctional phytohormone that controls both growth and senescence in plants [12]. It regulates the development of leaves, flowers and fruits and also promotes senescence depending upon the level of ethylene applied to plants [13–15]. It is known to regulate wide range of responses in plants namely viz., seed germination, cell expansion, cell differentiation, flowering, abscission, senescence etc. Some important physiological role of ethylene is described below (**Figure 4**).

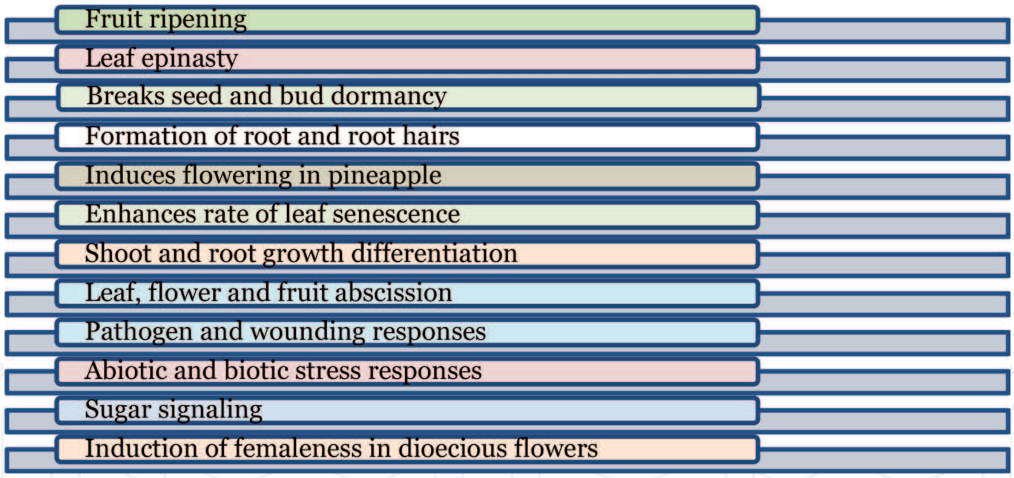

Figure 4.
Physiological role of ethylene in plants.

4.1 Ethylene as a fruit ripening hormone

The term fruit ripening involves changes in texture of fruit including softening due to enzymatic breakdown of cell wall, starch hydrolysis, accumulation of sugar and absence of phenolic compounds in fruits that makes the same ready to eat. Ethylene has been identified as ripening hormone since past long years and increase in concentration of ethylene in such fruits accelerates ripening phenomenon. The fruits can be categorized into two major classes on the basis of ethylene production i.e., climacteric and non-climacteric. Climacteric fruits are those which shows sudden characteristics respiratory rise when ripen in response to ethylene. As much treatment with ethylene causes fruit to produce additional amount of ethylene, referred to as autocatalytic. On the other hand, fruits which do not shows the rise in respiration rate upon treatment with ethylene called as non-climacteric fruits.

Climacteric	Non-climacteric
Fruits Apple, Pear, Plum, Peach, Mango, Banana, Avocado, Papaya, Guava, kiwifruit, apricot, sapota, passion fruit, persimmon	**Fruits** Cherry, Pineapple, Citrus fruits, Strawberry, Dates, pomegranate, grapes, Blackberry
Vegetables Tomato, muskmelon	**Vegetables** Brinjal, cucumbe, okra, watermelon, pea, leafy vegetables , bell pepper, summer squash

The respiratory climacteric and ethylene production are two most important and decisive parameters in ripening process. Change in ripening patterns determines the fact that whether fruits has ripen naturally or artificially upon ethylene exposure. Although artificial ripening of climacteric fruits enhances ripening process but also results in spoilage of products as well deteriorating market quality and demand [16]. The elevation in rates of respiration enhances fruit ripening and diminishes the postharvest life of both climacteric as well as non-climacteric fruits. In climacteric fruits, ethylene accelerates the time without modifying magnitude in order to achieve maximum respiration rates while in non-climacteric, since it lacks autocatalytic activities once ethylene is removed the respiration process slows down and respiration rates progresses in concentration dependent manner [17]. Furthermore, it is matter of fact that climacteric respiration does not always associated with increased ethylene responses instead depends on fruit species. Although the biochemical responses on the same are not fully understood yet but the several physiological and molecular studies enlighten that ethylene is a primary factor which is responsible for increased respiration rates and it can be thus concluded that climacteric respiration is an ethylene regulated event [18]. Ethylene on the other hand, does not primarily involved in ripening phenomenon in non-climacteric fruits. But it is noteworthy that despite of lack of climacteric ethylene, the ripening responses of non-climacteric fruits are responsive to ethylene. Thus, ethylene is a crucial regulator of ripening process in climacteric fruits and also plays an important role in regulating the same in non-climacteric fruits as well. The presence of ethylene is not always desirable in entertaining shelf life of post-harvest products. However, the extent of damage depends on ethylene concentration, length of exposure time and temperature of the product. Therefore, in order to achieve maximum postharvest benefits of produce the controlled use of ethylene is necessary in order to prevent damage and excessive ripening. Exposure of postharvest products to ethylene accelerates the rate of ageing and senescence (**Table 1**).

Ethylene biosynthesis in fruits occurs through Yang cycle. The biosynthesis of ethylene is governed by several multi genic families of ACS and ACO enzymes. The expression of ACS and ACO genes are controlled by several environmental & hormonal factors and it consists of positive and negative feedback regulation. As mentioned, fruits are divided into climacteric and non-climacteric on the basis of ripening phenomenon. In climacteric fruits like tomato normal fruit ripening involves ethylene burst. In climacteric plants, two systems of ethylene regulation are identified namely system I and system II. System I is ethylene auto-inhibitory, operates during vegetative growth and stimulates the synthesis of basal ethylene levels, detected in all tissues including non-climacteric fruits. System II remains functional during ripening of climacteric fruit and senescence phenomenon when ethylene synthesis is autocatalytic [19]. Tomato is a model plant to study ethylene biosynthesis and its perception in plants. Several ACS genes are also identified in other plants including apple, melon, pear, banana, citrus, papaya etc. In tomato, ripening results in change in fruit color from green to red, degradation of chlorophyll and accumulation of carotenoids. In tomato, 8 genes have been identified which includes LeACS1A, LeACS1B, LeACS2-7. LeACS2 and LeACS4 are greatly expressed during ripening process whereas LeACS1A and LeACS6 are expressed before onset of ripening. Further studies on mutants revealed that only LeACS6 is ethylene regulated while rest is unaffected. The genes LeACS1A and LeACS6 plays major part in ethylene production in SystemI whereas transition phase includes enhanced

Produce	Effect of ethylene on postharvest quality
Brinjal	Increase in decay process, abscission of calyx
Cucumber	Yellowing, Softening
Carrot	Development of bitter flavor
Cabbage	Yellowing, leaf abscission
Leafy vegetables	Chlorosis
Lettuce	Russett spotting
Potato	Sprouting
Watermelon	Off flavor, reduced firmness
Brocolli	Yellowing, abscission of florets

Source: Dhall, 2013 [11].

Table 1.
Effect of ethylene on postharvest quality.

expression of LeACS1A and LeACS6 is also induced. The positive feedback regulation of gene LeACS2 maintains System II phase which gets started during transition phase. Similarly, in banana MaACS1 gene is related to ripening phenomenon as its transcript and ACC content enhances during ripening. In *Actinidia chinensis*, the levels of ACS mRNA is upregulated during climacteric ethylene production but ACS itself does not gets affected by exogenous ethylene [20]. Although ACS genes are transcriptionally regulated but post-translation regulation has also been reported. ACC oxidase (ACO) is another crucial enzyme which plays important role in ethylene biosynthesis. The level of ACO is enhanced in pre climacteric fruit before rise in ACS enzyme activity. ACO gene transcripts have been studied in various fruits like tomato, kiwi, pear, apple, banana etc. which regulated temporal and spatial expression. In tomato, so far 3 ACO genes have been identified namely LeACO1 (expressed in ripening fruit), LeACO2 (anther), LeACO3 mRNA (floral organs) with weak expression in fruit at breaker stage. In banana, MaACO1 mRNA is up regulated with onset of ripening but decreases during late ripening stage. In melon, Cm-ACO1 gene is highly expressed during ethylene production but Cm-ACO3 is induced in flowers only. In *A. chinensis*, exposure to ethylene induces up regulation of genes for ACO and Adometsynthetase enzyme and also before respiratory climacteric rise in ethylene biosynthesis.

4.2 Ethylene signaling in fruit ripening

It is well established fact that apart from several crucial role in plants ethylene also induces triple response in several species like Arabidopsis, pea and other plants. Triple response includes inhibition of hypocotyl elongation, initiation of swelling (radial elongation) & inhibition of root elongation and appearance of prominent apical hook. The pathway of ethylene signal transduction has been well established and extensively studied since long time and a number of genes have been identified. In Arabidopsis, the ethylene-resistant1 (ETR1) was first isolated ethylene receptor. Further, 5ethylene receptorsETR1, ETR2, ERS1, ERS 2 and EIN4 has been discovered. These ethylene receptors consist of 2 subfamilies with N-terminal transmembrane ethylene binding domain and C-terminal histidine kinase domain according to amino acid sequence. ETR2, ERS2 and EIN4 are subfamilies of ethylene receptors with extra N- terminal transmembrane domain. ERS1 has only histidine kinase activity whereas ERS1 has both histidine kinase and serine threonine kinase activity. Subfamily1 has major role in ethylene signal transduction as CTR1 (Constitutive triple response1) strongly interact with subfamily1 along with ETR1 & ERS1 as compared to subfamily2 [21, 22]. In tomato researchers have identified 6 ethylene receptors genes [LeETR1, LeETR2,LeETR3 (NR, never ripe), LeETR4-6] in which LeETR1-3 consists of type1 receptors while LeETR4-6 type2 receptor. Gene LeETR1 and LeETR2 are expressed constitutively during developmental phases, LeETR5 in fruits and flowers during biotic stress conditions, NR& LeETR4 in reproductive phase, fruit ripening and senescence. However, ethylene sensitivity can be affected by repression all above genes except LeETR4. Arabidopsis ETR1 forms dimer in endoplasmic reticulum where it requires copper as cofactor for ethylene binding. Responsive-to- antagonist1 (RAN1) acts as copper transporter and its mutation forms inactive receptors lacking copper. CTR1 is a negative regulator of ethylene signaling and shows homology with Raf family of mitogen activated protein kinase kinase kinase (MAPKKK). LeCTR1-4 are homologs of CTR1 identified in tomato where LeCTR1 functionally complement CTR1 in Arabidopsis. Mutation in EIN2 which lies downstream ethylene responses in Arabidopsis plant when ethylene binds to ligand, it trigger activities of several genes via EIN3 &ERF1 family of transcription factors. Primary ethylene response elements (PEREs) stimulate ethylene promoting genes and modulates ripening and senescence related genes as well. However, homodimer of EIN3 binds to PERE which in turn bind to GCC-box present in promoters of stress responsive gene, enabling downstream process. Four EIN3 (LeEIL1-4) genes are identified in tomato in which LeEIL3 functionally complement EIN3 mutation in Arabidopsis. In absence of ethylene, activated form of CTR1 binds to ethylene receptor &retards the downstream ethylene signal transduction process thus, suppressing ethylene stimulated gene expression. When ethylene binds to receptor, conformation change in receptor begins which enables dissociation of CTR1 and CTR deactivation, releasing downstream pathway from suppression. EIN2 protein gets activated to stimulate downstream signal transduction and perception [23].

5. Management of postharvest losses

Proper handling of food commodities and minimizing postharvest losses in fruits and vegetables are crucial means for maintain quality of produce, ensuring food security and combating poverty and hunger. The main cause of postharvest loss in developing countries occurs due to lack of infrastructure and agricultural operations while in developed countries it occurs at consumer stage. Postharvest management ensures quality management, increases market share and market value of commodities, ensures proper agricultural production and minimizes postharvest losses of fruits, vegetables, cereals, pulses, oilseeds and many other agricultural commodities. It also safeguards the nutritional quality and health benefits to consumers. Postharvest management of horticultural produces involves them to keep fresh and alive even after harvest to meet consumer demands by keeping a proper care on O_2 and CO_2 concentration. Postharvest handling of harvested produce plays a vital role in maintain quality of produce and managing losses (**Figure 5**).

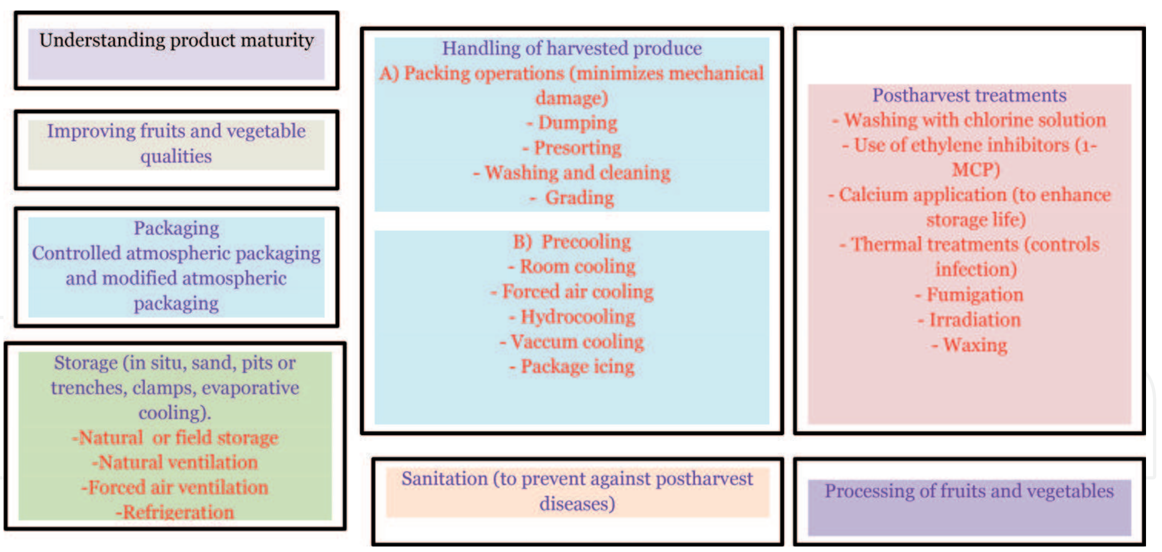

Figure 5.
Postharvest management methods.

5.1 Transgenic approaches in postharvest technology

Transgenic or genetically modified crops (GMO) are defined as those species whose DNA has been modified using genetic engineering techniques. Gene of interest is usually identified and taken from another and gets artificially inserted into desired crop species in order to develop genetically modified crop or transgenic crops. The aim is to develop a plant with traits that does not occur naturally in another plant species. The gene of interest which gets inserted in referred as transgene and it may be part of either unrelated plant or completely different plant species. The purpose of developing genetically modified crops is to create desirable and productive product [24]. Transgenic approaches have been commercially used in many plant species like tomato, corn, tobacco, potato, soybean, canola, banana, alfalfa, rice, squash, melon, papaya. Statistics reveals that about 18 million farmers are cultivating GMO crops with total area of 181.5 million hectares in 28 countries in 2014 [25] which has increased to 185.1 million hectares in 2016 and to 191.7 million hectares in 2018 globally. Transgenic crops possess several traits like higher yield, improving shelf life of commodities, quality improvement, resistance to insect-pest, tolerance to abiotic stresses like cold, drought, heat etc. They also possess industrial and pharmaceutical importance (**Figure 6**).

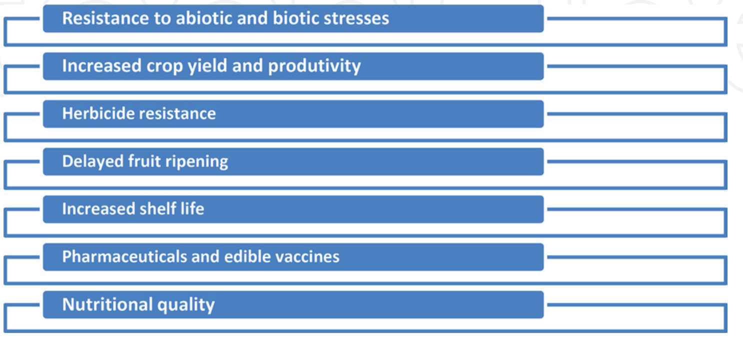

Figure 6.
Applications of transgenic in agriculture.

Transgenic plants are usually developed through genetic engineering by altering genetic makeup, adding one or more beneficial genes or removing detrimental genes in plant genome. The detail method for developing transgenic is described as (**Figure 7**).

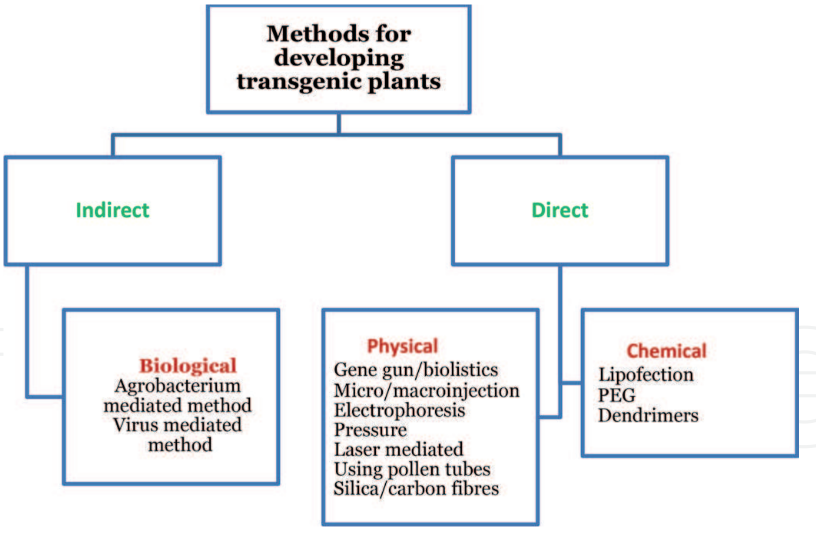

Figure 7.
Methods of developing transgenic crops.

Steps involved in developing transgenic plants.

1. Identification, isolation and cloning of genes of desired traits- It is the initial step of development of transgenic plants. Usually the gene of interest is identified, isolated and cloned in order to transfer to desired plant genome.

2. Design the gene construct for insertion- The gene construct or gene set must be designed having all possible DNA segment in order to integrate and express into plant genome. The gene construct must have following segments-

a. The promoter sequence- The promoter sequence is required for accurate expression of gene as it is on/off switch that controls the when and where the gene will be expressed. Promoter sequence is recognized by transcription factors in DNA transcription process. It is generally located at 5' upstream of gene. Promoters are usually classified as constitutive, tissue specific and inducible in nature. Constitutive promoters express themselves at almost all developmental stages and participate in moderate and constant gene expression. Tissue- specific promoters remains active facilitated gene expression during development- specific stage while inducible promoters are highly influence by environmental stimuli and facilitated gene expression in response to certain external factors Eg: CaMV35s promoter, NtHSP3A etc.

b. The transgene- It is generally modified to achieve greater expression in plant.

c. Termination sequence- It marks the end of gene sequence

d. The selectable marker gene- It is essential in order to identify the particular plant cells that have integrated transgene successfully. Common examples of selectable marker gene are nptII, hpt, acc3, bar, pat etc.

3. Transforming target plant with gene construct- Genetic transformation is the process of identifying the desired specific gene for a particular trait and isolation of the traits from various plant species. The target plant can be transformed by vector mediated gene transfer (indirect) or vector less gene transfer (direct).

4. Selection of transgenic plants- Plant tissues or cells are transferred to selective medium containing antibiotic or other components.

5. Regenerating transgenic plants- Plants are transferred into controlled environmental conditions in order to obtain whole plants of transgenic traits (**Figure 8**).

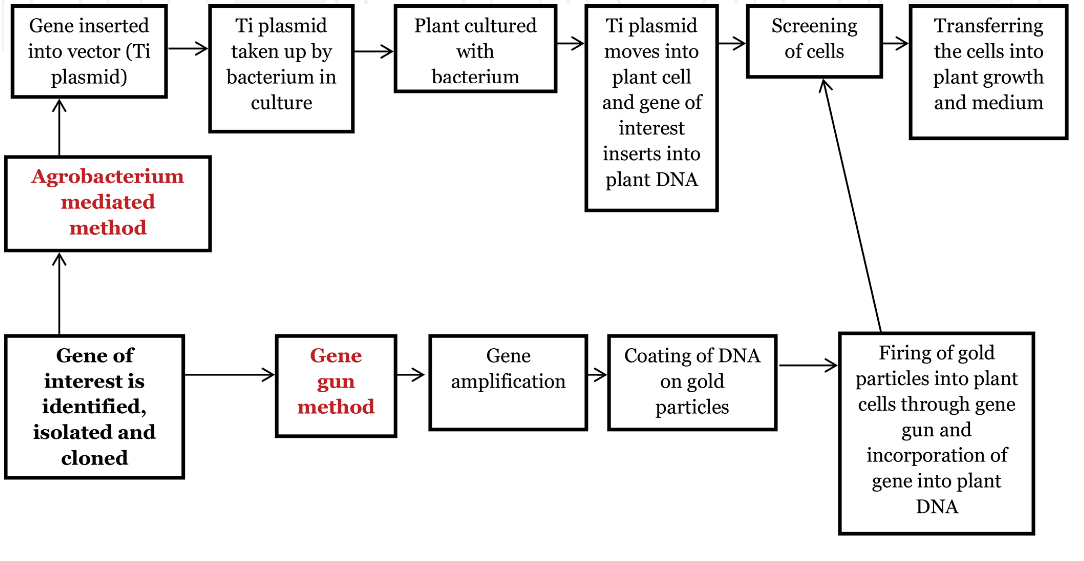

Figure 8.
Steps for developing transgenic plant.

Biotechnology and genetic transformation technology is an emerging technology and possess great impact on agricultural production and industry to meet the increasing world demand for food grains and agricultural commodities. The commonly used methods for reducing postharvest losses and increasing shelf life like early harvesting, modulating storage atmosphere, selection of late ripening genotypes are sometimes uneven and not satisfactory for most of the commodities and sometimes can cause loss of flavor, aroma and metabolites. Therefore, transgenic technologies are essential for production of improved crop varieties, enhancing plant tolerance to several biotic and abiotic stresses, securing nutritional status of crop species and many more. Transgenic plants are most promising means to improve crop productivity and production of metabolites and plant products as compared to conventional approaches. Postharvest deterioration of agricultural commodities on the other hand is the matter of great concern globally. As discussed, the main reasons behind this reduced shelf life of food products, rapid spoilage and softening of fruits and vegetables, low temperature, pathogen infection etc. They not only decrease quality of products but also affect their availability adversely. Biotechnological methods for enhancing postharvest factors of food products mainly aims at following perspective i.e., resistance to biotic and abiotic stresses, genetic transformation, secondary metabolites, simultaneous ripening for proper harvesting, improvement in shelf life and organoleptic taste and enhancing nutritional quality [26]. Also, biotechnological approaches are used to create new crop varieties which can acclimate in existing climate change, tolerant to postharvest diseases and acquires extended shelf life. It is well acquainted that the physiological, biochemical and molecular mechanism of plants are directly related to postharvest attributes of fruits and vegetables i.e. storage and shelf life of products. Hence, these traits can be genetically examined and manipulated through molecular and transgenic approaches [27]. Ripening is considered as destructive stage in development of fruits and vegetables and most essential aspect in postharvest study. Ripening process is extremely modulated by various environmental condition and changes in hormone levels but ethylene is a primary hormone that regulate ripening and senescence phenomenon. However, non-climacteric fruits are independent of ethylene production unlike climacteric fruits. Researchers therefore, attempt to manipulate the influence of ethylene on ripening of produce by suppressing it at different growth stages in order to increase storage and shelf life. Ripening in tomato can be delayed by silencing ethylene biosynthetic genes, manipulating ethylene signaling and transduction, discouraging ABA biosynthetic genes etc. TILLING approach is widely used to enhance shelf life of produce.

6. Genome editing technology

Genetic engineering plays a magnificent role in creation of crops with desired qualities, crop improvement and identification of gene functions. Genome editing technology has been emerged as a new and powerful approach over past few decades which involves the precise editing of gene of interest and utilizes nucleases that target the specific sequence to generate double strand break. The application certain genome editing technologies viz., sequence-specific nucleases (SSN) like ZNFs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases), CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system is a promising and advance technologies that increases crop yield and also confers tolerance to various stress conditions. TALENs has been successfully employed for genetic modification in tomato [28] and potato [29, 30] crops. CRISPR/Cas9 technology has been successfully used as genome editing tool in various vegetables and fruits. In tomato, it has been used with the purpose to increase shelf life and manipulates ripening using RIN, SLALC, lncRNA1459 genes [31–33] increased resistance to powdery mildew by involving SlMlo1 gene [34] (**Table 2**) (**Figure 9**).

Genome editing technique	Species	Gene edited	Physiological responses	References
TALEN	Tomato	PROCERA (PRO)	GA metabolism	Lor *et al*., 2014 [28]
TALEN	Tomato	LEAFY COTYLEDON1-LIKE4	Pleiotropic response	Hilioti *et al*., 2016 [35]
Crispr/Cas9	Tomato	RIPENING INHIBITOR(RIN)	Ripening response	Ito *et al*., 2015, 2017 [33, 36]
Crispr/Cas9	Kiwifruit	AcPDS	Carotenoid synthesis	Wang *et al.*, 2018 [37]
Crispr/Cas9	Apple	PDS	Carotenoid synthesis	Nishitani *et al.,* 2016 [38]
Crispr/Cas9	Apple	DIPM1,2 &4	Pathogen resistance	Malnoy *et al*., 2016 [39]
Crispr/Cas9	Cucumber	eIFGE	Virus resistance	Chandrasekaran *et al*., 2016 [40]

Table 2.
Genome editing techniques and their role in various plant species.

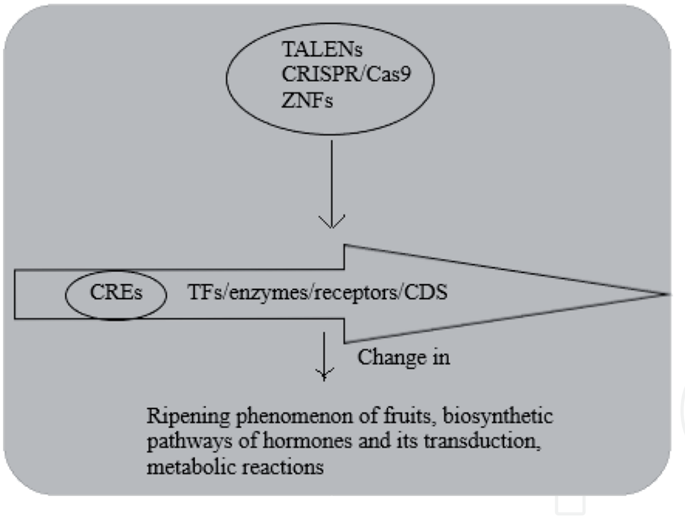

Figure 9.
Genome editing technology for postharvest factors (Source: Pizzaro et al., 2018.) [41].

6.1 RNA interference

RNA interference is a promising gene regulatory approach in which the molecules of RNA inhibit gene expression or translation process by neutralizing target mRNA molecules. It is also known as post- translational gene silencing (PTGS), quelling. It permits down regulation of gene expression more precisely without affecting the function of other genes. RNAi has been widely utilized nowadays in enhancing nutritional value of quality, increasing shelf life or perishables, imparting insect-pest, virus and pathogen resistance and abiotic stress tolerance. The fruits and vegetables are highly susceptible to postharvest losses which deteriorate their quality and economic value.

The storage life of such produces can be enhanced by RNA interference approach. Transgenic tomatoes containing ACC oxidase dsRNA showed delayed ripening and increased shelf life due to suppression of genes for ethylene synthesis. RNAialso is involved in the enhancing of nutritional quality of food products. In tomato, SINCED1 gene which is important for ABA biosynthesis is suppressed by RNAi and those fruit showed enhanced accumulation of lycopene and beta-carotene [42]. RNAi technology is widely used in Brassica napus to increase the carotenoid content by downregulating the lycopene epsilon cyclase gene (Ɛ-CYC) and possess increased content of β-carotene, violaxanthin and lutein [43]. Jiang et *al.*, 2013 [44] reported that RNAi can be successfully utilized in soybean to increase isoflavone content by silencing flavanone 3-hydroxylase (F3H) gene and the flavone synthase II (GmFNSII) gene, thus regulating flavone and isoflavone production in hairy roots. RNA interference is also applied to produce virus resistant plants. Transgenic tomato produces dsRNA which showed resistance to potato spindle tuber viroid [45]. Transgenic tobacco which expresses the coat protein (CP) gene from Tobacco mosaic virus showed resistance against the same and this technology is used for other crops also like potato against Potato virus Y [46], *Prunus domestica* resistant to *Plum pox virus.* The RNAi has been successfully implemented in common bean to induce resistance against Gemini virus *Bean golden mosaic virus* [47]. Ethylene is a major fruit ripening hormone in postharvest physiology. One of the major factor which is used to extend shelf life of harvested produce is delay in ripening process by downregulation of ethylene action. This can be achieved through antisense RNA technology. In this process, the protein coding region of a gene is inverted in reference to its promoter and thus antisense gene is constructed. The mRNA formed has similar sequence as of antisense gene of natural DNA. Endogenous and antisense RNA present on the same nucleus, their transcription results into antisense and sense RNA transcript. They are complementary to each other and forms RNA molecules having double stranded nature. The duplex thus formed inactivated mRNA and inhibits protein synthesis. Antisense RNA technology suppresses ACC oxidase and ACC synthase genes and plants possessing such techniques are resistant to over ripening and postharvest losses (**Figure 10**).

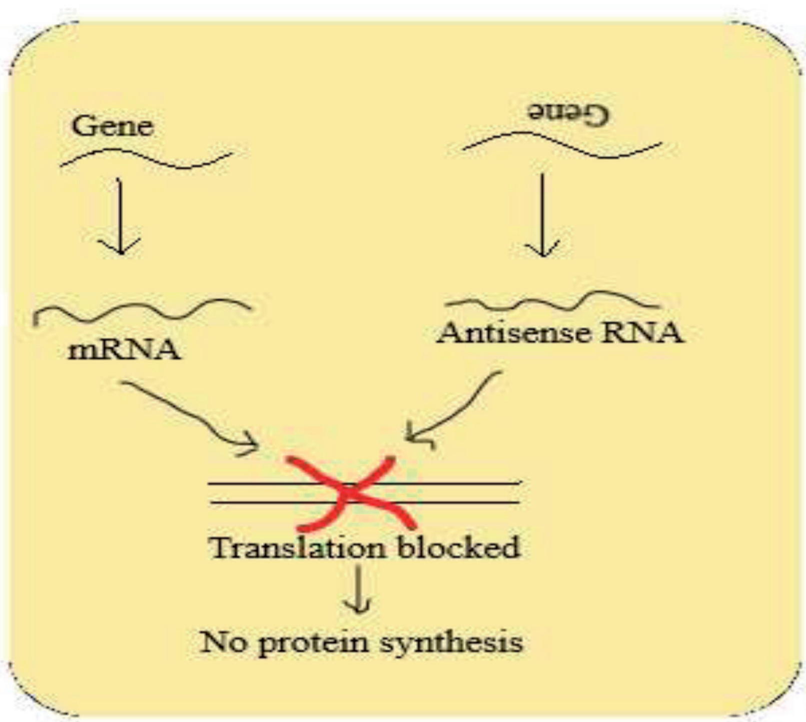

Figure 10.
Antisense RNA technology in post harvest physiology (Source: Gupta et al.*, 2011) [48].*

6.2 Breeding methods

Fruits and vegetables along with other agricultural commodities are perishable in nature and they have limited shelf life after harvest. Maintaining freshness and shelf life of produce is great challenge for researchers globally in order to achieve maximum economic value. Modern methodologies like breeding approaches play a vital role in minimizing postharvest losses and management. They include domestication, polyploidy breeding, mutation breeding, selection, hybridization etc. Domestication can be defined as bringing the wild plant species under human cultivation. In includes a series of genetic practices that involves storage and shelf life of species as compared to wild counterparts. Introduction of plant species includes widening of genetic base and many species have been bought through introduction includes Kinnow mandarin, Solo papaya, Jonathan apple etc. Mutations either induced or spontaneous are effective way to extend shelf life and reduce postharvest losses. Mutation has been induced in pear in order to increase shelf life of produce. Polyploids on the other hand also possess greater shelf life and polyploidy is an efficient approach to induce desired traits in plants. Sunny Rogue, a variety of grape possess tolerance to pre and postharvest disease resistance and better shelf life in storage conditions. Hybridization is also an efficient technique to minimize postharvest losses. It not only extend shelf life of fruits but also enhances nutritional quality. Several fruits and vegetables have been developed using this technique which includes mango, banana, papaya, onion, tomato etc.

6.3 Proteomics

Proteins plays an important role in various plant processes including development, growth, ripening and senescence, metabolic functions, resistance to abiotic and biotic stresses. Proteomics and omics approaches has been studied over past few decades and opens a new avenue in understanding ripening and senescence, development, postharvest responses of perishable commodities in many crops like apple, banana, citrus, grapes, strawberry, tomato, peach, papaya, mango etc. They also are crucial in understanding plant mechanisms during pathogen infection as well. Study on proteomics reveals that proteins involved in defense mechanism, energy metabolism and antioxidant system are crucial for food produce in stored condition and elicitor responses [49]. The study of proteomics in postharvest science involves 2-D electrophoresis (classical and/or differential electrophoresis, DIGE) for separating and quantifying proteins based on LC-MS/MS technique either by cross species identification or by species-specific database search. Several postharvest studies and researches also include gel based approach for quantification and identification of proteins. Furthermore, the proteomic research in fruits and vegetables are needed to be extended to other quantitative proteomic approaches like iTRAQ, TMT, peptides demethylation labeling, ICAT, MRM, label-free strategies or the integration of above mentioned approaches with LC–MS/MS. Abdi *et al.,* 2002 [50] studied the postharvest proteomics based on 2D- PAGE related to optimal harvest of peaches. Postharvest proteomics are essential for minimizing postharvest losses by selection of desired quality of cultivars with desired quality of traits. Selection of traits like low susceptibility to bruising and chilling injury to post harvest food products are decisive factors for modulating shelf life of processed products. The combination of transcriptomics, metabolomics and proteomics has been used to study the quality of citrus in storage. Chilling injury is detrimental to produce in storage as it not only affects degradation of proteins, polysaccharides but also affect organic acids and causes injury to postharvest produce (**Figure 11**).

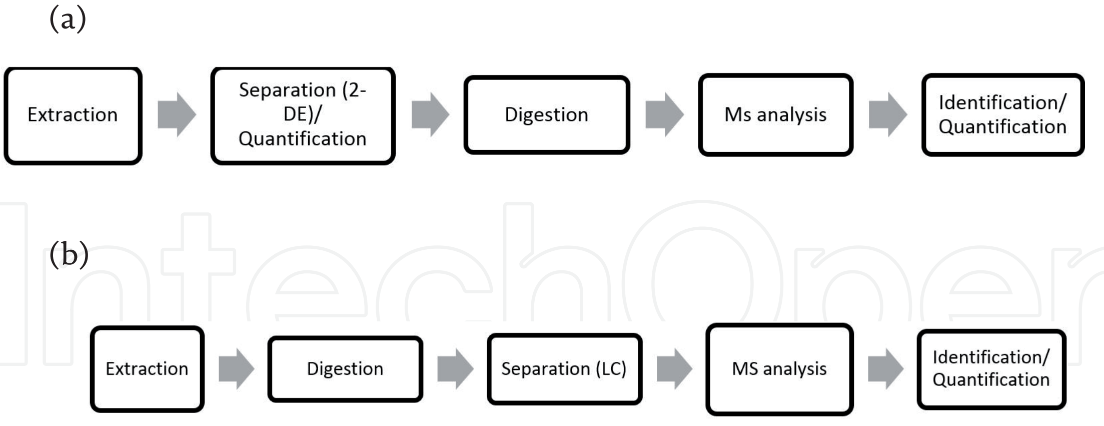

Figure 11.
(a) Gel based approach and (b) gel free approach. Source: Pedreschi et al., 2013 [51].

7. Conclusion

India stands second after China, in global production of fruits and vegetables but one–third of agricultural produce available for human consumption in the world per year gets wasted. Agricultural commodities after harvest are liable to decay thus, causing loss of quality and quantity Different factors are responsiple for causing post harvest losses. Ethylene as a gaseous hormone, plays an important physiological role in fruit ripening as well as accelerates the rate of aging and senescence. It also acts as a signaling molecule and controls ripening process in plants. Different transgenic approaches and breeding methods are developed in order to prevent Post harvest losses. Therefore, the significance of post harvest physiology is utmost important in the field of agriculture in developing Genetically modified crops.

Author details

Binny Sharma[1] and Asha Kumari[2]*

1 Department of Plant Physiology, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

2 ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

*Address all correspondence to: asha.sasrd@gmail.com

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