



Biotechnological Approaches to Improve Nutritional Quality and Shelf Life of Fruits and Vegetables

Ernest Ekow Abano^{1,3}, John Nelson Buah²

¹Department, of Agricultural Engineering, School of Agriculture, University of Cape Coast, Cape Coast, Ghana

²Department, of Crop Science, School of Agriculture, University of Cape Coast, Cape Coast, Ghana

³Department of Food Science and Engineering, School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China

ABSTRACT

The objective of this review was to provide comprehensive overview of the application of biotechnological approaches to improve the nutritional quality and shelf life of fruits and vegetables. In doing so, the following issues were critically assessed: the origin and definitions of plant biotechnology; successful application of biotechnology in fruit and vegetables production; the dynamics of ripening and perishability in fruits and vegetables; the understanding of the fundamental processes that influence fruit set, maturation, and ripening; the effect of the biotechnological application on the nutritional quality and shelf life of fruit and vegetables; the challenges associated with the commercialization of biotech fruits and vegetables; the need for biotechnology in the production of fruits and vegetables in the 21st century, and the new paradigm shift necessary to improve nutrient quality and shelf life of biotech fruits and vegetables. The available scientific literature shows that the developed biotechnological approaches have the potential to enhance the yield, quality, and shelf-life of fruits and vegetables in the mist of the global climate change, water scarcity, population increase, and ever-increasing demand for food. To make sure that the current debates and complexities surrounding the registration, commercialization, human safety, environmental considerations such as non-target safety, gene flow, biodiversity and associated risks of biotech fruits and vegetables are adequately addressed, various stakeholders in the industry-policy makers, private sectors, agriculturalists, biotechnologists, scientists, extension agents, farmers, and the general public must be engaged in policy formulations, seed-embodiments, and products developments.

Keywords: *Biotechnology, Fruits and vegetables, Nutritional quality, Shelf-life, Ripening, Ethylene.*

1. INTRODUCTION

The importance of fruits and vegetables (F&V) in the diet of mankind cannot be over emphasized. Many reviews have reported the wide range of determinants of desirable quality attributes in fruits and vegetables such as nutritional value, flavor, colour, texture, processing qualities and shelf-life (Bapat *et al*, 2010; Vadivambal and Jayas, 2007). The understanding of the fundamental processes that influence fruit set, maturation, and ripening are required to manipulate fruits and vegetable yield and quality. Biotechnology has played a significant role in this respect. Report by Bapat *et al.*, (2010) revealed the constraints surrounding the extensive reproductive cycle in some fruits and vegetables that have long juvenile periods, the complex reproductive biology, high degree of heterozygosity, inter and intra incompatibility and sterility of breeding of fruits and vegetables plants such as tomatoes, orange etc. for improvement. Typically, biotechnology technique such as genetic modification is used in F& V to enable plants tolerate the biotic and abiotic stresses, and plant resistances to problematic pests and disease, which may provide higher nutritional contents, and extend the shelf-life of the produce. The objective of this study was to provide a critical review of the use of biotechnological approaches to improve nutritional quality and shelf life of fruits and vegetables. Particularly, a great deal of attention was given to the dynamics of ripening and perishability in fruits and

vegetables, ripening studies with tomato as a model system; the effect of the biotechnological application on the nutritional quality and shelf life of fruit and vegetables; the challenges associated with the commercialization of biotech fruits and vegetables, and the new paradigm shift necessary to improve nutrient quality and shelf life of biotech fruits and vegetables.

Biotechnology: Origin and Definitions

A review by Uche (2004) well documents the origin and various definitions of the word biotechnology. The term biotechnology is viewed today as the novel technique capable of reshaping global agriculture Buttel, (1989) even though it has been practiced by ancient farmers. Evidence support the fact that as far back as 6000BC, yeasts were used in baking and brewing, and the use of living organisms such as bacteria and molds for fermentation was indispensable in the preparation of diet by people in ancient civilization (Bud, 1991). Therefore, ancient farmers could be thought of as the first biotechnologists (SPORE, 1996). According to Uche (2004), the word biotechnology was used by a Hungarian pig farmer, Karl Ereky (1878-1952) in a 1917 article written in German, which described his industrialized pig-fattening plants during the World War I, where 50,000 pigs converted sugar-beet into meat. Ereky used this analogy with chemical technology to suggest that biotechnology covers the area of technology associated with living beings (Raghava, 2002). The broader definition of biotechnology refers to commercial techniques

that use living organisms to make or modify a product, including techniques for improving the characteristics traits of plants and animals, and development of microorganisms that act on the environment. In another definition, OTA (1989) described biotechnology as ‘any technique that uses living organisms, or substances from these organisms to make or modify a product, to improve plant and animals, or to develop microorganisms for specific uses’. Additionally, Persley (1992) view of traditional biotechnology covers well established and widely used technologies in brewing, food fermentation, conventional animal vaccine production, and many others based on the commercial use of living organisms. In recent times, advanced biotechnology techniques involve the use of induced mutations, marker-assisted selection, homologous recombination, genomics, and genetic modifications (Gellatly and Deniss, 2011) The major ones are

the tissue or cell culture, cell fusion, embryo transfer, recombinant DNA, and age-old fermentation technique. Table 1 presents the biotechnological approaches developed so far for crop production. Today, plant biotechnology has been defined as comprising a range of advanced methods, which lead to a variety of improvement, true reproduction, and a very large number of individual plants, which are exactly the same as the parent variety (SPORE, 1996). In the mist of recent global challenges such as increasing population, increasing demand for food, climate change, and water scarcity, plant biotechnology has become a necessity tool for growth and yield performance to meet the food needs of today. The production of quality fruits and vegetables with improved shelf-life is no exception.

Table 1: Biotechnological approach developed for crop production

Technology	Application
Meristem and bud culture	Micro propagation for commercial purpose, genetic conservation, and exchange of material.
Zygotic embryo culture	Inter-specific crosses
Anther and microspore culture	Haploid production
Cell and tissue culture	In vitro selection, somaclonal variation, somatic embryogenesis, artificial seeds
Chromosome engineering	Zn gametes for inter-specific crosses
Protoplast culture	Fusion for somatic hybridization
Genetic engineering	Gene transfer
Molecular markers (RFLPs)	Aid to breeding programmes
Monoclonal antibodies	Diagnosis of plant diseases (pathogens)
Recombinant DNAs	DNA transfer
Induced mutations	Inter-specific DNA crosslinks
Marker-assisted selection	Aid breeding programmes
Homologous recombination	DNA transfer
Genetic modification	Improve crop varieties using molecular biology and plant breeding techniques
Genomics	Cell or tissue at the DNA, mRNA or protein levels

(Source: Unche, 2004; Gellatly and Dennis, 2011)

The need for biotechnology in fruits and vegetable production

A number of challenges have called for the application of biotechnology in the production of fruits and vegetables. These are population increase, water shortages, climate change, high perishability or postharvest decays, and short shelf-life associated with fruits and vegetables. Fruits and vegetables by their intrinsic properties require more water and in the face of water scarcity throughout the world, biotechnology will be

required to develop fruits and vegetables that can withstand water stress and still be able to produce good crop of high quality and yield. For instance, during the last century, world population rose from 1.6 to 6 billion creating huge challenges for agriculture. However, new technologies increased crop yields drastically so the predicted catastrophic starvation and resulting conflicts did not occur. There are still serious challenges to be faced. World population is anticipated to rise to 10 billion by 2050. Freshwater, vital for agricultural productivity, is becoming scarce and climate change could

increase temperature, drought, and uncertainty. New crop varieties need to be developed quickly to meet these challenges and biotechnology will be needed to enhance existing technologies to achieve this. So far, biotechnology has been successfully used to develop insect and herbicide resistance in a limited number of crops such as corn. In the future, the actual metabolism of crop plants will be altered to produce new varieties or species that are tolerant against environmental stresses. In addition, the nutritional value of crops such as rice will be enhanced. Crops will also be used to harvest the sun for biofuels to replace fossil fuels and reduce the emission of CO₂. Some of these new crop plants are already in field trials and will be available to farmers in the near future (Gellatly and Dennis, 2011).

Postharvest decay of fruits and vegetables are a major challenge throughout the world. The degree of postharvest loss through decay is well documented. In the industrialized countries, it is estimated that about 20– 25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling (Sharma *et al.*, 2009; Singh and Sharma, 2007, Droby, 2006; Zhu, 2006; El-Ghaouth *et al.*, 2004). The situation is far more exasperating in the developing countries, where postharvest decays are often times over 35% , due to inadequate storage, processing and transportation facilities (Abano and Sam-Amoah, 2011). The use of synthetic fungicides such as benomyl and iprodione to control postharvest diseases of fruits and vegetables is well known in scientific literature (Zhang *et al.*, 2007; Singh and Sharma, 2007; Korsten, 2006; Zhu, 2006; El-Ghaouth *et al.*, 2004; Fan *et al.*, 2000). The health and environmental concerns associated with the continuous use of synthetic fungicides have alarmed legal enforcers and consumers to demand greener technology and quality products from the food industry as well as the scientific community. In the past 20 years, microbial antagonists like yeasts, fungi, and bacteria have been used with limited successes to reduce postharvest decays in fruits and vegetables (Sharma *et al.*, 2009; Zhang *et al.*, 2007; Droby, 2006; Korsten, 2006; Zhang *et al.*, 2005; Janisiewicz &

Korsten, 2002; Roberts, 1990;; Droby *et al.*, 1991; Wisniewski and Wilson, 1992). For instance, fungal diseases like grey mould, powdery mildew, and downy mildew in grapes do notable only cause losses in yield but also reduce wine quality (GMO Compass, 2006). However, the advances in biotechnology can be employed to develop fruits and vegetables with improved quality and shelf-life. According to Lers (2012), the ability to maintain the quality of stored F&V during postharvest storage is highly related to the physiological, biochemical, and molecular traits of the plant from which they derive. These traits are genetically determined and can be manipulated using genetic breeding and/or biotechnology. Published research results have revealed potential genes, which when manipulated can be used to improve postharvest qualities of crop plants. The application of this biotechnological knowledge should not only lead to major improvements in postharvest storage of fresh fruits and vegetables but as well improved human food supply.

The Dynamics of Ripening and Perishability in Fruits and Vegetables

Fruit ripening and softening are major attributes that contribute to perishability in both climacteric and non-climacteric fruits. Fruits and vegetables such as tomato, banana, mango, avocado etc. take about a few days after which it is considered inedible due to over-ripening. The spoilage includes excessive softening and changes in taste, aroma and skin color. This unavoidable process brings significant losses to both farmers and consumers alike. Even though ripening in F&V can be delayed through several external procedures, the physiological and biochemical changes associated with ripening is an irreversible process and once started cannot be stopped (Prasanna *et al.*, 2007; Martínez-Romero *et al.*, 2007). Ethylene has been identified as the major hormone that initiates and controls ripening in fleshy fruits and vegetables. Influencing ethylene biosynthesis during ripening in fleshy commodities has been the foremost attempt for combating post-harvest deterioration.

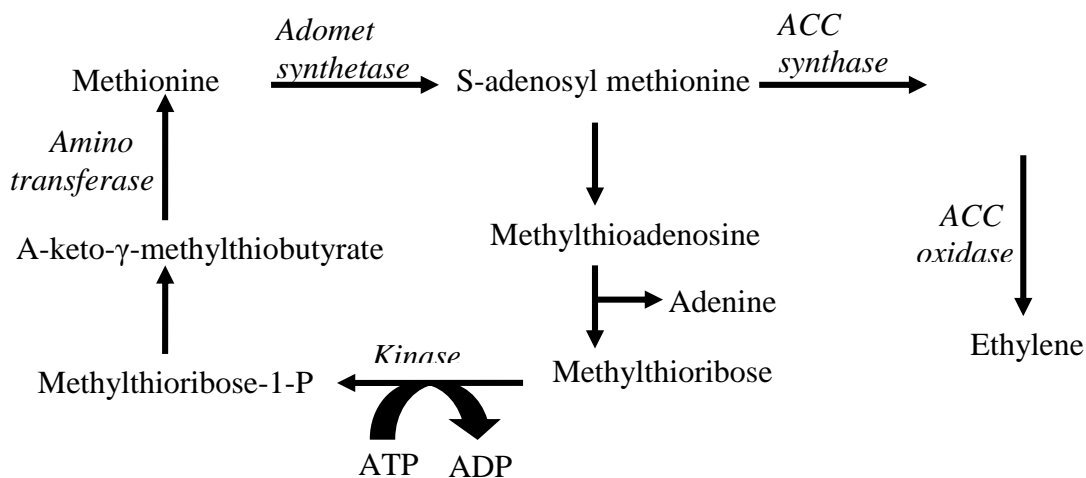


Fig. 1. Model for ethylene signal transduction

Fig. 1. Model for ethylene signal transduction depicting activities of various signal molecules during fruit ripening. ETR; Ethylene receptor, CTR; Constitutive triple response, MAPK; Mitogen activated protein kinase, MAPKK; Mitogen activated protein kinase kinase, EIN2; Ethylene insensitive 2, EIN3; Ethylene insensitive 3, EIL; Ethylene insensitive like, ERE; Ethylene-responsive element, ERF; Ethylene response factor. (Source: Bapat et al, 2010)

Figure 1 shows the ethylene biosynthesis. The perception by the target cells through receptors (ETRs), signal transduction cascade involving both positive and negative regulators (CTR, EIN2, EIN3 etc.) and finally regulation of target gene expression by transcription factors such as ethylene response factors (ERFs) is depicted in Fig. 2. The target gene or set of genes which control fruit firmness, taste, color and aroma are regulated by specific set of genes which in turn may be regulated by a single or set of transcription factors (Nath *et al.*, 2007). Two distinct ethylene biosynthesis systems have been described. The first system corresponds to low ethylene production before the ripening stage when the respiration rate is low and is present throughout the development and ripening

of non-climacteric F&V. The second system refers to an auto-stimulated increased ethylene production, called 'autocatalytic synthesis' and is specific to climacteric fruit. Therefore, Bouzayen *et al.* (2009) reported that the major distinctive characteristics of climacteric and non-climacteric fruit are the presence or absence of autocatalytic ethylene production. However, external application of ethylene during ripening to non-climacteric F&V may hasten the process in some cases. According to Bapat *et al.* (2010), most of the information on the role of ethylene in fleshy F&V ripening is based on the studies on tomatoes. Ripening mutants of tomato like Nr, rin, nor, Cnr etc., have proven very valuable in unraveling how the developmental and ethylene signal is transduced to cause ripening in F&V (Giovannoni, 2004, 2007). Genes encoding cell wall degradation, ethylene production and pigment biosynthesis enzymes were among the first ethylene-responsive genes which have been isolated from tomato fruit. As the role of ethylene in ripening of fruit is most distinctly described in climacteric or fleshy fruit, fruit mostly from this category are chosen for case studies with tomato as model fruit for understanding ripening in fleshy fruit.

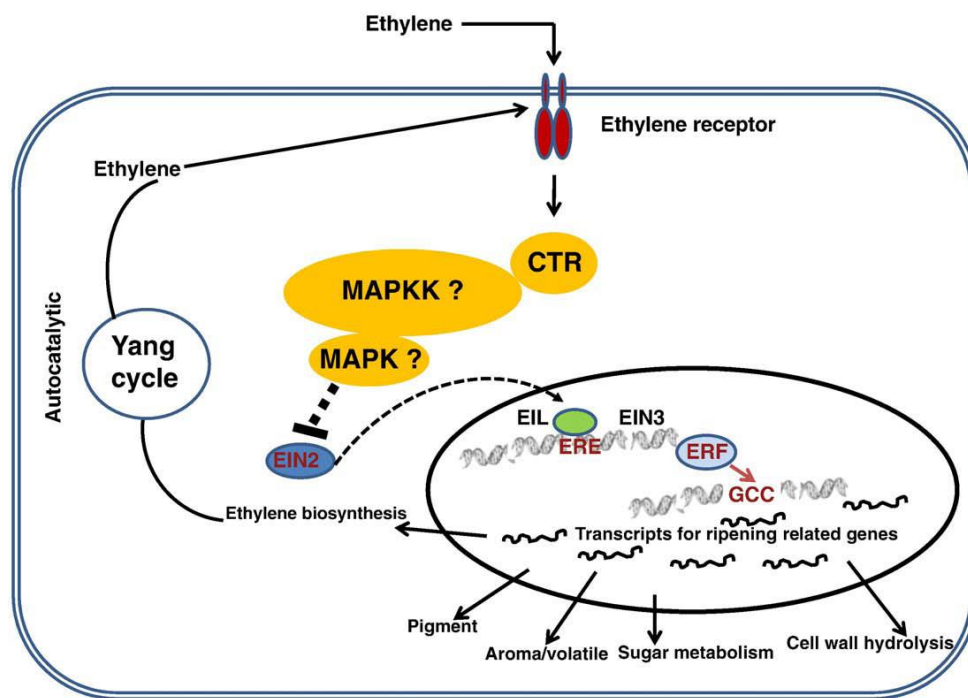


Fig 2 : Ethylene biosynthesis pathway (Source, Bapat et al, 2010)

Biotechnological approaches applied to fruits and vegetables

The transfer of genetic material from one organism into the DNA of another called transgenic application has been widely used in fruits and vegetables. Tolerant plants to biotic and abiotic stress, higher nutritional contents and extended shelf-life are some of the advantages of transgenic plants. In addition, once a useful transformant is obtained, vegetative propagation, which is the normal method of multiplying in several fruit plants, provides unlimited production of the desired transgenic lines. Recently, reports indicate that recombinant DNA technology has been used by scientists to

delay ripening in fruits and vegetables in order for farmers to have the flexibility in marketing their produce and ensure consumers good quality produce from their farms (Bapat *et al.*, 2010). Transgenic grapes were developed for modified auxin production, fungal and virus resistance as well as fruit quality and color modifications (DeFrancesco, 2008). Costantini *et al.* (2007) transformed grape cultivar Thompson Seedless with an ovule-specific auxin-synthesizing (DefH9-iaaM) gene and observed that average number of inflorescence per shoot in transgenic grape lines was doubled as compared to control.

Binnie and McManus (2009) identified three ACO genes from apple and showed that all three genes express differently. MdACO1 is restricted to fruit tissues, with optimal expression during fruit ripening, MdACO2 expression occurs more predominantly in younger fruit tissue, with some expression in young leaf tissue, while MdACO3 is expressed predominantly in young and mature leaf tissue. Recently detailed studies on anti-ACO transgenic fruits have been carried out (Johnston *et al.*, 2009). They have shown that antisense suppression of *M. domestica* ACC oxidase (MdACO1) resulted in a fruit with very low ethylene production. Exposure of these fruit to different concentrations of exogenous ethylene showed that various ripening parameters like pulp softening, biosynthesis of volatile aroma compounds, and starch degradation, had different ethylene sensitivities. Their results suggested that the conversion of starch to sugars (an early ripening event) showed a low dependency on ethylene, but a high sensitivity to low concentrations of ethylene. On the other hand, late ripening events such as pulp softening and ester volatile production showed a high dependency on ethylene but were less sensitive to low ethylene concentrations.

In a related development, Schaffer *et al.* (2007) have identified 17 candidate genes that were likely to be the control points for ethylene with respect to aroma production. However, not all components of fruit quality were under the direct control of ethylene. Similarly, two MdERFs (ethylene response factors) were isolated from ripening apple fruit by Wang *et al.* (2007). MdERF2 expressed exclusively in ripening fruit whereas MdERF1 was expressed predominantly in ripening fruit with a small degree of expression in non-fruit tissues. The transcription of MdERFs was regulated positively by the ethylene signaling system. Recently Liu *et al.* (2009) reported involvement of a banana MADS-box transcription factor gene (MuMADS1) in ethylene induced fruit ripening. In their study, Mu- MADS1 is induced by ethylene during post harvest ripening. In naturally ripened banana, a rise in its expression was noted after 6DPH (days post harvest) and rose further till it reached the maximal level on the same day when ethylene production peaked. Initiatives have been made to sequence complete *Musa* genome recently. Huge data has been collected by Global *Musa* Genomics Consortium (<http://www.musagenomics.org>, accessed January 10, 2012). Establishment of complete sequence will lead to the understanding of genes and their regulation involved in banana fruit ripening.

Nora *et al.* (2001) constructed a gene having an antisense of apple ACC oxidase (pAP4) and transformed melon leaves. The pAP4 gene detected in transformed leaves and fruits showed a low ethylene production. Perin *et al.* (2002) showed that two independent loci Al 3 and Al 4 controlled ethylene production and fruit abscission in melon fruits. Moreno *et al.* (2008), however, demonstrated that two ACOs, two ACS and ERS genes localized on the melon genetic map did not exhibit co localization with Al3 and Al4. Carbohydrate metabolism is correlated to the process of ripening in melon and a single recessive gene *suc* controlled sucrose levels in melon (Burger *et al.*, 2002; Burger and Schaffer, 2007).

Ripening studies with tomato as a model system

A long existing history of physiological, biochemical, and molecular research in genetics and molecular tool kits of tomato species have been used for fruits and vegetables development and ripening mainly due to its short generation time. Besides this, Bapat *et al.* (2010) reported that the transient and stable transformation system, deep expression sequence tag (EST) resources, microarrays and ongoing genome sequencing effort, availability of large number of germ plasm, well-characterized mutants, and high-density genetic maps, have assisted in the understanding of development and ripening in F&V to a large extent. In recent times, molecular biology of ripening has metamorphosed into the identification and study of gene sequences in the DNA of organism to reveal insights into ripening control of ethylene, ripening-related signal transduction systems and downstream metabolic networks, even though earlier research focused on ethylene synthesis (Theologis, 1992) and modification of cell wall and proteins structure (Rose *et al.*, 2004). A phenomenon in which a single gene from tomato determines two or more apparently unrelated characteristics of the same organism in ripening mutations have added to the understanding of ripening in fresh F&V. The pleiotropic in tomatoes include colorless non-ripening (Cnr), ripening-inhibitor (*rin*), Never-ripe (Nr), Green-ripe (Gr) and high-pigment (*hp-1* and *hp-2*). Positional cloning and genetic mapping of mutant loci and candidate genes have made it possible to characterize in details the various tomato ripening mutations.

The Cnr and *rin* mutations are recessive and dominant mutations, respectively, and effectively block the ripening process. This was attributed to failure to produce elevated ethylene or to respond to exogenous ethylene during ripening (Vrebalov *et al.*, 2002; Manning *et al.*, 2006). These mutant loci encode putative transcription factors were revealed to provide the first insights into dedicated fruit-specific transcriptional control of ripening. The *rin* was reported to encode a partially deleted MADS-box protein of the SEPELATTA clade, while Cnr is a genetic gene control unassociated with DNA change but alters the function of the promoter methylation of a SQAMOSA promoter binding (SPB) protein. The Nr mutation revealed an ethylene receptor gene, and Gr has been found to encode a novel component of ethylene signalling. GR was cloned by positional cloning of the gene underlying a dominant ripening mutation (Barry and Giovannoni, 2006). The biochemical nature of the GR remains unclear, but amino acid sequence suggests its membrane localization and possible copper-binding activities. Apart from studies carried out on various mutants, development of transgenic tomato fruit with different genes has provided a better insight of fruit ripening and genes involved with this process. SAMDC has been isolated from different plants and has been utilized to modify fruits with an idea that over-expression of SAMDC, might enhance the flux of SAM through the polyamine pathway (Fig. 1), thus reducing the amount available for ethylene biosynthesis. The rate of ethylene production in transgenic tomatoes with yeast SAMDC gene under the control of E8 promoter inhibitor was reported to be lower than in the non-transgenic control fruit, suggesting that polyamine and ethylene biosynthesis pathways may act simultaneously in ripening tomato fruit (Mehta *et al.*, 2002). Both ACO and ACS are encoded by a multigene family of five and nine members, respectively in tomato, whose

expressions are differentially regulated during fruit development and ripening. In tomato, the antisense copy of one member of ACS gene family with its untranslated region was used to develop transgenic plants. Transgenic tomatoes showed 99.5% decrease in ethylene production and did not ripen without exogenous treatment of ethylene. In another attempt, anti-ACS containing transgenic tomato plants showed a 30% decrease in ethylene production by fruits.

Effect of biotechnological approaches on nutritional quality of fruits and vegetables

Many reviews have reported the wide range of determinants of desirable quality attributes in fresh fruits and vegetables such as nutritional value, flavor, colour, texture, processing qualities and shelf-life. (Bapat *et al.*, 2010; Vadivambal and Jayas, 2007). Studies found that tomato plants transformed with yeast SAMDC gene under the control of E8 promoter showed improvement in tomato lycopene content, better fruit juice quality, and vine life (Bapat *et al.*, 2010). Fruit coloration and softening were essentially unaffected, and all the seedlings from first generation seed displayed a normal triple response to ethylene. Over-expression of Nr (wild-type) gene, in tomato using constitutive 35S promoter produced plants that were less sensitive to ethylene (Ciardi *et al.*, 2000). As ethylene receptors belong to a multi-gene family, antisense reduction in expression of individual receptors did not show a major effect on ethylene sensitivity possibly due to redundancy except in case of LeETR4. Antisense plants developed using LeETR4 under the control of CaMV35S promoter exhibited a constitutive ethylene response and were severely affected (Tieman *et al.*, 2000). When antisense plants were developed, using this receptor with fruit-specific promoter, fruits showed early ripening (Kevany *et al.*, 2008). Hackett *et al.* (2000) developed transgenic Nr plants by inhibition of the mutant Nr gene. In these transgenic plants, normal ripening of Nr fruit was restored and fruit achieved wild-type levels of expression of ripening related (PSY1 and ACO1) and ethylene-responsive (E4) genes. Their study confirmed receptor inhibition as one of the mode of action of the NR (receptor) protein as in case of Arabidopsis. Fruit softening is one of the most prominent parameter in climacteric fruits. Softening of fruit occurs due to solubilization and depolymerization of cell wall hemicelluloses and pectin by various cell wall hydrolases (Rose *et al.*, 2004; Brummell and Harpster, 2001). Due to accelerated fruit softening, excessive spoilage occurs which needs to be checked. Transgenic rin plants which accumulated reduced amount of endogenous PG provided clue to develop antisense PG transgenic under the control of E8 promoter. These transgenic produced fruit with PG enzyme activity that was 60% of wild-type however, this did not affect softening much.

Down-regulation of PG mRNA accumulation by constitutive expression of an antisense PG transgene driven by the cauliflower mosaic virus 35S promoter yielded transgenic fruits, retaining only 0.5–1% of wild-type levels of PG enzyme activity though overall fruit ripening and softening was not affected (Rose *et al.*, 2003; Saladié *et al.*, 2007). Suppression of PME activity in tomato by introducing antisense PME2/PEC2 transgenes under the control of the constitutive CaMV35S promoter modulated degree of pectin methyl esterification. In transgenic antisense PME fruit esterification

was higher than controls throughout ripening, but the fruit otherwise ripened normally (Nath *et al.*, 2006). In another study, antisense suppression of pectinesterase under CaMV35S promoter produced fruits with reduced PE activity and suppression in the rate of softening during ripening (Phan *et al.*, 2007). In tomato, a large and divergent multigene family encodes EGases (cellulases), which consists of at least eight members. mRNA accumulation of the highly divergent EGases LeCel1 and LeCel2 was suppressed individually by constitutive expression of antisense transgenes (Rose *et al.*, 2003). In both cases, most suppressed lines showed decreased mRNA accumulation in fruit pericarp by 99% as compared to wild-type, without affecting the expression of the other EGase and fruit softening. Galactosidases in tomato are encoded by a multigene family having seven members (TBG1–7). These members show differential expression patterns during fruit development (Smith and Gross, 2000). Transgenic plants have been developed using members of this family to reduce softening process. Sense suppression by a short gene specific region of TBG1 cDNA reduced TBG1 mRNA abundance to 10% of wild-type levels in ripe fruit, but did not reduce total exo-galactanase activity and did not affect cell wall galactose content or fruit softening (Carey *et al.*, 2001). Antisense tomato beta-galactosidase 4 (TBG4) and 7 (TBG7) cDNAs driven by the CaMV35S promoter resulted in transgenic tomatoes with modulated fruit firmness in comparison to control fruit (Moctezuma *et al.*, 2003). Ethylene response factors (ERFs) play important role in modulating ethylene induced ripening in fruits. These ERFs belong to multigene family and are transcriptional regulators. These mediate ethylene-dependent gene expression by binding to the GCC motif found in the promoter region of ethylene-regulated genes. Modulation of expression of these individual ERFs in tomato has demonstrated their role in plant development and ripening. The sense and antisense LeERF1 transgenic tomato under the control of CaMV35 promoter were developed. Over-expression of LeERF1 in tomato caused the typical ethylene triple response on etiolated seedling. Antisense LeERF1 fruits showed longer shelf-life compared with wildtype tomato (Li *et al.*, 2007). Over-expression of the Sl-ERF2 gene in transgenic tomato lines resulted in premature seed germination and enhanced hook formation of dark-grown seedlings, which is indicative of increased ethylene sensitivity (Pirrello *et al.*, 2006). The expression of the mannanase 2 gene was up-regulated in Sl-ERF2- over-expressing seeds, suggesting that Sl-ERF2 stimulated seed germination through the induction of the mannanase 2 gene. In 2007, Rose *et al.* studied previously unreported cultivar of tomato. Fruits of this cultivar named as Delayed Fruit Deterioration (DFD) undergo normal ripening but remain firm and show no loss of integrity for at least six months. Ripening DFD fruit interestingly showed minimal water loss by transpiration and elevated cellular turgor whereas expression of genes associated with wall disassembly were similar as in other cultivars (Saladié *et al.*, 2007). Based on biochemical and bio-mechanical analyses, this group has proposed a model in which softening of tomato fruit is affected by cuticle directly by providing physical support and by regulating fruit water status. Candidate gene/genes are not yet identified for this trait but once identified would be of much interest for biotechnological purposes. A new and important set of genes regulating different developmental processes involve micro RNAs (miRNAs) (Jones-Rhoades *et al.*, 2006). Though miRNAs and their targets have been identified in

number of plant species not much work has been carried out in relation to their involvement in fruit development and ripening. Recently Yin *et al.* (2008) and Zhang *et al.* (2008) identified a set of miRNA and their targets from tomato that were associated with the phase change from vegetative to generative growth. In addition, high throughput Pyrosequencing has revealed micro RNAs targeting genes that are involved in fruit ripening (Moxon *et al.*, 2008).

In apples, Dandekar *et al.* (2004) reported differential regulation of ethylene with respect to fruit quality components. A direct correlation between ethylene and aroma production during apple ripening has been reported (Wang *et al.*, 2007). Schaffer *et al.* (2007) identified 17 candidate genes that were likely to be the control points for ethylene with respect to aroma production. However, not all components of fruit quality are under the direct control of ethylene. Two MdERFs (ethylene response factors) were isolated from ripening apple fruit (Wang *et al.*, 2007). MdERF2 expressed exclusively in ripening fruit whereas MdERF1 was expressed predominantly in ripening fruit with a small degree of expression in non-fruit tissues. The transcription of MdERFs was regulated positively by the ethylene signaling system.

In a related study with two cultivars of apple, Zhu *et al.* (2008) characterized the expression patterns of AAT and ACS gene family members in order to examine the relationship with volatile ester production during on-tree and post harvest ripening. They found that differential expression of AAT genes contributed to phenotypic variation of volatile ester biosynthesis in the apple cultivars. The climacteric expression of MdACS1 greatly enhanced the expression levels of MdAAT1 and MdAAT2 genes was reported as the plausible reason for the emission of aromatic volatile esters. It was also suggested that the expression of MdACS3 might play a role on induction of AAT genes expression during early fruit development as it expresses prior to MdACS1. Grumet *et al.* (2007) found enhanced sugar and carotenoid accumulation whereas Katzir *et al.* (2008) reported a considerable reduction in aroma production for ACO1 antisense melons. In a study of ethylene-regulated and ethylene independent ripening pathways by Silva *et al.* (2004) in wild-type and AS3 transgenic melons, the AS3 transgenic melon fruits were reported to be firmer and higher in chlorophyll levels and acidity than their wild-type counterparts with no changes in carotenoid contents in both types. In a related research, Nishiyama *et al.* (2007) found that there was expressed suppression of the ACO gene of transgenic melon fruit when they examined the cell wall polysaccharide depolymerization and the expression of the wall metabolism-related genes. There was also a complete inhibition of softening in the transgenic melon fruits but were restored by exogenous ethylene treatment. Post harvest application of 1-MCP after the onset of ripening completely suppressed subsequent softening, suggesting that melon fruit softening is ethylene-dependent. There were however, partial fragmentations (1038 bp cDNA) of melon invertase expressed in antisense orientation under the CaMV35S promoter observed by Yu *et al.* (2008). The transgenic melon fruits were 60% smaller in size and recorded increased sucrose and acidity invertase levels, with degraded chloroplast as a result of decreased photosynthetic rate than the control.

In another study involving avocado fruits, Tateishi *et al.* (2007) found that three cloned members of β -galactosidases (PaGAL2, PaGAL3 and PaGAL4) played a significant role in the cell wall metabolism during fruits growth and ripening as well as AV-GAL1. The study of expression pattern of the isozymes by the same authors during avocado ripening found that the accumulation pattern of the gene transcripts and the response to ethylene gave a correlation between AV-GAL1 transcript and isozyme AV-GAL III. The authors therefore speculated that AV-GAL1, might have encoded the AV-GAL III and might be important for post harvest fruit softening while PaGAL2 was responsible for galactose metabolism both in expanding tissue and cell wall disassembly during ripening. In their research, they observed that PaGAL3 and PaGAL4 expression were strongly inhibited by ethylene and ripening signals suggesting that PaGAL2, PaGAL3 and PaGAL4 might have been involved in galactose metabolism of cells or cell walls during development and ripening. This could be the reason why post harvest biotechnology of avocado has been strongly limited in spite of the fact that it provided early clues to the ripening mechanism in fleshy fruit.

Costantini *et al.* (2007) transformed grape cultivar Thompson Seedless with an ovule-specific auxin-synthesizing (DefH9-iaaM) gene and observed that average number of inflorescence per shoot in transgenic grape lines was doubled as compared to the control. In their studies, they reported that auxin enhanced fecundity in grapes, thus resulting in increased yield with lower production costs. Similarly, Symons *et al.* (2006) have shown that brassinosteroids (steroidal hormones) might be implicated in ripening of non-climacteric fruits. The group isolated Brassinosteroid-6-oxidase gene homolog from grape and its function was checked by transgenic complementation of the tomato dwarf (dx/dx) mutant. The study showed that grape ripening was significantly promoted by exogenous application of brassinosteroids (BRs) and ripening could be delayed by brassinazole, an inhibitor of BR biosynthesis. Since exogenous BRs have also been shown to promote ripening in tomato it was speculated that common regulatory mechanisms might be operating early in the ripening processes of both climacteric and non-climacteric species involving brassinosteroids.

Recent advances in recombinant DNA technology and genetic engineering have opened up the possibility to manipulate ripening in fast perishable fruits like banana. Towards this, many genes involved in ripening have been cloned and characterized (Kesari *et al.*, 2007; Gupta *et al.*, 2006). Ripening in banana is characterized by a biphasic ethylene production with a sharp early peak followed by a post climacteric small peak (Pathak *et al.*, 2003). During banana fruit ripening ethylene production triggers a developmental cascade that is accompanied by a huge conversion of starch to sugars, an associated burst of respiratory activity and an increase in protein synthesis. Other changes include fruit softening. Banana fruit softening is attributed to activities of various cell wall hydrolases. Lohani *et al.* (2004) reported participation of various cell wall hydrolases in banana softening during ripening. The enhancing and suppressive effects of ABA and IAA respectively on activities of different cell wall hydrolases during ethylene induced ripening in banana were also discussed. Decline in polyphenols, increase in activity of alcohol acetyl transferase, chlorophyll degradation etc. have been earlier reported during ripening in

banana. Liu *et al.* (1999) have analyzed the expression of ACC synthase gene in association with ethylene biosynthesis and ripening in banana. Huang *et al.* (2006) have shown the presence of many isoforms of ACS other than MA-ACS1 (Musa acuminata ACC synthase 1) in banana. Clendennen and May (1997) reported a number of up-regulated endochitinase, β -1, 3-glucanase, and BanTLP (thaumatin like protein and metallothionin) as well as down-regulated genes (class III chitinase and jacalin-related lectins) during ripening. Class III chitinase was postulated to fulfill a storage role in banana pulp. It is supposed to serve as an important source of amino acids for the synthesis of ripening associated proteins (Peumans *et al.*, 2002). The role of expansin (Trivedi and Nath, 2004; Asha *et al.*, 2007) and polygalacturonase genes during banana fruit ripening has been investigated (Asif and Nath, 2005).

Effect of biotechnological approaches on the shelf life of fruits and vegetables

The shelf-life of transgenic tomato fruits was reported to last for at least 60 days at room temperature without significant change in hardness and color. After 15–20 days of treatment of the transgenic fruits with ethylene, most of the tomatoes reached the ripe stage. Antisense transgenic lines of tomato have also been raised with anti-ACO gene to alter ethylene biosynthesis (Nath *et al.*, 2006). RNAi technique has also been used to produce tomato fruit with delayed ripening using ACO gene. According to Xiong *et al.* (2005) transgenic tomato fruits had a prolonged shelf-life of at least 120 days. In another study with apples, Wang *et al.* (2009) showed that null mutation in MdACS3 gene leads to longer shelf-life. Out of the three genes in the MdACS3 family (a, b, and c) two of them (MdACS3b and MdACS3c) possessed 333-bp transpose on-like insertion in their 5' flanking region, which was reported to may have prevented transcription of these genes during ripening. A single nucleotide polymorphism in the coding region of MdACS3a resulted in an amino acid substitution (glycine-289 → valine) in the active site that inactivated the enzyme. A review by Bapat *et al.* (2010) reported that two ripening-related genes (MaMads-rin and MaExp2) have been used for banana transformation to increase shelf-life and fruit quality. Results indicated increment in shelf-life both on plant and at post harvest.

Challenges associated with commercialization of biotech fruits and vegetables

My research revealed that even though biotechnological approaches is seen by the scientific community as a panacea to solve recent increased demands for fruits and vegetables, the technology is more of a scientific jargon than a commercially viable entity. This is because;

dilemma and uncertainties remain up to today regarding the consumption of biotechnological fruits and vegetables. The impasse has created challenges with consumption of genetically modified fruits and vegetables in many countries and some continents mainly due to the complexities surrounding its use.

Although the first biotech crop to be commercialized was a genetically modified tomato for processing as a consumer tomato paste, there have been comparatively few introductions of biotech fruits and vegetables since then (Anthony and

Ferroni, 2011). Reported cases with potential benefits for farmers in developing countries include virus resistant papaya in China, now commercially grown, and, more recently, the high profile case of Bt eggplant, or brinjal, in India (Choudhary and Gaur, 2009). Because of the susceptibility of brinjal to the fruit and shoot borer insect, multiple insecticide applications are required to prevent uneconomic losses of yield in this crop. In India, the Indian Genetic Engineering Appraisal Committee recommended the commercial release of Bt brinjal (Event EE1) in 2010, but no authorization was given by the Ministry of Environment and Forestry (Jayaraman, 2010). A wide array of vegetables such as tomato, broccoli, cabbage and okra are also under development in India (James, 2010).

According to James (2010), since 1996, over 900 registration approvals have been granted for 183 events in 24 crop species, mainly for events in broad acre crops (CERA. GM Crop Database; ISAAA GM Approval Database). Very few registrations have been given for commercialization of modified fruits or vegetables since the 1990s. Approvals of suitably developed and stewarded high-value vegetables and fruits could carry significant benefits for small farmers, because of the relatively high prices of these crops on the market.

In a study involving 77 fruits and vegetables and other specialty crops, Miller and Bradford (2010) attempted to understand the factors driving the lack of traits for commercialization. They reported that during 2003–2008 over 300 research papers were published describing over 250 unique transgenic events for these kinds of crops of which some 20% of the papers were from China and India. The various researches addressed not just input traits such as herbicide tolerance and insect resistance but also output traits such as yield, postharvest quality, and modifications to compositions of oil, starch, protein and nutrients. The primary conclusion was that the traits did not reach the market not because of poor performance or lack of grower interest but because of regulatory approval uncertainty and prohibitively high and uneconomic development and regulatory costs—a de facto barrier for technology deployment for smallholder farmers, even for high-value crops.

In recent surveys by private sector companies during 2008–2012, it was established that the cost of intervention, development, and registration of a new traits for internationally traded crops such as maize and soybean was as high as \$136 million for cultivation in two countries and for import approvals in at least five others. The breakdown cost analysis for regulatory scientific studies, registration and regulatory affairs accounted for 25.8% of this total, \$35.1 million. More so, McDougall (2011) reported that the time taken for registration has also increased, from a mean of 3.7 years for events sold before 2002 to a current estimate of 5.5 years.

A review by (Anthony and Ferroni, 2011) has argued that opinion and debate on acceptance of transgenic agricultural biotechnology remains polarized both 'for and against' and is often not aligned with rigorous review and balanced, empirically grounded assessment of socio-economic and community benefits, human safety, environmental considerations such as non-target safety, gene flow, biodiversity and associated risks. At a time when biotech crops

have been grown extensively in the Americas and Asia for over 13 years, the precautionary principle prevails in many countries even for the traits embodied in these crops. In the European Union (EU) for example, genetically modified fruits and vegetables are not allowed on the market and none of the GM plants currently authorized in the EU are intended for direct consumption (GMO Compass, 2006). For instance, in the case of GM tomatoes, they are lurking in grocery stores in the USA and never received authorization in the EU. The situation is the same for biotech bananas, apples, wine grapes, and papaya production. Recent reports in the EU member states indicate that while countries like Finland, Germany, and Greece have strongly opposed commercialization of GM crops including fruits and vegetables; Spain and UK do not fundamentally oppose cultivating GM crops but have used the precautionary principle. So the question remains that 'is biotechnology in fruit and vegetable plant production a commercial activity or simply a research jargon?' A pragmatic approach proposed by Godfray *et al.* (2010) move the debate forward saying 'genetic modification is a potentially valuable technology whose advantages and disadvantages need to be considered rigorously on an evidential, inclusive, case-by-case basis'. Genetic modification should neither be privileged nor automatically dismissed. In addition to governments' policy on regulation, key factors influencing future availability of biotech fruits and vegetables in developing countries are stewardship capability, and liability of technology providers. Excellence through stewardship (2009) reveals that stewardship biotechnology of fruits and vegetables includes not just management of biosafety and compliance with regulatory authorities' requirements but also product quality and integrity along the whole product life cycle right from early research ideas to the withdrawal of crop varieties. The need for stewardship is fully founded. Unapproved events entering the trade channel can have serious consequences. In 2006, the co-mingling of the herbicide tolerant research event LLRice601 led to the reduction of US rice trade to Europe to only 10% of normal levels with major economic and international consequences (Stein and Rodriguez-Cerezo, 2009). Multi-million dollar lawsuits from rice growers followed. With the expected rise in numbers of commercialized events around the world (Dunwell, 2011), including potentially Bt rice in China (Bennet, 2010), concern is growing about the potential for low-level presence of numerous events in trade channels and the food chain in countries without regulatory authorizations (Stein and Rodriguez-Cerezo, 2010). International harmonized solutions need to be found. Otherwise the private sector will remain very cautious about supporting technology releases to the public sector to assist smallholder farmers in developing countries, especially for food crops that could cross national borders or enter international trade channels. Additionally, little impact has been realized to date with fruits and vegetables because of development timescales for molecular breeding and development and regulatory costs and political considerations facing biotech crops in many countries.

The new paradigm shifts for commercialization of biotech fruits and vegetables

Biotechnological approaches offer much potential to increase the development and introduction of improved varieties and as an enabler for greater genetic diversity, but the full benefits are

yet to be established. Constraints to the development and adoption of technology-based solutions to reduce yield gaps need to be overcome. The new paradigm shift proposed includes the (1) integration of broader thrust that galvanizes public and private investment for the development and provision of technology with the creation of seed systems and markets supported by agricultural extension and other services for farmers; (2) a commitment to increase and sustain funding of agricultural R&D; (3) the need to break barriers at the policy and operational level to enable formation of public-private partnerships for transformational change in research, product development, and the delivery of seed-embodied technology to farmers; (4) the integration of low risk chemical fungicides, natural anti-microbial substances, and physical means such as hot water treatment, irradiation with ultraviolet light, microwave, and infrared treatment in the postharvest biocontrol process; (5) the enhancement in the expression of crucial recombinant DNA genes and/or combining genes from different agents in the mass production, formulation and storage, or in response to exposure and contact with parent plant tissue after application; (6) the use of genetically modified organisms as biocontrol agents to enhance the postharvest quality and shelf-life of fruits and vegetables; (7) the research towards discovering new DNA genes instead of the ones currently used in practices in that only a small portion of the earth micro flora has been identified and characterized.

2. CONCLUSION

The application of biotechnological approaches to improve nutritional quality and shelf life of fruits and vegetables were reviewed. It was evident that developed biotechnological approaches have the potential to enhance the yield, quality, and shelf-life of fruits and vegetables to meet the demands of the 21st century. However, the developed biotech approaches for fruits and vegetables were more of academic jargon than a commercial reality. To make sure that the current debates and complexities surrounding the registration and the commercialization of genetically modified fruits and vegetables are adequately addressed, various stakeholders in the industry (policy makers, private sectors, agriculturalists, biotechnologists, scientists, extension agents, farmers, and the general public must be engaged in policy formulations, seed embodiments, and products development. The full benefit of the knowledge can be reaped if there are total commitment by all stakeholders regarding increased and sustained funding, increase agricultural R&D, and less cost and time for registration and commercialization of new traits.

REFERENCES

- Abano, E.E., and Sam-Amoah, L.K. (2011). Effects of different pretreatments on drying characteristics of banana slices. *Journal of Engineering and Applied Science*. Vol 6, Article 3.
- Anthony, VM, Ferroni M. (2011). Agricultural biotechnology and smallholder farmers in developing countries, *Curr Opin Biotechnol* (2011), doi:10.1016/j.copbio.2011.11.020
- Asha, Sane VA, Sane AP, Nath P (2007). Multiple forms of alpha-expansin genes are expressed during banana fruit

- ripening and development. *Postharvest Biol Technol*;45: 184–92.
- Asif MH, Nath P. (2005) Expression of multiple forms of polygalacturonase gene during ripening in banana fruit. *Plant Physiol Biochem*;43:177–84.
- Bapat, V. A. Trivedi, P. K. Ghosh, A. Sane, V. A. Ganapathi T. R., Nath, P. (2010). Ripening of fleshy fruit: Molecular insight and the role of ethylene. *Biotechnology Advances*, 28, 94–107
- Barry CS, Giovannoni JJ. (2006) Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. *Proc Natl Acad Sci U S A*;103:7923–88.
- Bennett J (2010) Biotech rice — present status and future prospects. *National Seed Association of India Magazine*; July–September:7-33.
- Binnie IE, McManus MT. (2009) Characterization of the 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase multigene family of *Malus domestica*. *Borkh Phytochem*;70:348–60.
- Bouzayen M, Latché A, Nath P, Pech JC. Mechanisms of fruit ripening. . 10.1007/978-3-642-02301-9_16 In: Pua EC, Davey MR, editors. *Plant Developmental Biology*–
- Brummell DA, Harpster MH. (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Mol Biol*;47:311–40.
- Bud R (1991). *Biotechnology in the twentieth century*. In: *Social studies of science*, vol. 21. London: Sage, p. 415–57.
- Burger Y, Sa'ar U, Katzir N, Paris HS, Yeselson Y, Levin I, et al. (2002) A single recessive gene for sucrose accumulation in *Cucumis melo* fruit. *J Am Soc Hortic Sci*;127: 938–43.
- Burger Y, Schaffer AA. (2007) The contribution of sucrose metabolism enzymes to sucrose accumulation in *Cucumis melo*. *J Am Soc Hortic Sci* 132:704–12.
- Buttel FH (1989). How epoch making are high technologies? The case of biotechnology. *Sociol Forum*, 4:247–60..
- Carey AT, Smith DL, Harrison E, Bird CR, Gross KC, Seymour GB, et al. (2001) Down-regulation of a ripening-related beta-galactosidase gene (TBG1) in transgenic tomato fruits. *J Exp Bot*; 52:663–8.
- Choudhary, B. Gaur, K. (2009). *The Development and Regulation of Bt Brinjal in India (Eggplant/Aubergine)*. ISAAA Brief No. 38. Ithaca, NY: ISAAA.
- Ciardi JA, Tieman DM, Lund ST, Jones JB, Stall RE, Klee HJ. (2000) Response to *Xanthomonas campestris* pv. *vesicatoria* in tomato involves regulation of ethylene receptor gene expression. *Plant Physiol*;123:81–92.
- Clendennen SK, May GD. (1997) Differential gene expression in ripening banana fruit. *Plant Physiol*;115:463–9.
- Costantini E, Landi L, Silvestroni O, Pandolfini T, Spena A, Mezzetti B. (2007) Auxin synthesis encoding transgene enhances grape fecundity. *Plant Physiol*;143:1689–94.
- Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kadar AA, et al. (2004) Effect of downregulation of ethylene biosynthesis on fruit flavor complex in apple fruit. *Transgenic Res*;3:373–84.
- DeFrancesco L (2008). *Vintage genetic engineering*. *Nat Biotechnol* , 26:261–3.
- Droby, S., (2006). Improving quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticulturae* 709, 45–51.
- Droby, S., Chalutz, E., Wilson, C.L., Wisniewski, M.E. (1991). Biological control of postharvest diseases: a promising alternative to the use of synthetic fungicides. *Phytoparasitica* 20, 1495–1503.
- Droby, S., Wisniewski, M., Macarisinb, D., Wilson, C. (2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm? *Postharvest Biology and Technology* 52, 137–145
- Dunwell J (2011). *Crop biotechnology: prospects and opportunities*. *J Agric Sci*, 149:17-27.
- El-Ghaouth, A., Wilson, C.L., Wisniewski, M.E. (2004). Biologically based alternatives to synthetic fungicides for the postharvest diseases of fruit and vegetables. In: Naqvi, S.A.M.H. (Ed.), *Diseases of Fruit and Vegetables*, vol. 2. Kluwer Academic Publishers, The Netherlands, pp. 511–535.
- Excellence Through Stewardship (2009): *Advancing Best Practices in Agricultural Biotechnology. Guide for Maintaining Plant Product Integrity of Biotechnology-Derived Plant Products*. Washington: ETS
- Fan, Q., Tian, S.P., (2001). Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. *Postharvest Biology and Technology* 21 (3), 341–350.
- Gellatly, K, Dennis, D.T. (2011). *Plant Biotechnology and GMOs*. *Comprehensive Biotechnology* (2nd Ed.) Vol 4, pp 9-22.
- Giovannoni J. (2004) Genetic regulation of fruit development and ripening. *Plant Cell*;16: S170–80 Suppl.
- Giovannoni J. (2007) Fruit ripening mutants yield insights into ripening control. *Curr Opin Plant Biol*;10:283–9.
- GMO Compass (2006). Also available at <http://www.gmo-compass.org>**
- Godfray C, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010). Food security: the challenge of feeding 9 billion people. *Science*, 327:812-818.

- Grumet R, Katzir N, Little HA, Portnoy V, Burger Y. (2007) New insights into reproductive development in melon (*Cucumis melo* L.). *Int J Plant Dev Biol*;1:253–64.
- Gupta SM, Srivastava S, Sane AP, Nath P. (2006) Differential expression of genes during banana fruit development, ripening and 1-MCP treatment: presence of distinct fruit specific, ethylene induced and ethylene repressed expression. *Postharvest Biol Technol*;42:16–22.
- Hackett RM, Ho CW, Lin Z, Foote HC, Fray RG, Grierson D. (2000) Antisense inhibition of the *Nr* gene restores normal ripening to the tomato Never-ripe mutant, consistent with the ethylene receptor-inhibition model. *Plant Physiol*;124:1079–86.
- Huang FC, Do YY, Huang PL. (2006) Genomic organization of a diverse ACC synthase gene family in banana and expression characteristics of the gene member involved in ripening of banana fruits. *J Agric Food Chem*;54:3859–68.
- James C (2010). *Global Status of Commercialized Biotech/GM Crops 2010*. ISAAA, Brief No. 42. Ithaca, NY: ISAAA.
- Jayaraman, K. (2010). Bt brinjal splits Indian cabinet. *Nat Biotechnol*, 28:296.
- Johnston JW, Gunaseelan K, Pidakala P, Wang M, Schaffer RJ. (2009) Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. *J Exp Bot*;60:2689–99.
- Jones-Rhoades MW, Bartel DP, Bartel B. (2006) MicroRNAs and their regulatory roles in plants. *Annu Rev Plant Biol*;57:19–53.
- Katzir N, Harel-Beja R, Portnoy V, Tzuri G, Koren E, Lev S, et al (2008). In: Pitrat M, editor. *Melon fruit quality: a genomic approach*. Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. Avignon (France): INRA; May 21–24th.
- Kesari R, Trivedi PK, Nath P. (2007) Ethylene-induced ripening in banana evokes expression of defense and stress related genes in fruit tissue. *Post Harvest Biol Technol*;46: 136–43.
- Kevany BM, Taylor MG, Klee HJ. (2008) Fruit-specific suppression of the ethylene receptor *LeETR4* results in early-ripening tomato fruit. *Plant Biotechnol J*;3:295–300.
- Korsten, L., 2006. Advances in control of postharvest diseases in tropical fresh produce. *International Journal of Postharvest Technology and Innovation* 1 (1), 48–61.
- Lers, A (2012). 27 - Potential application of biotechnology to maintain fresh produce postharvest quality and reduce losses during storage. *Plant Biotechnol and agriculture*, Pages 425–441
- Li Y, Zhu B, Xu W, Zhu H, Chen A, Xie Y, et al. (2007) *LeERF1* positively modulated ethylene triple response on etiolated seedling, plant development and fruit ripening and softening in tomato. *Plant Cell Rep*;26:1999–2008.
- Liu C, Tian Y, Shen Q, Jiang H, Ju R, Yan T, Liu C, Mang K. (1999) Cloning of 1-aminocyclopropane-1-carboxylate (ACC) synthetase cDNA and the inhibition of fruit ripening by its antisense RNA in transgenic tomato plants. *Chin J Biotech*;14:75–84.
- Liu J, Xu B, Hu L, Li M, Su W, Wu J, et al. (2009) Involvement of a banana MADS-box transcription factor gene in ethylene-induced fruit ripening. *Plant Cell Rep*;28:103–11.
- Liu X, Shiomi S, Nakatsuka A, Kubo Y, Nakamura R, Inaba A. (1999) Characterization of ethylene biosynthesis associated with ripening in banana fruit. *Plant Physiol*;121: 1257–66.
- Lohani S, Trivedi PK, Nath P. (2004) Changes in activities of cell wall hydrolases during ethylene induced ripening in banana: effect of 1-MCP, ABA and auxin. *Postharvest Biol Technol*;31:119–26.
- Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, et al. (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet*;38:948–52.
- Martínez-Romero D, Bailén G, Serrano M, Guillén F, Valverde JM, Zapata P, et al. (2007) Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: a review. *Crit Rev Food Sci Nutr*;47:543–60.
- McDougall P (2011). The cost and time involved in the discovery, development and authorization of a new plant biotechnology derived trait. A Consultancy Study for Crop Life International; Midlothian, Scotland.
- Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK. (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. *Nat Biotechnol*;20:613–8.
- Miller JK, Bradford KJ (2010). The regulatory bottleneck for biotech specialty crops. *Nat Biotechnol*, 28:1012–1014.
- Moctezuma E, Smith DL, Gross KC. (2003) Antisense suppression of a beta-galactosidase gene (*Tb G6*) in tomato increases fruit cracking. *J Exp Bot*;54:2025–33.
- Moreno E, Obando JM, Dos-Santos N, Fernández-Trujillo JP, Monforte AJ, Garcia-Mas J (2008). Candidate genes and QTLs for fruit ripening and softening in melon. *Theor Appl Genet*;116:589–602.
- Moxon S, Jing R, Szittyá G, Schwach F, Rusholme Pilcher RL, Moulton V, et al. (2008) Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. *Genome Res*;18:1602–9.
- Nath P, Sane AP, Trivedi PK, Sane VA, Asif M. (2007) Role of transcription factors in regulating ripening, senescence and organ abscission in plants. *Steward Postharvest Rev*;2:1–14.
- Nath P, Trivedi PK, Sane VA, Sane AP. (2006) In: Khan NA, editor. *Role of ethylene in fruit ripening*. Berlin Heidelberg: Springer-Verlog; p. 151–76.

- Nishiyama K, Guis M, Rose JK, Kubo Y, Bennett KA, Wangjin L, et al. (2007) Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. *J Exp Bot*;58:128–9.
- Nonis A, Ruperti B, Falchi R, Casatta E, Enferadi ST, Vizzotto G. (2007). Differential expression and regulation of a neutral invertase encoding gene from peach (*Prunus persica*): evidence for a role in fruit development. *Physiol Plant*;129:436–46.
- Nora FR, Peters JA, Schuch JA, Marda W, Lucchetta L, Marini L, et al. (2001). Melon regeneration and transformation using an apple ACC oxidase antisense gene. *Rev Bras De Agrociencia*, 7:201–4.
- OTA (1989). New developments in biotechnology: patenting life. Special report OTA-BA-370. Washington DC: US Government Printing Office, p. 25–48.
- Pathak N, Asif MH, Dhawan P, Srivastava MK, Nath P (2003). Expression and activities of ethylene biosynthesis enzymes during ripening in banana fruits and effect of 1-MCP treatment. *Plant Growth Regul*; 40:11–9.
- Persely, G.J. (1992). Biotechnology in agriculture, G. Thottappilly, L.M. Monti, D.R. Mohan Raj, A.W. Moore, Editors, *Biotechnology: enhancing research on tropical crops in Africa*, IITA, Ibadan (Nigeria), pp. 11–12.
- Peumans WJ, Proost P, Swennen RL, Van Damme EJ (2002). The abundant class III chitinase homolog in young developing banana fruits behaves as a transient vegetative storage protein and most probably serves as an important supply of amino acids for the synthesis of ripening-associated proteins. *Plant Physiol*; 130: 1063–72.
- Phan TD, Bo W, West G, Lycett GW, Tucker GA (2007). Silencing of the major salt-dependent
- Pirrello J, Jaimes-Miranda F, Sanchez-Ballesta MT, Tournier B, Khalil-Ahmad Q, Regad F, et al (2006). Sl-ERF2, a tomato ethylene response factor involved in ethylene response and seed germination. *Plant Cell Physiol*;47:1195–205.
- Prasanna V, Prabha TN, Tharanathan RN (2007). Fruit ripening phenomena—an overview. *Crit Rev Food Sci Nutr*;47:1-19.
- Raghava C.R. and E. Haribabu (2002). Biotechnology and the industrialization of horticulture in India. *Outlook Agric*, 31 3, pp. 187–192.
- Roberts, R.G., (1990). Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. *Phytopathology* 80, 526–530.
- Rose JK, Saladié M, Catalá C (2004). The plot thickens: new perspectives of primary cell wall modification. *Curr Opin Plant Biol*;7:296–301.
- Rose JKC, Catalá C, Gonzalez-Carranza CZH, Roberts JA (; 2003). Plant cell wall disassembly. In: Rose JKC, editor. *Plant Cell Wall*, Vol 8. Oxford: Blackwell Publishing Ltd. p. 264–324.
- Saladié M, Matas AJ, Isaacson T (2007). A reevaluation of the key factors that influence tomato fruit softening and integrity. *Plant Physiol*;144:1012–28.
- Schaffer RJ, Friel EN, Souleyre EJ, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma JH, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD (2007). A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiol*;144:1899–912.
- Sharma, R.R., Singh D., Singh R. (2009) Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review, *Biological Control*, 50 (2009) 205–221.
- Silva JA, da Costa TS, Lucchetta L, Marini LJ, Zanuzo MR, Nora L, et al (2004). Characterization of ripening behavior in transgenic melons expressing an antisense 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene from apple. *Postharvest Biol Technol*;32:263–8.
- Singh, D., Sharma, R.R. (2007). Postharvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), *Sustainable Pest Management*. Daya Publishing House, New Delhi, India.
- Smith DL, Gross KC (2000). A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. *Plant Physiol*;123:1173–83.
- SPORE (1996). The promise of biotechnology in agriculture. In: CTA Information for agricultural development in ACP countries, no 66, Nov–Dec 1996, p. 1–4.
- Stein AJ, Rodriguez-Cerezo E (2009). The Global Pipeline of New GM Crops. Implications of Asynchronous Approval for International Trade. European Commission. Joint Research Centre Scientific and Technical Report.
- Stein AJ, Rodriguez-Cerezo E (2010): Low-level presence of new gm crops; an issue on the rise for countries where they lack approval. *AgBioForum*, 13:173-182.
- Symons GM, Davies C, Shavrukov Y, Dry IB, Reid JB, Thomas MR (2006). Grapes on steroids. Brassinosteroids are involved in grape berry ripening. *Plant Physiol*;140: 150–8.
- Tateishi A, Shiba H, Ogihara J, Isobe K, Nomura K, Watanabe K, et al (2007). Differential expression and ethylene regulation of beta-galactosidase genes and isozymes isolated from avocado (*Persea americana* Mill.) fruit. *Postharvest Biol Technol*;45:56–65.
- Theologis A (1992). One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. *Cell*;70:181–4.
- Tieman DM, Taylor MG, Ciardi JA, Klee HJ (2000). The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proc Natl Acad Sci U S A*;97:5663–8.

Trivedi PK, Nath P (2004). MaExp1. An ethylene-induced expansin, from ripening banana fruit. *Plant Sci*;167:1351–8.

Uche, C. A. (2004). Plant biotechnology and food crop development in Sub-Saharan Africa, , **Technology in society, Vol 26, Issue ., pp537-550**

Vadivambal, R., and Jayson, D.S. (2007). Changes in quality of microwave-treated agricultural products- A review. *Journal of Biosystems Engineering*, 98: 1-16.

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, et al (2002). AMADS-Box gene necessary for fruit ripening at the tomato Ripening-Inhibitor (Rin) locus. *Science*;296:343–6.

Wang A, Tan D, Takahashi A, Li TZ, Harada T (2007). MdERFs, two ethylene-response factors involved in apple fruit ripening. *J Exp Bot*;58:3743–8.

Wang A, Yamakake J, Kudo H, Wakasa Y, Hatsuyama Y, Igarashi M, et al (2009). Null mutation of the MdACS3 gene, coding for a ripening-specific 1-aminocyclopropane-1-carboxylate synthase, leads to long shelf life in apple fruit. *Plant Physiol*; 151:391–9.

Wisniewski, M., Wilson, C.L., Chalutz, E., Hershberger, W. (1992). Biological control of postharvest diseases of fruit: inhibition of Botrytis rots on apples by an antagonistic yeast. *Proceedings of the Electron Microscopic Society of America* 46, 290–291.

Xiong AS, Yao QH, Peng RH, Li X, Han PL, Fan HQ. (2005) Different effects on ACC oxidase gene silencing triggered by

RNA interference in transgenic tomato. *Plant Cell Rep*;23:639–46.

Yin Z, Li C, Han X, Shen F. (2008) Identification of conserved microRNAs and their target genes in tomato (*Lycopersicon esculentum*). *Gene*;414:60–6.

Yu X, Wang X, Zhang W, Qian T, Tang G, Guo Y, et al. (2008) Antisense suppression of an acid invertase gene (MAI1) in muskmelon alters plant growth and fruit development. *J Exp Bot*;59:29 69–77.

Zhang , H., Zheng , X., Yu, T., (2007). Biological control of postharvest diseases of peach with *Cryptococcus laurentii*. *Food Control* 18, 287–291.

Zhang J, Zeng R, Chen J, Liu X, Liao Q. (2008) Identification of conserved microRNAs and their targets from *Solanum lycopersicum* Mill. *Gene*;423:1–7.

Zhang, H., Zheng, X., Fu, C., Xi, Y., (2005). Postharvest biological control of gray mold rot of pear with *Cryptococcus laurentii*. *Postharvest Biology and Technology* 35 (1), 79–86.

Zhu YM, Rudell DR, Mattheis JP. (2008) Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1-carboxylate synthase gene expression and volatile ester emission during apple fruit maturation and ripening. *Postharvest Biol Technol*;49:330–9.

Zhu, S.J. (2006). Non-chemical approaches to decay control in postharvest fruit. In: Nouredine, B., Norio, S. (Eds.), *Advances in Postharvest Technologies for Horticultural Crops*. Research Signpost, Trivandrum, India, pp. 297–313.