CHAPTER 13

Genetic Modification in Fruits and Vegetables for Improved Nutritional Quality and Extended Shelf Life

Khalid Z. Masoodi*, Saba Mir*, Shabir H. Wani,†, Farheena Shah*, Minu B. Balkhi‡, Sajad M. Zargar***

*Division of Plant Biotechnology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

**Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, Wadura, Sopore, Jammu and Kashmir, India

†Michigan State University, East Lansing, MI, United States

‡GHHS Mirgund, J&K Govt., Srinagar, Jammu and Kashmir, India

1 INTRODUCTION

Fruits and vegetables are grown worldwide and make up a major portion of the human diet in many parts of the world. They play a significant role in human nutrition, especially as sources of vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber, and phytochemicals (Dias and Ryder, 2011). Fruits and vegetables in the daily diet have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases, such as diabetes, and some forms of cancer (Keatinge et al., 2010). Fruit and vegetable consumption worldwide is rising, reflecting the consumer's increased income, desire of diversity, and awareness of nutritional benefits. A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays, 2011; Kays and Dias, 1995). Leafy vegetables—of which the leaves or young leafy shoots are consumed—were the most often utilized (53% of the total), followed by vegetable fruits (15%), and vegetables with belowground edible organs comprised 17%. Many vegetable crops have more than one part used. Most of the vegetables are marketed fresh with only a small proportion processed because most vegetables are perishable. Consumption shortly after harvest guarantees optimal vegetable quality. About 3 billion people in the world are malnourished due to imbalanced diets (Keatinge et al., 2010; Pfeiffer and McClafferty, 2007). Underconsumption

Preharvest Modulation of Postharvest Fruit and Vegetable Quality
http://dx.doi.org/10.1016/B978-0-12-809807-3.00013-5

of vegetables and fruits is among the top 10 risk factors leading to micronutrient malnutrition and is associated with the prevalence of chronic diseases (Dias, 2011; WHO, 2003). More than 70% of malnourished children live in Asia. At least half of the preschool children and pregnant women are affected by micronutrient deficiencies in Bangladesh, Cambodia, Nepal, and the Philippines (Talukder et al., 2010). Fruits and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases, such as cardiovascular disease (CVD) and certain cancers. According to *The World Health Report 2002*, low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of strokes worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased. Recommendations in this direction tend to complement and reinforce other valid messages based on the long-known health benefits of consuming vegetables and fruit as dietary sources of fiber, proteins, and protective micronutrients. The recent Joint FAO/WHO Expert Consultation on diet, nutrition, and the prevention of chronic diseases, recommended the intake of a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases, such as heart disease, cancer, diabetes, and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less-developed countries. The recommendation thus adds to the already strong case for the health benefits to be gained from the consumption of fruits and vegetables

2 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, low nutritional quality, climate change, high perishability or postharvest decays, and short shelf life associated with fruits and vegetables. Genetic engineering has the potential to address some of these most challenging constraints faced by vegetables and fruit growers, which are not easily addressed through conventional plant breeding alone. Many vegetables exhibit a very short life span after harvesting and require very elaborate measures to expand their life. Reducing the rate of senescence in these crops is not an easy task either by conventional or biotechnological methods. The main obstacle to devising new technologies is the complexity of the problem

and lack of basic knowledge about the biochemical and cellular processes accompanying postharvest-induced senescence. This is accentuated by the extraordinary variety of tissue types that are commercialized. Early attempts to use genetic manipulation to alter senescence have been based on hormone physiology, either enhancing cytokinin production or blocking ethylene production or perception. In order to extend the postharvest life of leafy vegetables we first need to focus on the events that occur in regular leaves during senescence.

Most fruits ripen, deteriorate in appearance and eating quality, and succumb to postharvest diseases very rapidly after harvest. Poor postharvest characteristics, such as deficient flavor development, very short shelf life, quick softening, easy spoilage, sensitivity to low temperatures (chilling injury), and easy pathogen attack (fungi, etc.), are major constraints to profitability for the domestic market, and to the expansion of existing and new export markets. Among all fruits, tropical fruits are notorious for their poorer-than-average postharvest qualities. Two major obvious targets to improve the postharvest characteristics of fruits are (1) extension of shelf life and (2) resistance to pathogen attack. The ripening process involves a large number of biochemical pathways in the fruit that will result in marked changes in texture, taste, and color. At the molecular level there are a large number of genes involved and they are tightly regulated in order to induce the right changes at the right time in a highly coordinated process. In general, fruits are classified as climacteric or nonclimacteric depending on their patterns of respiration and ethylene synthesis during ripening. Climacteric fruits are characterized by an increased respiration rate at an early stage in the ripening process accompanied by autocatalytic ethylene production whereas nonclimacteric fruits show a different respiration pattern and display a lack of autocatalytic ethylene synthesis. Many of the economically important fruit crops are climacteric; therefore, a large amount of research has been devoted to studying the biochemical and molecular pathways operating during the climacteric ripening of fruits. Most of the genetic engineering approaches attempted in order to improve the shelf life and general appearance of fruits have centered on the set of genes controlling fruit firmness (membrane and cell wall properties) and the ripening rate (ethylene production or perception). These approaches have targeted endogenous genes with vital functions in the ripening process aiming to downregulate their activity by gene silencing. 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 (Barkai-Golan, 2001; Droby, 2006; Sharma et al., 2009). The situation is far more exasperating in the developing countries, where postharvest decays are often more than 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 (Korsten, 2006; Singh and Sharma, 2007; Zhang et al., 2007). 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 (Barkai-Golan, 2001; Sharma et al., 2009; Singh and Sharma, 2007; Zhang et al., 2005, 2007). For instance, fungal diseases like gray mold, powdery mildew, and downy mildew in grapes do notably only cause losses in yield but also reduce wine quality (Compass, 2006). However, the advances in biotechnology can be employed to develop fruits and vegetables with improved quality and shelf life. The ability to maintain the quality of stored fruits and vegetables during postharvest storage is highly related to the physiological, biochemical, and molecular traits of the plant from which they derive (Lers, 2012). 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 shelf life and nutritional qualities of fruits and vegetables. Moreover, the nutritional value of fruits and vegetables depends on their composition, which shows a wide range of variation depending on the species, cultivar, and maturity stage. The composition of fruits and vegetables includes a great number of metabolites. It could be predicted that no single commodity might be rich in all these constituents, which might be one of the reasons that consumption fruit and vegetable is still below the dietary guideline goal. However, the biotechnological approaches have the potential to overcome these limitations, which is not possible by conventional breeding and the knowledge of these biotechnological approaches have not only led to major improvements in the extended shelf life of fruits and vegetables but improved nutritional quality as well.

3 TOMATO AS AN IMPORTANT MODEL SYSTEM FOR FLESHY FRUIT RIPENING

Tomato is the centerpiece system for genetic and molecular research in the family Solanaceae has emerged as a model for fleshy fruit ripening. It is due to its facilitating attributes including simple genetics, numerous characterized mutants, cross fertile wild germplasm to promote genetic studies and routine transformation technology. Recently it has been taken for genome sequencing by an international consortium currently funded and supported by 10 contributing countries. From the perspective of genetic and molecular research, tomato has advantages, such as ease of seed and clonal propagation, short generation time (approximately 45–100 days), efficient cross- and self-pollination ability, and year-round growth potential in the greenhouse has made tomato a plant of choice for fruit-ripening studies as well.

4 TOMATO RIPENING STAGES

Once the tomato fruit completes its development and attains final size then it is in mature green (MG) stage. The fruit then stops growing and starts ripening by sequential stage transition. Ripening process in tomato sequentially passes through six stages, based on the percentage of the external color: MG (no external red coloration), breaker (<10% red color at blossom end), turning (10%–30% of fruit surface having red color), pink (30%–60% of fruit surface having red shade), light red or orange (60%–90% of fruit surface having red color), and red (at least 90%–95% of fruit surface having red color). The key regulator for all the changes during ripening is the climacteric rise of ethylene observed in breaker stage.

Fruit ripening is a developmental process that is exclusive to plants whereby mature seed-bearing organs undergo physiological and metabolic changes that promote seed dispersal (Seymour, 1993). Anatomically, fruits are swollen ovaries that may also contain associated flower parts. Their development follows fertilization, and occurs simultaneously with seed maturation. Initially, fruits enlarge through cell division and then by increasing cell volume. The embryo matures and the seed accumulates storage products, acquires desiccation tolerance, and loses water. The fruit then ripens. Fruit ripening is a highly coordinated, genetically programmed, and an irreversible phenomena involving a series of physiological, biochemical, and organoleptic changes that finally lead to the development of a soft edible ripe fruit with desirable quality attributes (Seymour et al., 2002).

During maturation stage several structural and biochemical changes occur in fruit, which confers on them specific organoleptic qualities, such as modifications in the external aspect, texture, and flavor of the fruit. Although the specific biochemical programs resulting in ripening phenomena vary among species, changes typically include:

1. modification of color through the alteration of chlorophyll, carotenoid, and/or flavonoid accumulation;
2. textural modification via alteration of cell turgor and cell wall structure and/or metabolism;
3. modification of sugars, acids, and volatile profiles that affect nutritional quality, flavor, and aroma; and
4. generally enhanced susceptibility to opportunistic pathogens (likely associated with the loss of cell wall integrity).

The series of cell divisions followed by a phase of cell expansion stops after reaching maturity. The tomato maturation process is accompanied with alterations in the texture of the fruit, more specifically the loss of firmness, due to structural changes in the principle cell wall components (cellulose, hemicellulose, and pectin). The change in the color of tomato fruit results from transformation of chloroplasts into chromoplasts and from the degradation of chlorophyll, as well from the accumulation of pigments, such as carotenes and lycopenes, which are responsible for the orange and red color of the fruit (Gray et al., 1994). Finally, the accumulation of sugars, such as glucose and fructose and organic acids in vacuoles and the production of complex volatile compounds are responsible for the aroma and flavor of the fruit (Seymour, 1993).

5 BIOTECHNOLOGICAL APPROACHES FOR SHELF LIFE AND NUTRITIONAL QUALITY OF FRUITS AND VEGETABLES

Biotechnological approaches enable plant breeders to bring favorable genes, often previously inaccessible, into already elite cultivars, improving their value considerably and offering unique opportunities for extending the shelf life and improving nutritional quality of the produce (Dias et al., 2013). In this chapter we describe several advances of transgenic vegetables and fruits to nutritional quality and shelf life, very important for consumers. Many reviews have reported the wide range of determinants of desirable quality attributes in fresh fruits and vegetables, such as nutritional value, flavor, color, texture, processing qualities, and shelf life (Bapat et al., 2010; Vadivambal and Jayas, 2007). By regulating the activity of enzymes involved

in senescence of vegetables and fruit ripening, such as cell wall–degrading enzyme polygalacturonase, or ethylene biosynthesis, it is possible to control or delay the vegetable senescence and fruit softening allowing the vegetable and fruit to stay longer on the plant for greater flavor and texture development, and improving its shelf life. The shelf life of transgenic tomato fruits was reported to last for at least 60 days at room temperature without significant change in hardiness and color. After 15–20 days of treatment of the transgenic fruits with ethylene, most of the tomatoes reached the ripe stage. RNAi technique has also been used to produce tomato fruit with delayed ripening using ACO gene.

Overexpression of Nr (wild-type) gene, in tomato using constitutive 35S promoter produced plants that were less sensitive to ethylene. As ethylene receptors belong to a multigene 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 (Ciardi et al., 2000). Antisense plants developed using LeETR4 under the control of CaMV35S promoter exhibited a constitutive ethylene response and were severely affected (Tieman et al., 2000). The antisense plants that were developed using this receptor with fruit-specific promoter, fruits showed early ripening (Hackett et al., 2000; Kevany et al., 2008), 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 modes 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 (Brummell and Harpster, 2001; Rose et al., 2004). Due to accelerated fruit softening, excessive spoilage occurs, which needs to be checked. Transgenic rin plants, which accumulated reduced amounts of endogenous PG, provided clues to develop antisense PG transgenic under the control of E8 promoter. These transgenics produced fruit with PG enzyme activity that was 60% of wild-type and did not affect softening much. Downregulation 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., 2004; 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 the 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, Phan et al. (2007) found antisense suppression of pectinesterase under CaMV35S promoter produced fruits with reduced PE activity and suppression in the rate of softening during ripening. In tomato, a large and divergent multigene family encodes EGases (cellulases), which consists of at least eight members. Rose et al. (2004) reported that mRNA accumulation of the highly divergent EGases LeCel1 and LeCel2 was suppressed individually by constitutive expression of antisense transgenes. 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 levels during fruit development (Smith and Gross, 2000). Transgenic plants have been developed using members of this family to reduce the 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 exogalactanase 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). Overexpression 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 upregulated in Sl-ERF2- overexpressing seeds, suggesting that Sl-ERF2 stimulated seed germination through the induction of the mannanase 2 gene. Fruits of this cultivar, called delayed fruit deterioration (DFD) undergo normal ripening but remain firm and show no loss of integrity for at least 6 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). Ethylene response factors (ERFs) play an important role in modulating ethylene-induced ripening in fruits. These ERFs belong to a 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. Overexpression of LeERF1 in tomato caused the typical ethylene triple response on etiolated seedling. Antisense LeERF1 fruits showed longer shelf life as compared to wild-type tomato (Li et al., 2007). Based on biochemical and biomechanical 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 involves micro-RNAs (miRNAs) (Jones-Rhoades et al., 2006). Though miRNAs and their targets have been identified in the 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; 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 was reported between ethylene and aroma production during apple ripening (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 nonfruit 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 postharvest 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 that 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. 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. Postharvest 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 postharvest 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 postharvest biotechnology of avocado has been strongly limited in spite of the fact that it provided early clues to the ripening mechanism in fleshy fruit. Symons et al. (2006) have shown that brassinosteroids (BRs) (steroidal hormones) might be implicated in ripening of nonclimacteric fruits. The group isolated BR-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 BRs and ripening

could be delayed by brassinozole, 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 nonclimacteric species involving BRs. Recent advances in recombinant DNA technology and genetic engineering have opened up the possibility to manipulate ripening in fast perishable fruits like banana. Toward this, Kesari et al. (2007) and Gupta et al. (2006) reported many genes involved in ripening have been cloned and characterized. Ripening in banana is characterized by a biphasic ethylene production with a sharp early peak followed by a postclimacteric 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) found 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, and so on, 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 upregulated endochitinase, β-1,3-glucanase, and BanTLP (thaumatin like protein and metallothionein) as well as downregulated 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 (Sane et al., 2007; Trivedi and Nath, 2004) and polygalacturonase genes during banana fruit ripening has been investigated (Asif and Nath, 2005). 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 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 postharvest. Fraser et al. (2002) investigated an increase in tomato fruit carotenoids phytoene, lycopene, β-carotene, and lutein in cultivar "Ailsa Craig." Phytoene synthase from the bacterium Erwinia uredovora (crtB) has been overexpressed in tomato cultivar. Fruit-specific expression was achieved by using the tomato polygalacturonase promoter, and the CRTB protein was targeted to the chromoplast by the tomato phytoene synthase-1 transit sequence. Total fruit carotenoids of primary transformants [T(0)] were 2- to 4-fold higher than the controls, whereas phytoene, lycopene, β-carotene, and lutein levels were increased 2.4-, 1.8-, and 2.2-fold, respectively. The biosynthetically related isoprenoids, tocopherols, plastoquinone, and ubiquinone were unaffected by changes in carotenoid levels. The progeny T(1) and T(2) generations inherited both the transgene and phenotype. Ripe tomato fruits accumulate large amounts of lycopene and small amounts of β-carotene (provitamin A). Lycopene is transformed into β-carotene by the action of lycopene beta-cyclase (beta-Lcy). Rosati et al. (2000) introduced, via *Agrobacterium*-mediated transformation, DNA constructs aimed at upregulating (OE construct) or downregulating (AS construct) the expression of the beta-Lcy gene in a fruit-specific fashion. Three tomato transformants containing the OE construct show a significant increase in tomato fruit β-carotene content. The tomato fruits from these plants display different color phenotypes, from orange to orange-red, depending on the lycopene/beta-carotene ratio. Fruits from AS transformants show up to 50% inhibition of beta-Lcy expression, accompanied by a slight increase in lycopene content. Leaf carotenoid composition is unaltered in all transformants. In most transformants, an increase in total carotenoid content is observed with respect to the parental line. This increase occurs in the absence of major variations in the expression of endogenous carotenoid genes. Current advances in genetic engineering of brassicas have enabled the production of plants with alterations in a range of vitamins or amino acids for improved human nutrition. 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. Vitamin E is a lipid-soluble

antioxidant, which includes tocopherols, have α, β, γ, and δ isoforms of tocopherol with relative vitamin E potencies of 100, 50, 10, and 3%, respectively. Conversion of γ-tocopherol to α-tocopherol in vegetable crops could increase their value and importance in human health because vitamin E reduces the risk of several serious disorders (e.g., cardiovascular diseases and cancer), slows aging, and enhances the function of the immune system. Cho et al. (2005) developed transgenic lettuce plants of the cultivar "Chungchima" expressing a cDNA encoding γ-tocopherol methyltransferase to improve tocopherol composition from *Arabidopsis thaliana*. Transgene inheritance and expression in transformed plants increased enzyme activity and conversion of γ-tocopherol to the more potent α form. Wahlroos et al. (2005) produced oilseed *Brassica rapa* with increased histidine content. Folate deficiency, which is regarded as a global health problem, causes neural tube defects and other human diseases. Folates are synthesized from pteridine, *p*-aminobenzoate (PABA), and glutamate precursors. (de La Garza et al., 2007; Díaz et al., 2004) developed transgenic tomatoes by engineering fruit-specific overexpression of GTP cyclohydrolase I that catalyzes the first step of pteridine synthesis and amynodeoxychorismate synthase that catalyzes the first step of PABA synthesis.Vine-ripened fruits contained on average 25-fold more folate than controls by combining PABA- and pteridine-overproduction traits through crossbreeding of transgenic tomato plants. The achieved folate level provides a complete adult daily requirement with less than one standard serving. Grumet et al. (2007) reported enhanced sugar and carotenoid accumulation whereas Katzir et al. (2008) reported a considerable reduction in aroma production for ACO1 antisense melons. Vegetables also offer consumers a diverse mixture of nutrients that promote human health more beneficially than dietary supplements. However, the ingestion of plant-based diets rather than diets that rely primarily on animal products could limit the intake of essential nutrients, such as calcium (Ca). Consequently, genetically engineering vegetables containing increased Ca levels may boost Ca uptake, thereby reducing the incidence of Ca deficiencies, such as osteoporosis. In this regard, Park et al. (2004) modified carrots to express increased levels of the plant Ca transporter sCAX1. These carrot lines were fertile and displayed no adverse phenotypes. Further, mice and human feeding trials demonstrated increased Ca absorption from sCAX1-expressing transgenic carrots vis-à-vis controls (Morris et al., 2008). This research supports alternative means of biofortifying vegetables with bioavailable Ca. Zinc, which is also an essential element in human nutrition, as its deficiency severely impairs organ function. In experiments to fortify

lettuce with this element, Xiaofeng et al. (2002) used *Agrobacterium*-mediated gene delivery of a mouse metallothionein mutant β-cDNA in the lettuce cultivar "Salinas 88." The concentration of zinc in the lettuce transgenic plants increased to 400 μg/g dry weight, which is considerably higher than in wild-type plants. Flavonoids are polyphenols whose dietary intake has the potential to prevent chronic diseases. Schijlen et al. (2006) introduced heterologous, flavonoid pathway genes—stilbene synthase, chalcone synthase, chalcone reductase, chalcone isomerase, and flavone synthase—to produce novel flavonoids in tomato fruit. These novel flavonoids-flavones and flavonols increased threefold, mostly in the Q12 peel, which had higher total antioxidant capacity. These findings add further support to the potential of engineering tomato fruit for accumulation of high levels of beneficial nutrients. Similarly, the polyphenol resveratrol, a stilbene, shows cancer chemopreventative activity and may prevent coronary heart disease and arteriosclerosis. Liu et al. (2006) in a quantitative analysis showed that resveratrol in transgenic lettuce plants was 56.0 ± 5.52 μg/g leaf fresh weight, which is comparable to that in the skin of grape fruit (*Citrus* × *paradisi* Macfad.). Flavonoids, such as anthocyanins are known as antioxidants in vitro and can reduce the risk of many diseases related to aging. However, some vegetable brassicas, such as cauliflower, are low in anthocyanins. In an attempt to manipulate pigment biosynthesis to increase the health benefits of brassica vegetables, the effect of a regulatory locus of flavonoid content was assessed. *Agrobacterium tumefaciens*—mediated transformation of a *Brassica oleracea* line—selected for high transformation ability by Sparrow et al. (2004), was used to produce plants transgenic for the maize lc (leaf color) locus. Lc is a regulatory gene in the anthocyanin pathway, and it is expected that its presence will increase the flavonoid content. Seedling explants were cocultivated with *A. tumefaciens* strain LBA4404 containing a binary vector Q27 with a neomycin phosphotransferase II (NPTII) gene. Under tissue culture conditions, lc-containing plants were green with no visible increase in anthocyanin production. However, after transfer to the greenhouse, the exposure to high-light intensity led to visible signs of pigmentation within 1 week. Increased pigmentation was apparent in stems, petioles, main leaf veins, and sepals. Lc-containing lines had 10–20 times higher levels of total anthocyanins than controls. In addition, antioxidant activity of lc-containing lines was 1.5 times higher than that of controls (Braun et al., 2006). The unique flavor and odor of alliums is derived from the hydrolysis of organosulfur compounds, which produces pyruvate, ammonia, and volatile sulfur compounds (Randle and Lancaster, 2002). This reaction is catalyzed by the

enzyme alliinase, which is contained in vacuoles within cells and released upon disruption of the tissue (Lancaster and Collin, 1981). Variations in the ratios of these volatile sulfur compounds are responsible for the difference in flavors and odors between *Allium* species (Randle and Lancaster, 2002). Along with health and nutritional benefits associated with these compounds, these thiosulfides are also major contributors to the bitter taste of some onions (Almeida, 2006; Randle and Lancaster, 2002). Three sets of transgenic onion plants containing antisense alliinase gene constructs (a CaMV 35S-driven antisense root alliinase gene, a CaMV 35S-driven antisense bulb alliinase, and a bulb alliinase promoter-driven antisense bulb alliinase) have been recently produced (Eady et al., 2003). Results from the antisense bulb alliinase lines have been much more encouraging, and three lines were produced with barely detectable bulb alliinase levels and activity. Progress has been confounded by the poor survival of transgenic plants. Crossing a nontransgenic open-pollinated parental line with a transgenic parental plant carrying a single transgene in the hemizygous state has conducted to a transgenic hybrid onion seed from these transgenic lines. Some resulting seeds produced by the nontransgenic parents will be hemizygous for the transgene and can be selected to give F1 heterozygous individuals containing the transgene. Self-fertilization of these individuals produces homozygous, hemizygous, and null F2 progeny for the transgene locus. These homozygous individuals can then be used to generate the bulk seed required for the production of commercial transgenic onion lines with less bitter taste. It was reported that tomato plants transformed with yeast SAM-DC gene under the control of E8 promoter showed improvement in tomato lycopene content, better fruit juice quality, and vine life (Bapat et al., 2010).

6 CHALLENGES ASSOCIATED WITH GENETICALLY MODIFIED FRUITS AND VEGETABLES

It is revealed that although biotechnological approaches are seen by the scientific community as a panacea to solve recent increased demands for fruits and vegetables, still the technology is more of a scientific jargon than a commercially viable entity. This is because of the dilemma and uncertainties that remain up to today regarding the consumption of biotechnological fruits and vegetables. Although the first biotech crop to be commercialized was a genetically modified tomato for processing as a consumer tomato paste, since then there have been comparatively few introductions of biotech fruits

and vegetables (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). Due to 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). 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 2003–08 more than 300 research papers were published describing more than 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. It was established in surveys by private sector companies during 2008–12, that the cost of intervention, development, and registration of 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. Further 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. 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, "Is biotechnology in fruit and vegetable plant production a commercial activity or simply research jargon?"

7 CONCLUSIONS

The 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, nutritional quality, and shelf life of fruits and vegetables to meet the demands of the 21st century and make important contributions to sustainable vegetable and fruit production by overcoming limiting factors, which are not easily addressed through conventional vegetable breeding alone. However, the biotechnological approaches for fruits and vegetables were more of academic jargon than a commercial reality. A barrier to the successful use of transgenic techniques might be the acceptance or lack thereof of transgenic fruit and vegetable crops by the public. 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 commitments by all stakeholders regarding increased and sustained funding, increased agricultural research and development, and less cost and time for registration and commercialization of new traits.

REFERENCES

Abano, E., Sam-Amoah, L., 2011. Effects of different pretreatments on drying characteristics of banana slices. APRN J. Eng. Appl. Sci. 6, 121–129.

Almeida, D., 2006. Manual de Culturas HortícolasEditorial Presença, Lisboa.

Anthony, V.M., Ferroni, M., 2011. Agricultural biotechnology and smallholder farmers in developing countries. Curr. Opin. Biotechnol., doi:10.1016/ j.copbio.2011.11.020.

Asif, M.H., Nath, P., 2005. Expression of multiple forms of polygalacturonase gene during ripening in banana fruit. Plant Physiol. Biochem. 43 (2), 177–184.

Bapat, V.A., et al., 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnol. Adv. 28 (1), 94–107.

Barkai-Golan, R., 2001. Postharvest disease initiation. Elsevier, Amsterdam, pp. 3–24 (Chapter 2).

Braun, R., et al., 2006. *Agrobacterium* mediated transformation of *Brassica oleracea* with the Lc locus. International Association for Plant Tissue Culture and Biotechnology, Beijing, p. 115.

Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47, 311–340.

Carey, A.T., et al., 2001. Down-regulation of a ripening-related β-galactosidase gene (TBG1) in transgenic tomato fruits. J. Exp. Bot. 52 (357), 663–668.

Cho, E.A., et al., 2005. Expression of γ-tocopherol methyltransferase transgene improves tocopherol composition in lettuce (*Latuca sativa* L.). Mol. Cells 19 (1), 16–22.

Choudhary, B., Gaur, K., 2009. The Development and Regulation of Bt Brinjal in India (Eggplant/Aubergine). ISAAA Brief No. 38. ISAAA, Ithaca, NY.
Ciardi, J.A., et al., 2000. Response to *Xanthomonas campestris* pv. vesicatoria in tomato involves regulation of ethylene receptor gene expression. Plant Physiol. 123 (1), 81–92.
Clendennen, S.K., May, G.D., 1997. Differential gene expression in ripening banana fruit. Plant Physiol. 115 (2), 463–469.
Compass, G., 2006. GMO database. Genetically modified food and feed: authorization in the EU, p. 13. Available from: http://www.gmo-compass.org/eng/gmo/db
Dandekar, A.M., et al., 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Trans. Res. 13 (4), 373–384.
de La Garza, R.I.D., Gregory, J.F., Hanson, A.D., 2007. Folate biofortification of tomato fruit. Proc. Natl. Acad. Sci. USA 104 (10), 4218–4222.
Dias, J.S., 2011. World Importance, Marketing and Trading of Vegetables. International Society for Horticultural Science (ISHS), Leuven, Belgium.
Dias, J.S., Ryder, E.J., 2011. World vegetable industry: production, breeding, trends. Horticultural Reviews vol. 38, pp. 299–356.
Dias, J.S., et al., 2013. Transgenic vegetables for Southeast Asia. SEAVEG 2012 High Value Vegetables in Southeast Asia: Production, Supply and Demand, p. 361.
Díaz, d.l.G.R., et al., 2004. Folate biofortification in tomatoes by engineering the pteridine branch of folate synthesis. Proc. Natl. Acad. Sci. USA 101 (38), 13720–13725.
Droby, S., 2006. Improving Quality and Safety of Fresh Fruits and Vegetables After Harvest by the Use of Biocontrol Agents and Natural Materials. International Society for Horticultural Science (ISHS), Leuven, Belgium.
Eady, C., et al., 2003. *Agrobacterium tumefaciens*-mediated transformation and regeneration of herbicide resistant onion (*Allium cepa*) plants. Ann. Appl. Biol. 142 (2), 213–217.
Fraser, P.D., et al., 2002. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc. Natl. Acad. Sci. USA 99 (2), 1092–1097.
Gray, J., et al., 1994. The use of transgenic and naturally occurring mutants to understand and manipulate tomato fruit ripening. Plant Cell Environ. 17 (5), 557–571.
Grumet, R., et al., 2007. New insights into reproductive development in melon (*Cucumis melo* L.). Int. J. Plant Dev. Biol. 1, 253–264.
Gupta, S.M., et al., 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 (1), 16–22.
Hackett, R.M., et al., 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 (3), 1079–1086.
Huang, F.-C., Do, Y.-Y., Huang, P.-L., 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 (11), 3859–3868.
James, C., 2010. Global Status of Commercialized Biotech/Gm Crops 2010. ISAAA, Brief No. 42. ISAAA, Ithaca, NY.
Jayaraman, K., 2010. Bt brinjal splits Indian cabinet. Nat. Biotechnol. 28, 296.
Jones-Rhoades, M.W., Bartel, D.P., Bartel, B., 2006. MicroRNAs and their regulatory roles in plants. Annu. Rev. Plant Biol. 57, 19–53.
Katzir, N., Harel-Bega, R., Protnoy, V., Tzuri, G., Koren, E., Lev, S., Bar, E., Tadmor, Y., Burger, Y., Lewinsohn, E., Fei, Z., 2008. Melon Fruit Quality: A Genomic Approach. INRA, Monfavet, Avignon, France.
Kays, S.J., 2011. Cultivated Vegetables of the World: A Multilingual Onomasticon. University of Georgia. Wageningen Academic Publishers, The Netherlands, p. 184.
Kays, S.J., Dias, J.C.S., 1995. Common names of commercially cultivated vegetables of the world in 15 languages. Econ. Botany 49 (2), 115–152.

Keatinge, J.D.H., et al., 2010. Relearning old lessons for the future of food—by bread alone no longer: diversifying diets with fruit and vegetables. Crop Sci. 50 (Suppl. 1), S-51–S-62.
Kesari, R., Trivedi, P.K., Nath, P., 2007. Ethylene-induced ripening in banana evokes expression of defense and stress related genes in fruit tissue. Postharvest Biol. Technol. 46 (2), 136–143.
Kevany, B.M., Taylor, M.G., Klee, H.J., 2008. Fruit-specific suppression of the ethylene receptor LeETR4 results in early-ripening tomato fruit. Plant Biotechnol. J. 6 (3), 295–300.
Korsten, L., 2006. Advances in control of postharvest diseases in tropical fresh produce. Int. J. Postharvest Technol. Innov. 1 (1), 48–61.
Lancaster, J.E., Collin, H., 1981. Presence of alliinase in isolated vacuoles and of alkyl cysteine sulphoxides in the cytoplasm of bulbs of onion (*Allium cepa*). Plant Sci. Lett. 22 (2), 169–176.
Lers, A., 2012. Potential application of biotechnology to maintain fresh produce postharvest quality and reduce losses during storage. In: Altman, A., Hasegawa, P.M. (Eds.), Plant Biotechnology and Agriculture: Prospects for the 21st Century. Academic Press, San Diego, CA, pp. 425–441.
Li, 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 (11), 1999–2008.
Liu, X., et al., 1999. Characterization of ethylene biosynthesis associated with ripening in banana fruit. Plant Physiol. 121 (4), 1257–1265.
Liu, S., et al., 2006. High content of resveratrol in lettuce transformed with a stilbene synthase gene of *Parthenocissus henryana*. J. Agric. Food Chem. 54 (21), 8082–8085.
Lohani, S., Trivedi, P.K., Nath, P., 2004. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA. Postharvest Biol. Technol. 31 (2), 119–126.
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.
Miller, J.K., Bradford, K.J., 2010. The regulatory bottleneck for biotech specialty crops. Nat. Biotechnol. 28, 1012–1014.
Moctezuma, E., Smith, D.L., Gross, K.C., 2003. Antisense suppression of a β-galactosidase gene (TB G6) in tomato increases fruit cracking. J. Exp. Bot. 54 (390), 2025–2033.
Morris, J., et al., 2008. Nutritional impact of elevated calcium transport activity in carrots. Proc. Natl. Acad. Sci. USA 105 (5), 1431–1435.
Moxon, S., et al., 2008. Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. Genome Res. 18 (10), 1602–1609.
Nath, P., Trivedi, P.K., Sane, V.A., Sane, A.P., 2006. In: Khan, N.A. (Ed.), Role of Ethylene in Fruit Ripening. Springer-Verlog, Berlin Heidelberg, pp. 151–176.
Nishiyama, K., et al., 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J. Exp. Bot. 58 (6), 1281–1290.
Park, S., et al., 2004. Increased calcium in carrots by expression of an *Arabidopsis* H^+/Ca^{2+} transporter. Mol. Breed. 14 (3), 275–282.
Pathak, N., et al., 2003. Expression and activities of ethylene biosynthesis enzymes during ripening of banana fruits and effect of 1-MCP treatment. Plant Growth Regul. 40 (1), 11–19.
Peumans, W.J., et al., 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 (2), 1063–1072.
Pfeiffer, W.H., McClafferty, B., 2007. HarvestPlus: breeding crops for better nutrition. Crop Sci. 47 (Suppl. 3), S-88–S-105.

Phan, T.D., et al., 2007. Silencing of the major salt-dependent isoform of pectinesterase in tomato alters fruit softening. Plant Physiol. 144 (4), 1960–1967.
Pirrello, J., et al., 2006. Sl-ERF2, a tomato ethylene response factor involved in ethylene response and seed germination. Plant Cell Physiol. 47 (9), 1195–1205.
Randle, W.J., Lancaster, J., 2002. Sulphur compounds in alliums in relation to flavour quality. CABI Publishing, UK, p. 329 (Chapter 14).
Rosati, C., et al., 2000. Metabolic engineering of beta-carotene and lycopene content in tomato fruit. Plant J. 24 (3), 413–420.
Rose, J.K., Saladié, M., Catalá, C., 2004. The plot thickens: new perspectives of primary cell wall modification. Curr. Opin. Plant Biol. 7 (3), 296–301.
Saladié, M., et al., 2007. A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiol. 144 (2), 1012–1028.
Sane, V.A., Sane, A.P., Nath, P., 2007. Multiple forms of α-expansin genes are expressed during banana fruit ripening and development. Postharvest Biol. Technol. 45 (2), 184–192.
Schaffer, R.J., Friel, E.N., Souleyre, E.J.F., Bolitho, K., Thodey, K., Ledger, S., Newcomb, R.D., 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 (4), 1899–1912.
Schijlen, E., et al., 2006. Pathway engineering for healthy phytochemicals leading to the production of novel flavonoids in tomato fruit. Plant Biotechnol. J. 4 (4), 433–444.
Seymour, G., 1993. Banana. Springer, pp. 83–106.
Seymour, G.B., et al., 2002. Genetic identification and genomic organization of factors affecting fruit texture. J. Exp. Bot. 53 (377), 2065–2071.
Sharma, R.R., Singh, D., Singh, R., 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. Biol. Control 50 (3), 205–221.
Silva, J.A., 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 (3), 263–268.
Singh, D., Sharma, R., 2007. Postharvest Diseases of Fruit and Vegetables and Their Management. Daya Publishing House, New Delhi, India.
Smith, D.L., Gross, K.C., 2000. A family of at least seven β-galactosidase genes is expressed during tomato fruit development. Plant Physiol. 123 (3), 1173–1184.
Sparrow, P., Dale, P., Irwin, J., 2004. The use of phenotypic markers to identify *Brassica oleracea* genotypes for routine high-throughput *Agrobacterium*-mediated transformation. Plant Cell Rep. 23 (1–2), 64–70.
Symons, G.M., et al., 2006. Grapes on steroids: brassinosteroids are involved in grape berry ripening. Plant Physiol. 140 (1), 150–158.
Talukder, A., et al., 2010. Homestead food production model contributes to improved household food security, nutrition and female empowerment: experience from scaling-up programs in Asia (Bangladesh, Cambodia, Nepal and Phillippines). Helen Keller International Nutrition Bulletin, 8 (1), p. 1.
Tateishi, A., et al., 2007. Differential expression and ethylene regulation of β-galactosidase genes and isozymes isolated from avocado (*Persea americana* Mill.) fruit. Postharvest Biol. Technol. 45 (1), 56–65.
Tieman, D.M., et al., 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. USA 97 (10), 5663–5668.
Trivedi, P.K., Nath, P., 2004. MaExp1, an ethylene-induced expansion from ripening banana fruit. Plant Sci. 167 (6), 1351–1358.
Vadivambal, R., Jayas, D., 2007. Changes in quality of microwave-treated agricultural products: a review. Biosyst. Eng. 98 (1), 1–16.
Wahlroos, T., et al., 2005. Increase of histidine content in *Brassica rapa* subsp. oleifera by overexpression of histidine-rich fusion proteins. Mol. Breed. 14 (4), 455–462.

Wang, A., et al., 2007. MdERFs, two ethylene-response factors involved in apple fruit ripening. J. Exp. Bot. 58 (13), 3743–3748.
Wang, A., 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 (1), 391–399.
WHO, 2003. Diet, nutrition and the prevention of chronic diseases. World Health Organ. Tech. Rep. Ser. 916, i–viii.
Xiaofeng, Z., et al., 2002. Expression of the mouse metallothionein mutant ββ-cDNA in the lettuces (*Lactuca sativa* L.). Chinese Sci. Bull. 47 (7), 558–562.
Yin, Z., et al., 2008. Identification of conserved microRNAs and their target genes in tomato (*Lycopersicon esculentum*). Gene 414 (1), 60–66.
Yu, X., et al., 2008. Antisense suppression of an acid invertase gene (MAI1) in muskmelon alters plant growth and fruit development. J. Exp. Bot. 59 (11), 2969–2977.
Zhang, H., et al., 2005. Postharvest biological control of gray mold rot of pear with *Cryptococcus laurentii*. Postharvest Biol. Technol. 35 (1), 79–86.
Zhang, H., Zheng, X., Yu, T., 2007. Biological control of postharvest diseases of peach with *Cryptococcus laurentii*. Food Control 18 (4), 287–291.
Zhang, J., et al., 2008. Identification of conserved microRNAs and their targets from *Solanum lycopersicum* Mill. Gene 423 (1), 1–7.
Zhu, Y., Rudell, D.R., Mattheis, J.P., 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 (3), 330–339.