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Biotechnological approaches for extended shelf life of horticultural commodities

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Abstract
Ethylene is a master regulator of ripening so ethylene production must be managed to optimize shelf-life. Altering ripening biology via refrigeration, chemicals, or other means to lengthen shelf-life, often unavoidably disrupts ripening outcomes and reduces quality. This leads to consumer rejection and postharvest waste. So, genetic solutions may be more effective. The goal of biotechnological technique is to extend shelf-life without loss of quality and therefore reduce postharvest loss and waste.

Keywords: Shelf life, quality, biotechnology, postharvest loss

Introduction

Harvested horticultural products are living tissues with continuing metabolism, and are subject to respiration, water loss and cell softening throughout the postharvest system. The main limiting factor for shelf-life and storage is excessive softening. To increase the shelf-life, plants modified for the expression and by varying the action of cell wall enzymes, which implicated in tissue softening and deterioration. Ethylene is known as fruit ripening hormone. Biosynthesis of ethylene has been disrupted for the delaying fruit ripening by inhibition of genes which are involved in ethylene biosynthetic pathway and shelf life increase can be achieved by maintaining resistance to ethylene.

Table 1: Relative storage potential of selected fruits and vegetables

Maximum storage	Fruits/ Vegetables
More than 12 months	Dried fruits and dried vegetables
6 to 12 months	Apple, European pear, carrot, garlic, ginger, pungent onion, late crop potato
3 to 6 months	Asian pear, pomegranate, kiwifruit, cabbage
1 to 3 months	Banana, mango, grape, lychee, cherry, plum, sweet lime, lemon, cauliflower, parsley, radish, sweet onion, pumpkin
Less than 1 month	Papaya, mandarin, guava, watermelon, sapota, apricot, melon, peach, nectarine, green bean, spinach, cucumber, lettuce, capsicum, chilly pepper, mature-green tomato
About 1 week	Brinjal, okra, green pea, ripe tomato

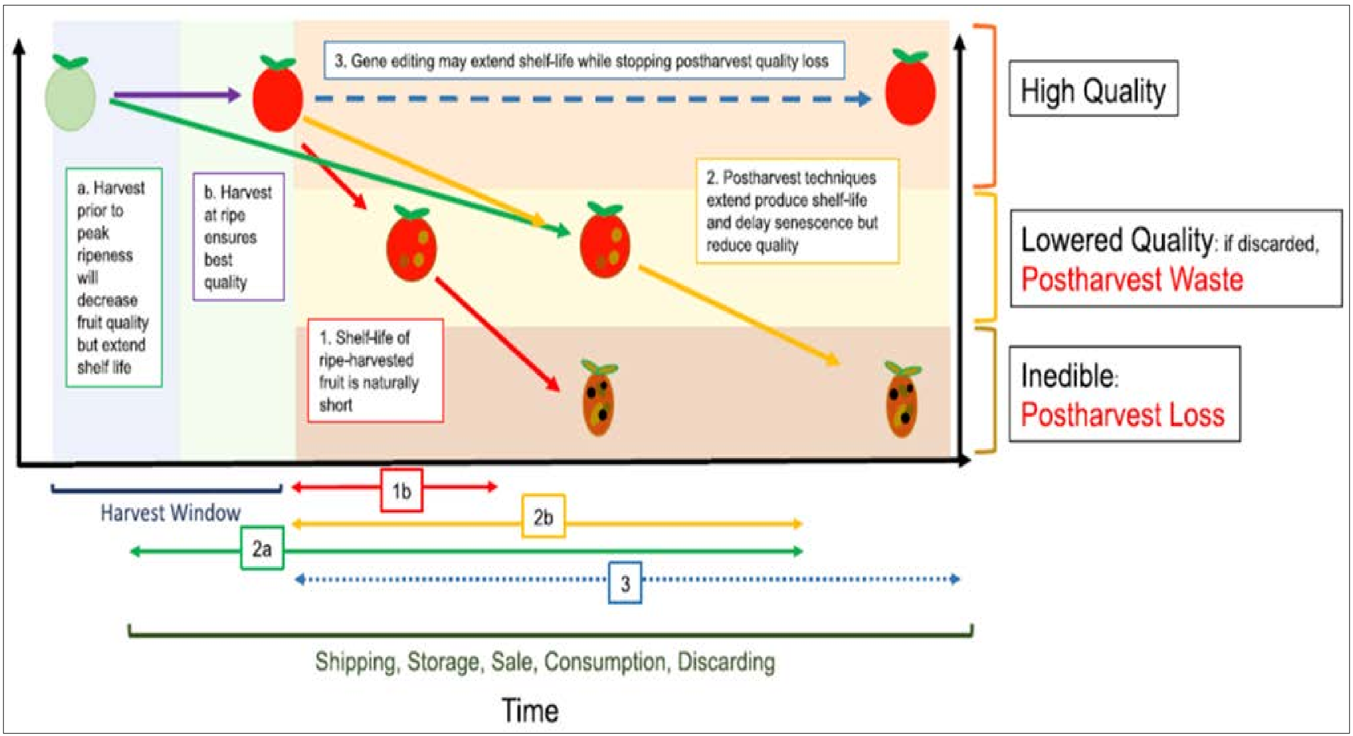
Source: www.nhb.gov.in [74]

Table 2: Recommended commercial storage conditions for important flowers at 90-95% RH

Storage	Crop	Storage temp. (°C)	Maximum storage period (days)
Dry	Carnation	0-1	16-24
	Chrysanthemum	0.5-1	21
	Gerbera	2	2
	Gladiolus	4-5	5-7
	Rose	0.5-2	7
Wet	Anthurium	13	14-28
	Carnation	0.5-1	21-28
	Dendrobium	5-7	10-14
	Gerbera	4	4-7
	Gladiolus	4-5	7
	Tuberose	7-10	3-5
	Rose	2-3	5-7

Source: www.ecoursesonline.iasri.res.in [73]

Need of biotechnological approach



Source: Shipman *et al.*, 2021 [64]

Fig 1: Potential postharvest outcomes for produce. Harvesting fruit prior to full ripeness will increase its shelf-life [a], but compromises quality during and after ripening [2a]. Fruit harvested at ripe [b] has a limited shelf-life before it declines in quality or rots [1b]. Postharvest intervention delays senescence and typically also results in some compromise of quality [2b]. The goal of gene editing is to extend shelf-life without loss of quality [3] and therefore reduce postharvest loss and waste [64].

Ethylene is a master regulator of ripening so ethylene production must be managed to optimize shelf-life. Altering ripening biology via refrigeration, chemicals, or other means to lengthen shelf-life, often unavoidably disrupts ripening outcomes and reduces quality. This leads to consumer rejection and postharvest waste. So, genetic solutions may be more effective. The goal of biotechnological technique is to extend shelf-life without loss of quality and therefore reduce postharvest loss and waste.

Table 3: Classification of horticultural commodities according to respiration rates

Class	Range at 15 °C (mg CO_2/Kg-hr)	Commodities
Very low	< 5	Dates, Nuts
Low	5-10	Apple, Celery, Citrus fruits, Garlic, Grape, Kiwifruit, Onion, Persimmon, Pineapple, Potato, Sweet potato, Watermelon
Moderate	10-20	Apricot, Cabbage, Cantaloupe, Carrot, Cherry, Cucumber, Pig, Gooseberry, Lettuce, Nectarine, Olive, Peach, Pear, Pepper, Plum, Tomato
High	20-40	Avocado, Cauliflower, Lima bean, Raspberry
Very high	40-60	Artichoke, Broccoli, Green onion, Snap beans
Extremely high	> 60	Asparagus, Mushroom, Parsley, Peas, Sweet corn

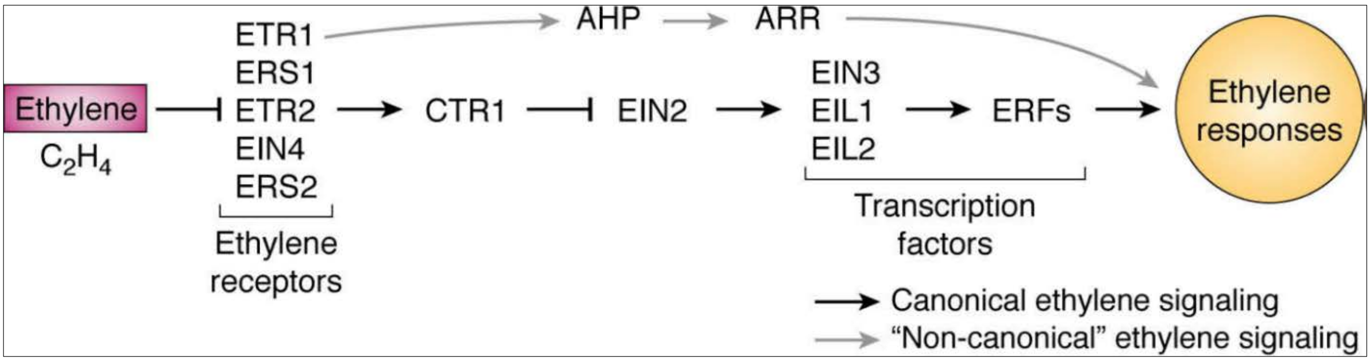
Source: Fallik and Aharoni, 2004 [19]

Table 4: Classification of horticultural commodities according to ethylene production rates

Class	Range at 20 °C (µl C_2H_4/Kg-hr)	Commodities
Very low	Less than 0.1	Artichoke, Asparagus, Cauliflower, Cherry, Citrus, Grape, Jujube, Strawberry, Pomegranate, Leafy vegetables, Root vegetables, Potato, Most cut flowers
Low	0.1-1	Blueberry, Cranberry, Cucumber, Eggplant, Okra, Olive, Pepper, Persimmon, Pineapple, Pumpkin, Raspberry, Watermelon
Moderate	1-10	Banana, Fig, Guava, Melon, Mango, Plantain, Tomato
High	10-100	Apple, Apricot, Avocado, Cantaloupe, Kiwifruit, Nectarine, Papaya, Peach, Pear, Plum
Very high	More than 100	Cherimoya, Passion fruit, Sapote

Source: Pesis, 2004) [57]

Ethylene regulation in ripening

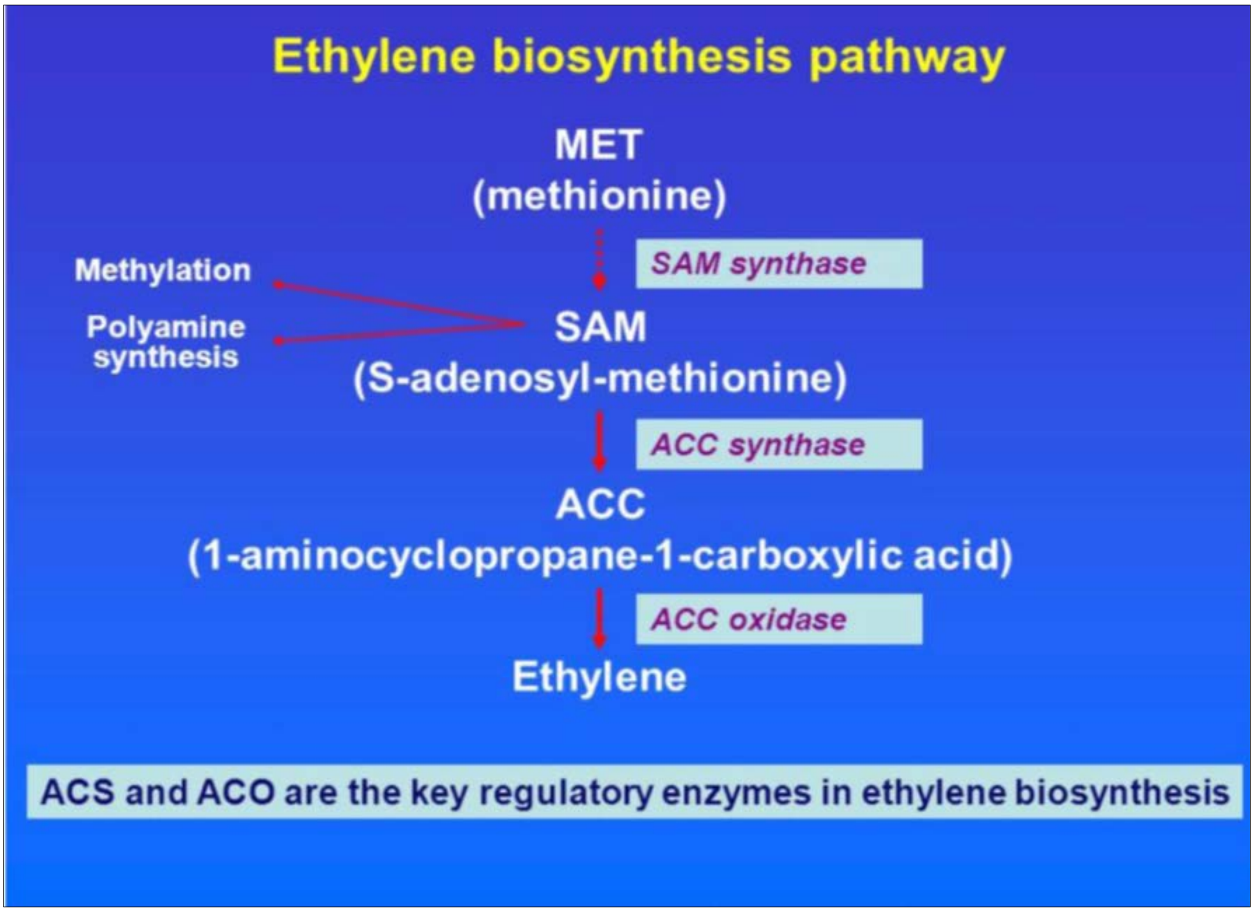


Source: Binder, 2020) [5].

Fig 2: Simple genetic model of ethylene signalling. In black is shown a model for ethylene signalling based on molecular genetic experiments in *Arabidopsis*. These experiments showed that ethylene signalling involves ethylene receptors (ETR1, ERS1, ETR2, EIN4, and ERS2), the protein kinase CTR1, and EIN2 that signals to the transcription factors EIN3, EIL1, and EIL2. These, in turn, signal to other transcription factors, such as the ERFs, leading to ethylene responses. This has long been considered the canonical signalling pathway. In this model, CTR1 is a negative regulator of signalling. Ethylene functions as an inverse agonist, where it inhibits the receptors, which leads to lower activity of CTR1 releasing downstream components from inhibition by CTR1. More recent evidence has shown the existence of an alternative, "no canonical" pathway (in gray), where ETR1 signals to histidine-containing AHPs and then to ARRs to modulate responses to ethylene

Involvement of other phytohormones in fruit ripening

ABA: The function of ABA as a ripening factor has recently been emphasized in several fruits. Zhang *et al.* (2009) [80] showed that endogenous ABA accumulated prior to the ethylene burst in tomato fruit, and exogenous ABA treatment promoted ethylene synthesis and fruit ripening (Source: Nath *et al.*, 2014) [50].



Ethylene biosynthesis pathway

MET
(methionine)

SAM synthase

Methylation

Polyamine synthesis

SAM
(S-adenosyl-methionine)

ACC synthase

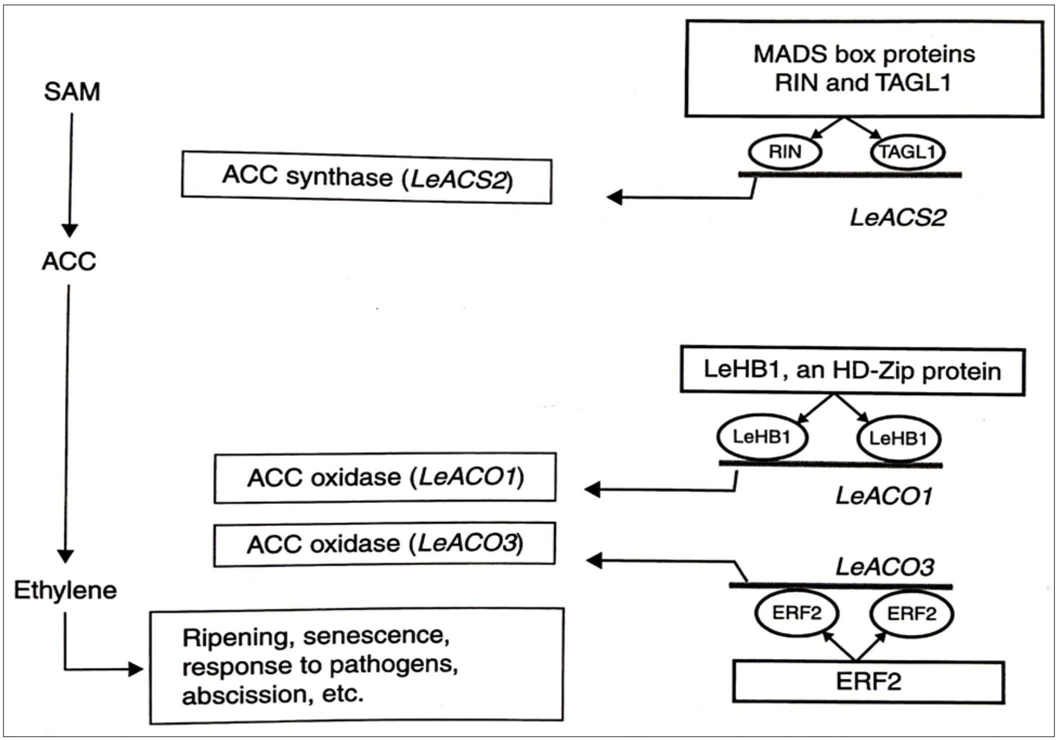
ACC
(1-aminocyclopropane-1-carboxylic acid)

ACC oxidase

Ethylene

ACS and ACO are the key regulatory enzymes in ethylene biosynthesis

Transcriptional regulation of ACO and ACS gene



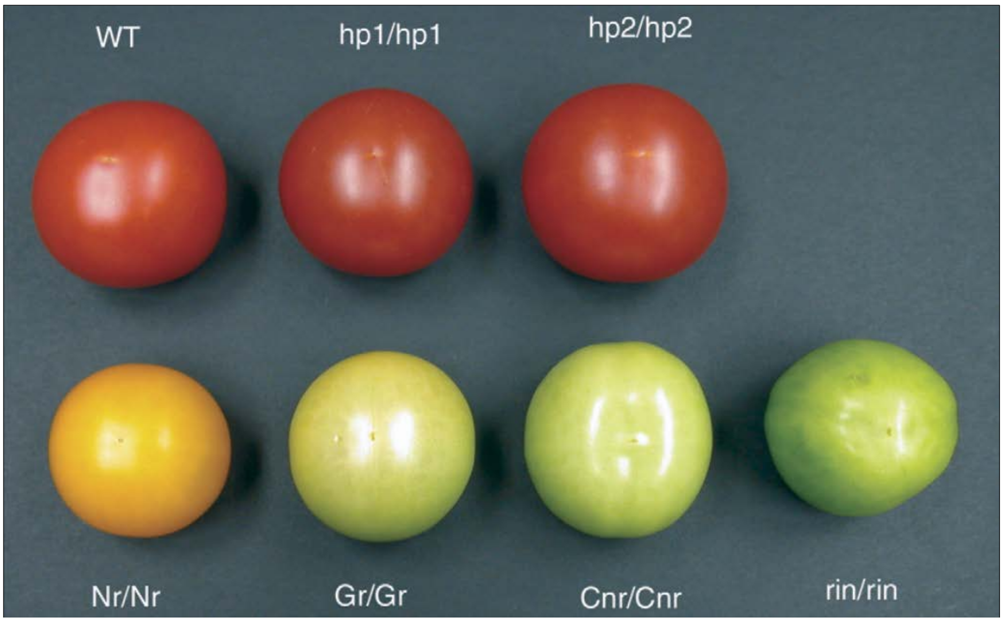
Source: Nath *et al.*, 2014 [50].

Fig 3: Transcription factors regulating *LeACS2, LeACO1* and *LeACO3* and enhanced ethylene synthesis in ripening tomato fruit

Transcription factors such as MADS box proteins *RIN* and *TAGL1* binds with *LeACS2*; *LeHB1* and *HD-Zip* Protein binds with *LeACO1*; *ERF2* binds with *LeACO3* enhanced ethylene synthesis in ripening tomato fruit.

Ripening Mutants: Master transcriptional regulators of ripening process

- 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*).
- 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.
- *rin* encodes a partially deleted MADS-box protein of the SEPELATTA clade, whereas *Cnr* is an epigenetic change that alters the promoter methylation of a SQAMOSA promoter binding (SPB) protein.



Source: Giovannoni, 2007) [22].

Fig 4: Normal and mutant tomato fruit. Normal tomato cultivar Ailsa Craig ripe fruit ten days post breaker and equivalent age fruit homozygous for the high-pigment 1 (hp1/hp1), high-pigment 2 (hp2/hp2), Never-ripe (Nr/Nr), Green-ripe (Gr/Gr), Colorless non-ripening (Cnr/Cnr) and ripening-inhibitor (rin/rin) mutations

Table 5: Application of genome editing techniques in horticultural crops to improve their shelf life

Fruit Crops	Gene(s)	System	Explants	Resistance in plants	References
Tomato	CRISPR/Cas9	A.T	*ALC*	Inhibit ethylene synthesis (SN1 is an insertion of an actual inhibitor gene *ALC*)	LÜ *et al.*, (2018) [43]
Tomato	CRISPR/Cas9 (SDN1)	A.T	*RIN*	Inhibit ethylene synthesis and specific biochemical processes related to fruit ripening	Jung *et al.*, (2018) [36]
Tomato	CRISPR/Cas9 (SDN2)	A.T	*ALC*	Inhibit ethylene synthesis (SN2 seems to be a knockout mutant of the *RIN* gene)	Yu *et al.*, (2017) [78]
Tomato	CRISPR/Cas9 (SDN1)	Not Mentioned	*SBP-CNR & NAC-NOR*	Transcription factor of ripening genes	Gao *et al.*, (2019) [21]
Potato	TALEN (SDN1)	PEG mediated	*Vinv*	Hydrolyzes the sucrose produced from starch breakdown into one molecule of glucose and one of fructose	Clasen *et al.*, (2016) [10]
Potato	CRISPR/Cas9 (SDN1)	PEG mediated	*StPPO2*	Catalyzes the oxidation of phenolic compounds into quinones (highly reactive form)	González *et al.*, (2020) [23]
Petunia	CRISPR/Cas9 (SDN1)	A.T.	*PhACO*	Catalyzes aminocyclopropane-1 carboxylic acid to ethylene in ethylene biosynthesis pathway	Xu *et al.*, (2020) [76]

Source: Kumari *et al.*, 2022 [41]

Table 6: Candidate genes in apple suitable for manipulation to extend shelf life

	Antisense/RNAi/sense technology	DNA markers
Cell-wall modification		
MdPG	Antisense: Increased firmness, reduced water loss (Atkinson *et al.*, 2012) [1]	Different SNPs correlated with softening phenotypes (Costa *et al.*, 2010) [11]
MdEXP7		Gene localized to a major QTL for firmness (Costa *et al.*, 2008) [13]
Ethylene biosynthesis		
MdACO1	Antisense: Increased firmness, reduced softening at room temperature. No change in TSS, reduced volatile esters Dandekar *et al.*, 2004 [16]; Johnston *et al.*, 2009 [35]; Schaffer *et al.*, 2007 [61]	Linkage group L10 *MdACO1-1* low ethylene (Zhu and Barritt, 2008) [82]
MdACS1	Antisense: Increased firmness, reduced softening at room temperature. No change in TSS, reduced volatile esters (Dandekar *et al.*, 2004) [16]	*MdACS1-1/MdACS1-2* high/low ethylene, respectively Costa *et al.*, 2005 [12]; Dougherty *et al.*, 2016 [17]; Harada *et al.*, 2000 [26]. *MdACS1-2* in late cultivars with better firmness Oraguzie *et al.*, 2004 [53]
MdACS3		*Mdacs3a/G289V* exists in firmer fruit (Wang *et al.*, 2009) [71]. Using restriction enzymes to identify *Mdacs3a* or G289V; *Mdacs3a* associated with delayed ethylene peak (Dougherty *et al.*, 2016) [17]
Transcription factors		
MdMADS8/9 (SEP)	Antisense: Inhibition of starch clearance, skin color change and volatile level changes. Fruit remained firm even with ethylene. Deformation of apple fruit shape (Ireland *et al.*, 2013[31]; Schaffer *et al.*, 2013) [62]	
MdMADS2.1 (FUL-AG)		Polymorphic repeat, (AT)n, in the 3′UTR of *MdMADS2.1* localized to linkage group 14 correlated with firmness (Cevik *et al.*, 2010) [8]

Source: Friedman, 2019 [20]

Table 7: Candidate genes in peach suitable for manipulation to extend shelf life

	Antisense/RNAi/ sense technology	DNA markers
Cell-wall modification		
PpPG		PG deletion caused non-melting phenotype (Lester *et al.*, 1996[42]; Peace *et al.*, 2005[56])
IAA biosynthesis		
PpYUC11		A microsatellite insertion causing stony hard phenotype (Pan *et al.*, 2015) [54]
Transcription factors		
PpPLENA (AG)	Overexpression in tomato: Enhanced ripening (Tadiello *et al.*, 2009) [66]	
PpNAC		QTL of SMF and MD co-localized and contain the gene *ppa008301m* encoding the SR phenotype (Eduardo *et al.*, 2015) [18]

Source: Friedman, 2019 [20]

Table 8: Identified candidate genes in strawberry suitable for manipulation to extend shelf life

	Antisense/RNAi/sense technology	DNA markers
Cell-wall modification		
FaPG	Antisense: firmer fruit (Quesada *et al*, 2009) [58]	Truncated PG was associated with higher fruit firmness (Villarreal *et al.*, 2008) [68]
FaPL	Antisense: Firm fruit also at overripe stage(Jiménez-Bermúdez *et al.*, 2002 [34]; Santiago-Domenech *et al.*, 2008 [59]	
FaβGAL	Antisense: Reduced fruit softening, higher sugar and smaller fruit (Paniagua *et al.*, 2015) [55]	
Upstream transcription factors		
FaSHP (AG)	Overexpression by transient expression: Enhanced ripening (Daminato *et al.*, 2013) [15]	
FaMADS9 (AG/PLE)	Antisense: Ripening delay and deformed fruit (Seymour *et al.*, 2011) [63]	

Source: Friedman, 2019 [3]

Table 9: Candidate genes in tomato suitable for manipulation to extend shelf life

	Antisense/RNAi/sense technology	DNA markers
Cell-wall modification		
SlPL	RNAi: Firmer fruit with no adverse effect on taste or color Uluisik *et al.*, 2016 [67]; Yang *et al.*, 2017 [77]	Localized to a major QTL for firmness (Uluisik *et al.*, 2016) [67]
SlEXP	Antisense and overexpression reduced and enhanced softening, respectively (Brummell *et al.*, 1999) [6]	Mutation in EXP1 by TILLING approach delayed softening (Minoia *et al.*, 2016) [47]
Slα-Man	RNAi: Suppression of tomato fruit softening (Meli *et al.*, 2010) [46]	
Slβ-Hex	RNAi: Suppression of tomato fruit softening (Meli *et al.*, 2010) [46]	
Ethylene biosynthesis		
SlACO1	Antisense: increased firmness, high TSS, flesh color maintained (Hamilton *et al.*, 1990) [25] RNAi: Delayed deterioration and color development (Xiong *et al.*, 2005) [75]	
SlACS2	Antisense: Delayed color development (Oeller and Min-Wong 1991) [52]	
Upstream transcription factors		
SlMADS-RIN (SEP)	Antisense: Reduced color development (Vrebalov *et al.*, 2002) [70]	
Slrin	CRISPR/Cas9: Enhanced ripening (Ito *et al.*, 2017) [33]	Deletion of C terminus of *SlRIN* and N terminus and *MC* creating a fusion gene. Heterozygote has a long shelf life and reduced lycopene accumulation (Kitagawa *et al.*, 2005) [38]. Homozygote has lower sugar and non-ripening phenotype (Mizrahi *et al.*, 1982) [48]
SlTAGL1 (AG)	Antisense, VIGS, CRES-T (SRDX): Reduced carotenoid and size, no effect on firmness (Itkin *et al.*, 2009[32]; Vrebalov *et al.*, 2009) [69]	
SlSBP-CNR		Hypermethylation of the CNR promoters caused delayed ripening (Kanazawa *et al.*, 2011[37]; Manning *et al.*, 2006[44])
SlNOR		Alc mutant shelf life up to 4 months. It contains an SNP in *NOR* (Casals *et al.*, 2012) [7]. *Dfd* mutant (Patent # US20150322537) has similar phenotype

Source: Friedman, 2019 [20]

Table 10: Candidate genes in melon suitable for manipulation to extend shelf life

	Antisense/RNAi/ sense technology	DNA markers
Cell-wall modification		
CmEXP		Independent QTLs for firmness harbour *EXP2*, *EXP3*, and *EXP1* genes (Moreno *et al.*, 2008) [49]
Ethylene biosynthesis/response		
CmACO1	Antisense: Increased firmness, TSS and flesh color not modified Ayub *et al.*, 1996[2]; Martínez-Madrid *et al.*, 2002[45]; Nuñez-Palenius *et al.*, 2007[51]), volatiles reduced (Bauchot *et al.*, 1998 [3]	Missense mutation by TILLING delayed softening (Dahmani *et al.*, 2010) [14]
CmACS5		Two SNPs colocalized to a no-ethylene-production QTL (Moreno *et al.*, 2008) [49]
CmEIL		Three genes of the family (*CmEIL1*, *CmEIL3*, and *CmEIL4*) are associated with 2 independent QTLs for firmness (Moreno *et al.*, 2008) [49]
Upstream transcription factors		
CmMADSRIN (SEP)	Antisense: Reduced softening and deterioration (Binzel and Giovannoni personal communication)	

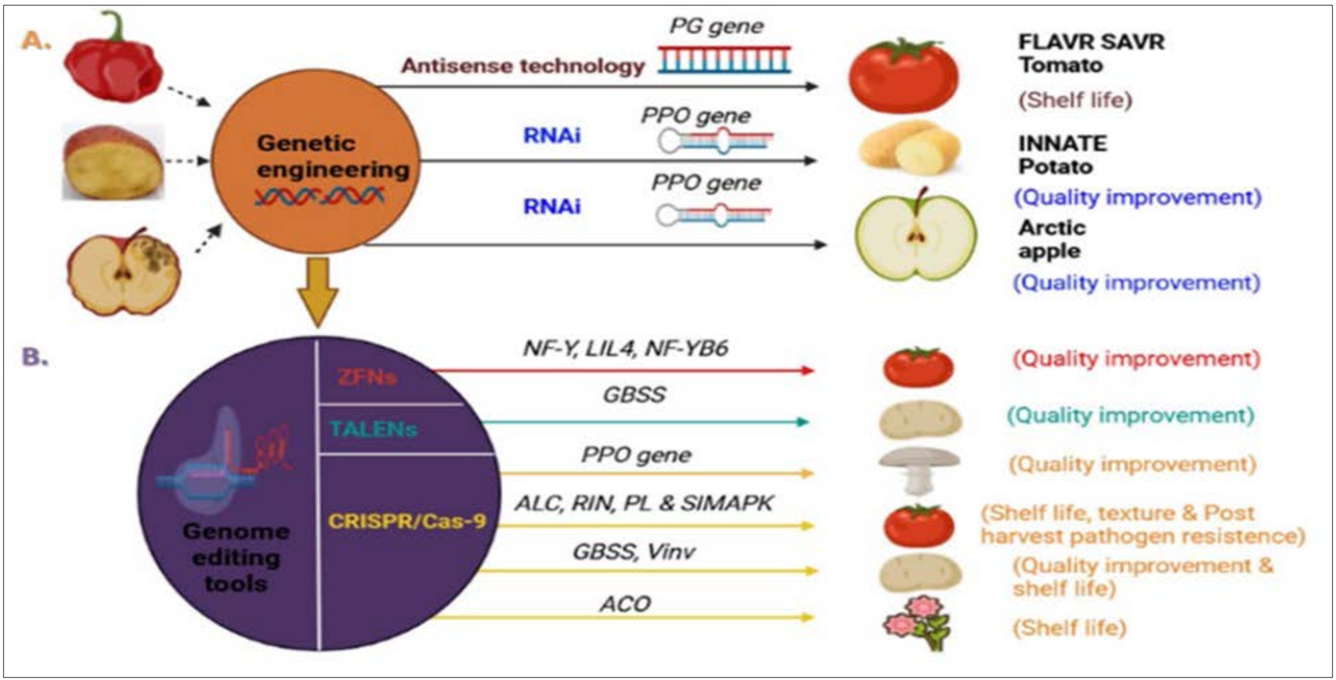
Source: Friedman, 2019 [20]

Table 11: Targets for enhanced shelf-life

Crop	Gene	Method	Phenotype	References
Tomato	*SlFSR*	RNAi	↓ Expression of cell wall modification enzymes in fruit	Zhang *et al.*, (2018) [49]
Tomato	*SlACO1*	RNAi	↓ Ethylene, ↓ firmness loss associated with ↓ PME and PG activities	Behboodia *et al.*, (2012)
Tomato	*PG*	Antisense RNA	↑ Fruit firmness, ↓ postharvest fungal infection	Kramer *et al.*, (1992)
Strawberry	*FaPG1*	RNAi	↑ Soluble solids, firmness and ↓ softening	Quesada *et al.*, (2009)
Tomato	*Del, Ros*	Ectopic Expression	Double shelf-life and ↓ susceptibility to *Botrytis*	Zhang *et al.*, (2013) [79]
Tomato	*SlALC*	CRISPR/Cas9	Extended shelf life	Yu *et al.*, (2017) [78]
Petunia (*Petunia hybrida* cv. "Mitchell diploid")	*Atetr1-1*	Ectopic Expression	Doubled vase-life	Wang *et al.*, (2013) [72]
Petunia (*Petunia hybrida* cv. Hort. Vilm.-Andr.)	*BoACO1*, *BoACS1*	Antisense	Delayed flower senescence, extended vase-life	Huang *et al.*, (2007) [29]
Petunia (*Petunia hybrida* cv. "Primetime Blue" and cv. "Mitchell Diploid")	*PhHD-Zip*	VIGS	↑ Vase-life by 20%	Chang *et al.*, (2014) [9]
Carnation (*Dianthus caryophyllus* L. cv. "Scania" and "White Sim")	*ACO*	Antisense	↑ Vase-life by 50%	Savin *et al.*, (1995) [60]
Petunia (*Petunia hybrida* cv. Mirage Rose	*PhACO1*	CRISPR	↑ Vase-life by 50%	Xu *et al.*, (2020) [76]

Source: Shipman *et al.*, 2021 [64]

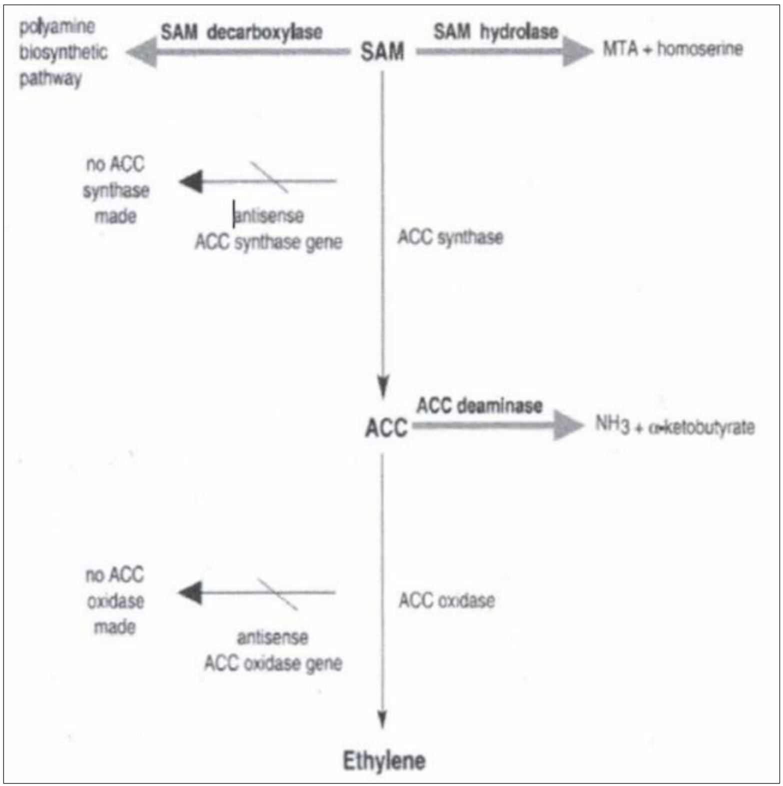
Biotechnological approaches



Source: Kumari *et al.*, 2022) [41]

Fig 5: Biotechnological approaches improved post-harvest shelf life and quality of many horticultural crops: (A) anti-sense RNA (asRNA) and RNA interference (RNAi) technologies were used to enhance shelf life and quality. The arrow from (A) to (B) depicts the transition from biotechnological tools, i.e., genetic engineering to modern genome editing tools. (B) In contrast, advanced biotechnological approach.

1) Down regulation/modification of ethylene metabolism
2) By manipulating cell wall metabolism

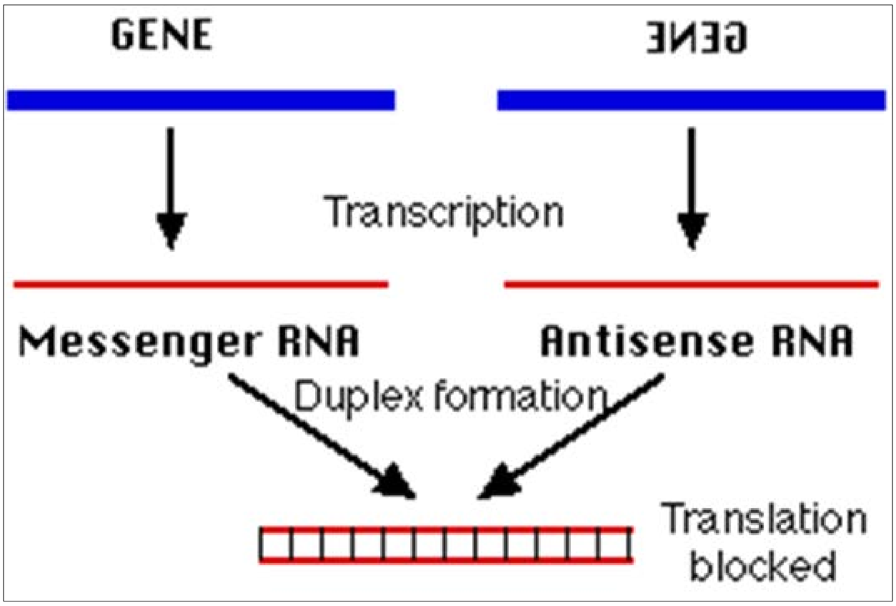


Source: Singh *et al.*, 2016) [65]

Fig 6: An overview of added or altered genes in the ethylene biosynthesis pathway.

Down regulation of ethylene metabolism
Antisense RNA technology

Antisense RNA technology has been used to suppress the expression of ACC synthase and ACC oxidase gene involved in ethylene biosynthesis and fruits in the silenced transgenic plants were found to be more resistant to over ripening and shriveling than control fruits.

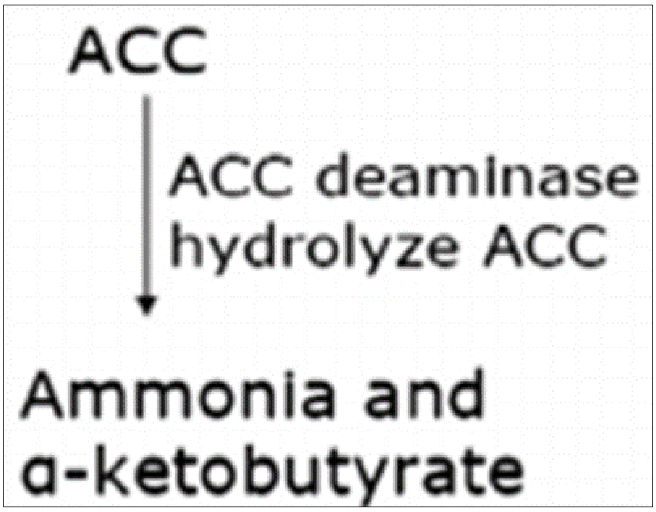


Modification of ethylene biosynthesis

- Over expression of ACC deaminase gene
- Over expression of SAM hydrolase gene
- Control of ethylene perception
- Use of Polyamine genes

Over expression of ACC deaminase gene

First discovered in soil microorganisms and shown to convert ACC to ammonia and α-ketobutyrate.

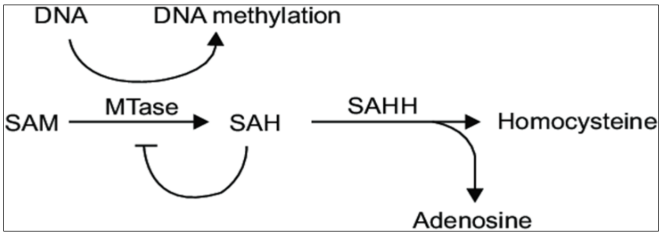


Example

- This gene has been isolated from Pseudomonas sp. and was expressed in transgenic tomato plants.
- This approach led to 90 to 97% inhibition of ethylene production during ripening in tomato.
- Fruits from these plants showed significant delays in ripening, and they remained firm for at least 6 weeks longer than the non-transgenic control fruits.

Over expression of SAM hydrolase gene

- SAMASE catalyzes the conversion of SAM to methylthioadenosine and homoserine thereby reducing the synthesis of ethylene.
- It has been used to control ethylene levels in both ripening fruit and ornamental crops.

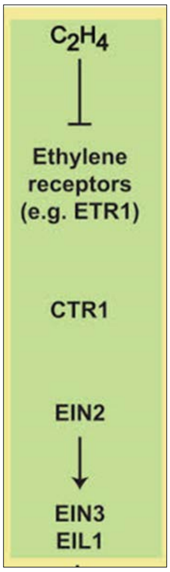


Example

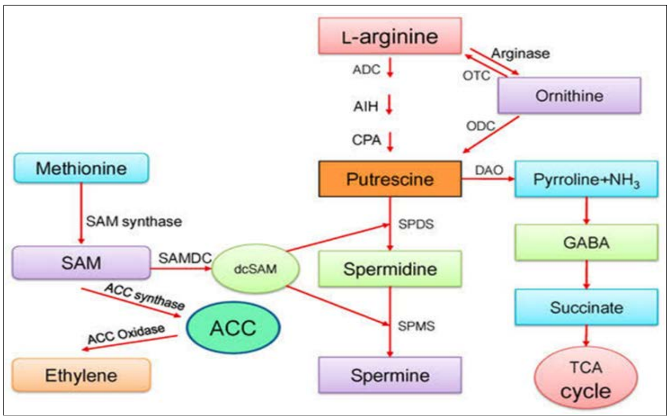
Gene from Bacteriophage T3 which encodes the enzyme S-adenosylmethionine hydrolase (SAMase) has been utilized to generate transgenic tomato plants that produce fruit with a reduced capacity to synthesize ethylene.

Control of ethylene perception

- Ethylene signals the onset of fruit ripening therefore delayed ripening in some plants can be achieved by modifying ethylene receptors.
- The ETR-1 (Ethylene receptor-1) encodes for ethylene binding protein.
- Plants modified with ETR1 lack the ability to respond to ethylene.



Use of polyamine genes



- Through utilization of SAM into polyamine biosynthesis pathway which reduce the biosynthesis of ethylene.
- Over expression of yeast spermidine synthase has been found to increase shelf life in tomato.

By manipulating cell wall metabolism

- Suppression of Polygalacturonase activity
- Use of n-glycan processing enzymes and cell wall modifying enzymes
- Down regulation of Expansins
- Over expression of genes of cytokinin biosynthesis

Suppression of Polygalacturonase activity

- Polygalacturonase (PG) is the enzyme responsible for the breakdown of pectin, the substance that maintains the integrity of plant cell walls.
- Pectin breakdown occurs at the start of the ripening process resulting in the softening of the fruit.
- To produce a fruit with delayed ripening trait scientists insert an anti-sense or a truncated copy of the PG gene into the plant's genome resulting in a dramatic reduction of the amount of PG enzyme produced, thereby delaying pectin degradation.
- This technology was used for the production of Flavr Savr tomatoes.

Flavr Savr tomato

- Developed by Calgene Company.
- 1st GM food for human consumption.
- Plants were transformed with the anti-sense Polygalacturonase (PG) gene, which is mRNA that base pair with mRNA that the plant produces, essentially blocking the gene from translation.
- These tomatoes make only 10% of the normal amount of the enzyme, thus delaying ethylene production.

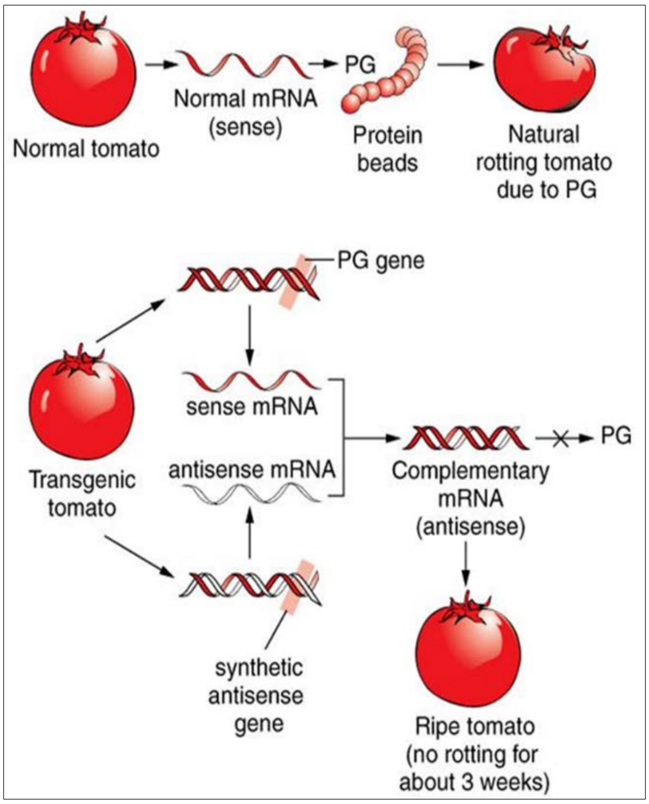
Fall of flavr savr tomato

These tomato could be shipped without refrigeration too far off places as it was capable of resisting rot for more than 3 weeks (double the time of a conventional tomato).

But there are some limitations

1. Transgenic tomatoes could not be grown properly in different parts of United States of America.
2. Yield of tomatoes was low.
3. Cost was high.

Therefore unfortunately, within a year after its entry, flavr savr was withdrawn and it is now almost forgotten.



Use of n-glycan processing enzymes and cell wall modifying enzymes

Suppression of two ripening specific N-glycoprotein modifying enzymes α-mannosidase and β-D-N Acetyl hexosaminidase resulted in down regulation of cell wall degradation and ripening related genes in transgenic fruits.

Example

In lettuce, down regulation of a cell wall modifying enzyme Xyloglycan endotransglucosylase/ hydrolase has also resulted in extended shelf life of crop.

Down regulation of Expansins

Expansins are proteins that cause cell wall loosening, and are involved in many aspects of cell wall modification during development.

Example

- In tomato, the expansion gene LeExp1 shows ripening-related accumulation of mRNA and protein, and transgenic silencing of the expression of this gene results in tomato fruit that are significantly firmer than corresponding controls throughout ripening.
- Examination of postharvest quality characteristics of fruit suppressed in accumulation of LeExp1 protein found that increased firmness resulted in significantly improved fruit integrity during storage at 13 °C.
- Based upon the first appearance of noticeable deterioration, fruit shelf life was extended by 5-10 days, depending upon the packaging.

Over expression of genes of cytokinin biosynthesis

- Isopentenyl transferase (*IPT* Gene) is a key enzyme in cytokinin biosynthesis. Cytokinins are known to delay floral yellowing of plants.
- This gene was linked to senescence associated gene promoter and transferred to broccoli.

- The *IPT* transformed lines had enhanced shelf life and acceptable yield and appearance.

World's first genetic modification spray to stop wilting

Monsanto (US company), an agriculture biotech firm, has patented a product that will stop flowers wilting by altering their DNA by strangles the EIN2 gene and stops the production of ethylene gas.

Transgenic rose showing, flower that won't wilt with petals that stay fresh for days (http://www.dailymail.co.uk) [28]

Extend shelf life of fruit

Hu *et al.* (2021) [30] demonstrated that CRISPR/Cas9-mediated genome editing of *MaACO1* promotes the shelf life of banana fruit-*Musa acuminata* (AAA group cv. Brazilian). The wild-type fruit was yellow with brown speckles at day 21; whereas *MaACO1*-disrupted fruit remained yellow or green with no speckles (little damage), even at day 60. Moreover, the ripening process of the *MaACO1*-disrupted fruit was delayed for about 1-2 days compared to that of wild-type fruit after ethephon treatment. Under natural ripening conditions, high amounts of ethylene were produced 18-21 days postharvest in wild-type fruits; whereas, in the *MaACO1*-disrupted line ethylene production was strongly delayed and reduced. Under ethephon treatment, ethylene production exhibited a similar trend between the wild type fruits and *MaACO1*-disrupted lines, but in the wild-type line, more ethylene was produced at day 3 compared with the *MaACO1*-disrupted line.

Extend shelf life of vegetable

Gupta *et al.* (2013) [24] demonstrated that delayed ripening in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. Fruits from RNAi-ACS lines liberated reduced levels of ethylene. Ethylene liberation was found to be least in RNAi-ACS60 and RNAi-ACS81, releasing only 4–5%; exhibit ∼50% reduction in respiratory activity in harvested fruits and on vine ripening period (BR to RR) was delayed for ∼45 days when compared with controls.

Extend vase life of flower

Kosugi *et al.* (2002) [39] revealed that flowers of NT control carnation line remained turgid until day 5, showed in-rolling of petals on day 6 and completed wilted on day 9. While flowers of sACO-1 line remained turgid without petal in-rolling until about 10, but began to show desiccation and discoloration in rim of petals on day 11 or later. And produce only a negligible amount of ethylene during natural senescence.

Wang *et al.* (2013) [72] demonstrated that delayed flower senescence on transgenic petunia cv. Mitchell diploid by inducing expression of *etr1-1*, a mutant ethylene receptor. In the presence of the inducer, wild-type flowers normally exhibited wilting by 6–7 days; however, flower longevity of E7H and E9G was extended by almost double, lasting average 12 days and 23 days, respectively. The peak of ethylene production came at about 4 days and 5 days on flowers of E7H and E9G with DEX, respectively, compared with 2.5 days and 3 days on flowers of E7H and E9G without DEX, and 2.6 days for wide-type, respectively.

Conclusion

Horticulture commodities are perishable, can respire and transpire even after harvest, resulting in excessive ripening-associated softening during post-harvest storage. Genetic engineering has the potential to be used as an efficient tool for developing the fruits and vegetables with improved storage life for extended availability in the market. Biotechnological approaches have a great significance to engineer fruits with delayed ripening character such as, RNAi; antisense RNA technology has been used to suppress the expression of ACC synthase and ACC oxidase gene involved in ethylene biosynthesis and fruits in the silenced transgenic plants were found to be more resistant to over ripening and shrivelling than control fruits. In spite of allied bio-safety issues, if planned and developed thoughtfully, it can assist to resolve the most important world problems of undernourishment and food insecurity in combination with conventional breeding programs.

References

1. Atkinson RG, Sutherland PW, Johnston SL, *et al.* Down-regulation of Polygalacturonase 1 alters firmness, tensile strength and water loss in apple (Malus × domestica) fruit. BMC Plant Biology. 2012;12:129.
2. Ayub R, Guis M, Amor MB, *et al.* Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nature Biotechnology. 1996;14:862-866.
3. Bauchot AD, Mottram DS, Dodson AT, *et al.* Effect of aminocyclopropane-1-carboxylic acid oxidase antisense gene on the formation of volatile esters in cantaloupe Charentais melon (cv. Vedrandais). Journal of Agricultural and Food Chemistry. 1998;46:4787-4792.
4. Behboodian B, Mohd AZ, Ismail I, Zainal Z. Post-harvest analysis of lowland transgenic tomato fruits harbouring hpRNAi-ACO1 construct. Science World Journal. 2012;439870. https://doi.org/10.1100/2012/439870.
5. Binder BM. Ethylene signalling in plants. Journal of Biological Chemistry. 2020;295(22):7710-7725.
6. Brummell DA, Harpster MH, Civello PM, *et al.* Modification of expansion protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell. 1999;11:2203-2216.
7. Casals J, Pascual L, Cañizares J, *et al.* Genetic basis of long shelf life and variability into Penjar tomato. Genetic Resources and Crop Evolution. 2012;59:219-229.
8. Cevik V, Ryder CD, Popovich A, *et al.* A fruit full-like gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.). Tree Genetics and Genomes. 2010;6:271–279.
9. Chang XX, *et al.* A petunia homeodomain-leucine zipper protein, Ph.D. Zip, plays an important role in flower senescence. PLOS One 2014;9:e88320.

10. Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, *et al.* Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnology Journal. 2016;14:169-176.
11. Costa F, Peace CP, Stella S, *et al.* QTL dynamics for fruit firmness and softening around an ethylene-dependent Polygalacturonase gene in apple (Malus × domestica Borkh.). Journal of Experimental Botany. 2010;61:3029-3039.
12. Costa F, Stella S, Van WW, *et al.* Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (Malus domestica Borkh). Euphytica. 2005;141:181-190.
13. Costa F, Van WW, Stella S, *et al.* Map position and functional allelic diversity of Md-Exp7, a new putative expansin gene associated with fruit softening in apple (Malus × domestica Borkh.) and pear (Pyrus communis). Tree Genetics and Genomes. 2008;4:575–586.
14. Dahmani F, Troadec C, Boualem A, *et al.* Engineering melon plants with improved fruit shelf life using the tilling approach. PLOS One. 2010;5:e15776.
15. Daminato M, Guzzo F, Casadoro G. A shatterproof-like gene controls ripening in non-climacteric strawberries, and auxin and abscisic acid antagonistically affect its expression. Journal of Experimental Botany. 2013;64:3775-3786.
16. Dandekar AG, Teo B, Defilippi, *et al.* Effect of down-regulation of ethylene biosynthesis on fruit flavour complex in apple fruit. Transgenic Research. 2004;13:373-384.
17. Dougherty L, Zhu Y, Xu K. Assessing the allelotypic effect of two aminocyclopropane carboxylic acid synthase-encoding genes MdACS1 and MdACS3a on fruit ethylene production and softening in Malus. Horticulture Research. 2016;3:16024.
18. Eduardo I, Picañol R, Rojas E, *et al.* Mapping of a major gene for the slow ripening character in peach: Co-location with the maturity date gene and development of a candidate gene-based diagnostic marker for its selection. Euphytica. 2015;205:627–636.
19. Fallik E, Aharoni Y. Postharvest Physiology, Pathology and Handling of Fresh Produce. Lecture Notes. International Research and Development course on Postharvest Biology and Technology. The Volcani Center, Israel; c2004. p. 30.
20. Friedman H. Candidate genes to extend fleshy fruit shelf life; c2019. p. 61-94. DOI: 10.1002/9781119616801.ch3.
21. Gao Y, Zhu N, Zhu X, Wu M, Jiang CZ, Grierson D, *et al.* Diversity and redundancy of the ripening regulatory networks revealed by the fruit ENCODE and the new CRISPR/Cas9CNR and nor mutants. Horticulture Research. 2019;6:39.
22. Giovannoni JJ. Fruit ripening mutants yield insights into ripening control. Current Opinion in Plant Biology. 2007;10(3):283-289.
23. González MN, Massa GA, Andersson M, Turesson H, Olsson N, Fält AS, *et al.* Reduced enzymatic browning in potato tubers by specific editing of a polyphenol oxidase gene via Ribonucleo protein complexes delivery of the CRISPR/Cas9 System. Frontiers in Plant Science. 2020;10:1649.
24. Gupta A, Pal RK, Rajam MV. Delayed ripening and improved fruit processing quality in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. Journal of Plant Physiology. 2013;170:987-995.
25. Hamilton A, Lycett G, Grierson D. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature. 1990;346:284.
26. Harada T, Sunako T, Wakasa Y, *et al.* An allele of the 1-aminocyclopropane-1-carboxylate synthase gene (Md-ACS1) accounts for the low level of ethylene production in climacteric fruits of some apple cultivars. Theoretical and Applied Genetics. 2000;101:742-746.
27. https://www.biotecharticles.com/Category-28/0/Biotech-Research.html, assessed on 2nd Oct 2022.
28. http://www.dailymail.co.uk, Assessed on 2nd Oct 2022.
29. Huang LC, *et al.* Delayed flower senescence of petunia hybrida plants transformed with antisense broccoli ACC synthase and ACC oxidase genes. Postharvest Biology and Technology. 2007;46:47–53.
30. Hu C, Sheng O, Deng G, He W, Dong T, Yang Q, *et al.* CRISPR/Cas9-mediated genome editing of *MaACO1* (Aminocyclopropane-1-carboxylate oxidase 1) promotes the shelf life of banana fruit. Plant Biotechnology Journal. 2021;19:654-656.
31. Ireland HS, Yao JL, Tomes S, *et al.* Apple Sepallata1/2-like genes control fruit flesh development and ripening. Plant Journal. 2013;73:1044-1056.
32. Itkin M, Seybold H, Breitel D, *et al.* Tomato Agamous-like 1 is a component of the fruit ripening regulatory network. Plant Journal. 2009;60:1081.
33. Ito Y, Nishizawa-Yokoi A, Endo M, *et al.* Re-evaluation of the RIN mutation and the role of RIN in the induction of tomato ripening. Nature Plants. 2017;3:866.
34. Jiménez BS, Redondo NJ, Muñoz BJ, *et al.* Manipulation of strawberry fruit softening by antisense expression of a pectatelyase gene. Plant Physiology. 2002;128:751-759.
35. Johnston JWK, Gunaseelan K, Pidakala, P, *et al.* Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. Journal of Experimental Botany. 2009;60.
36. Jung YJ, Lee GJ, Bae S, Kang KK. Reduced ethylene production in tomato fruits upon CRSPR/Cas9-Mediated LeMADS-RIN mutagenesis. Korean Journal of Horticultural Science and Technology. 2018;36:396-405.
37. Kanazawa A, Inaba JI, Shimura H, *et al.* Virus-mediated efficient induction of epigenetic modifications of endogenous genes with phenotypic changes in plants. Plant Journal. 2011;65:156–168.
38. Kitagawa MH, Ito H, Shiina T, *et al.* Characterization of tomato fruit ripening and analysis of gene expression in F1 hybrids of the ripening inhibitor (RIN) mutant. Physiologia Plantarum. 2005;123:331–338.
39. Kosugi Y, Waki K, Iwazaki Y, Tsuruno N, Mochizuki A, Yoshioka T, *et al.* Senescence and gene expression of transgenic non-ethylene-producing carnation flowers. Journal of Japanese Society for Horticultural Science. 2002;71(5):638-642.
40. Kramer M, Sanders R, Bolkan H, Waters C, Sheeny RE, Hiatt WR. Postharvest evaluation of transgenic tomatoes with reduced levels of Polygalacturonase: Processing, firmness and disease resistance. Postharvest Biology and Technology. 1992;1:241-255.
41. Kumari C, Sharma M, Kumar V, Sharma R, Sharma P, Kumar P, *et al.* Genome editing technology for genetic amelioration of fruits and vegetables for alleviating post-harvest loss. Bioengineering. 2022;9:176. Available at: https://doi.org/10.3390/bioengineering9040176.

42. Lester DR, Sherman WB, Atwell BJ. Endopolygalacturonases and the melting flesh (M) locus in peach. Journal of the American Society for Horticultural Science. 1996;121:231-235.
43. Lü P, Yu S, Zhu N, Chen YR, Zhou B, Pan Y, *et al.* Genome encode analyses reveal the basis of convergent evolution of fleshy fruit ripening. Nature Plants. 2018;4:784–791.
44. Manning K, Tor M, Poole M, *et al.* A naturally occurring epigenetic mutation in a gene encoding a SPB-box transcription factor inhibits tomato fruit ripening. Nature Genetics. 2006;38:949.
45. Martínez-Madrid MC, Flores F, Romojaro F. Behaviour of abscisic acid and polyamines in antisense ACC oxidase melon (*Cucumis melo*) during ripening. Functional Plant Biology. 2002;29:865-872.
46. Meli VS, Ghosh S, Prabha T, *et al.* Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:2413-2418.
47. Minoia S, Boualem A, Marcel F, *et al.* Induced mutations in tomato SlExp1 alter cell wall metabolism and delay fruit softening. Plant Science. 2016;242:195-202.
48. Mizrahi Y, Zohar R, Malis-Arad S. Effect of sodium chloride on fruit ripening of the no ripening tomato mutants nor and RIN. Plant Physiology. 1982;69:497-501.
49. Moreno E, Obando JM, Dos-Santos N, *et al.* Candidate genes and QTLs for fruit ripening and softening in melon. Theoretical and Applied Genetics. 2008;116:589-602.
50. Nath P, Bouzayen M, Mattoo AK, Pech JC. Fruit ripening: Physiology, signalling and genomics. (edt.). CAB International, UK; c2014.
51. Nuñez PHG, Huber DJ, Klee HJ, *et al.* Fruit ripening characteristics in a transgenic Galia male parental muskmelon (*Cucumis melo* L. var. reticulatus Ser.) line. Postharvest Biology and Technology. 2007;44:95–100.
52. Oeller PW, Min-Wong L. Reversible inhibition of tomato fruit senescence by antisense RNA. Science. 1991;254:437-439.
53. Oraguzie NC, Iwanami H, Soejima J, *et al.* Inheritance of the Md-ACS1 gene and its relationship to fruit softening in apple (Malus × Domestica Borkh.). Theoretical and Applied Genetics. 2004;108:1526–1533.
54. Pan L, Zeng W, Niu L, *et al.* PpYUC11, a strong candidate gene for the stony hard phenotype in peach (*Prunus persica* L. Batsch), participates in IAA biosynthesis during fruit ripening. Journal of Experimental Botany. 2015;66:7031–7044.
55. Paniagua C, Blanco-Portales R, Barceló-Muñoz M, *et al.* Antisense down-regulation of the strawberry β-galactosidase gene FaβGal4 increases cell wall GA Lactose levels and reduces fruit softening. Journal of Experimental Botany. 2015;67:619-631.
56. Peace CP, Crisosto CH, Gradziel TM. Endopolygalacturonases: A candidate gene for freestone and melting flesh in peach. Molecular Breeding. 2005;16:21-31.
57. Pesis E. Respiration and Ethylene. Lecture Notes. International Research and Development Course on Postharvest Biology and Technology. The Volcani Center, Israel; c2004.
58. Quesada MA, Blanco-Portales R, Posé S, *et al.* Antisense down-regulation of the FaPG1 gene reveals an unexpected central role for Polygalacturonase in strawberry fruit softening. Plant Physiology. 2009;150.
59. Santiago-Domenech, N, Jimenez-Bemudez S, Matas AJ, *et al.* Antisense inhibition of a pectatelyase gene supports a role for pectin depolymerisation in strawberry fruit softening. Journal of Experimental Botany; c2008. p. 59.
60. Savin KW, *et al.* Antisense Acc oxidase RNA delays carnation petal senescence. Hortscience. 1995;30:970-972.
61. Schaffer RJ, Friel EN, Souleyre EJF, *et al.* A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiology. 2007;144:1899–1912.
62. Schaffer RJ, Ireland HS, Ross JJ, *et al.* Sepallata1/2-suppressed mature apples have low ethylene, high auxin and reduced transcription of ripening-related genes. AoB Plants. 2013;5.
63. Seymour GB, Ryder CD, Cevik V, *et al.* A Sepallata gene is involved in the development and ripening of strawberry (Fragaria × Ananassa Duch.) fruit, a no climacteric tissue. Journal of Experimental Botany. 2011;62:1179-1188.
64. Shipman EN, Yu J, Zhou J, Albornoz K, Beckles DM. Can gene editing reduce postharvest waste and loss of fruit, vegetables, and ornamentals?. Horticulture Research. 2021;8:1: Available at: https://doi.org/10.1038/s41438-020-00428-4.
65. Singh S, Pandey PK, Singh MC, Singh A, Devi MB, Pandey AK. Delayed ripening of fruits and vegetables through genetic engineering. In National conference on Horticulture in North Eastern Region, January 16-18. College of Horticulture and Forestry Central Agricultural University, Pasighat, Arunachal Pradesh; c2016. p. 107-112.
66. Tadiello A, Pavanello A, Zanin D, *et al.* A PLENA-like gene of peach is involved in carpel formation and subsequent transformation into a fleshy fruit. Journal of Experimental Botany. 2009;60:651-661.
67. Uluisik S, Chapman NH, Smith R, *et al.* Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnology. 2016;34:950-952.
68. Villarreal NM, Rosli HG, Martínez GA, *et al.* Polygalacturonase activity and expression of related genes during ripening of strawberry cultivars with contrasting fruit firmness. Postharvest Biology and Technology. 2008;47:141-150.
69. Vrebalov J, Pan I, Matas A, *et al.* Fleshy fruit expansion and ripening are regulated by the tomato shatterproof gene, Tagl1. Plant Cell. 2009;21:3041.
70. Vrebalov J, Ruezinsky D, Padmanabhan V, *et al.* A mads-box gene necessary for fruit ripening at the tomato ripening-inhibitor (RIN) locus. Science. 2002;296:343-346.
71. Wang A, Yamakake J, Kudo H, *et al.* 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 Physiology. 2009;151:391-399.
72. Wang H, Stier G, Lin J, Liu G, Zhang Z, Chang Y, *et al.* Transcriptome changes associated with delayed flower senescence on transgenic petunia by inducing expression of *etr1-1*, a mutant ethylene receptor. PLOS One. 2013;8(7):e65800.

73. www.ecoursesonline.iasri.res.in, assessed on 7th October 2022.
74. www.nhb.gov.in, assessed on 5th October 2022.
75. Xiong AS, Yao QH, Peng RH, *et al.* Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. Plant Cell Reports. 2005;23:639–646.
76. Xu J, Kang B, Naing AH, Bae S, Kim J, Kim H, Kim CK. CRISPR/Cas9-mediated editing of 1-amino cyclopropane-1-carboxylate oxidase1 enhances petunia flower longevity. Plant Biotechnology Journal. 2020;18:287–297.
77. Yang L, Huang W, Xiong F, *et al.* Silencing of SlPL, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould. Plant Biotechnology Journal. 2017;15:1544-1555.
78. Yu Q, Wang B, Li N, Tang Y, Yang S, Yang T, *et al.* CRISPR/Cas9-Induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Scientific Reports. 2017;7:11874.
79. Zhang HW, *et al.* Genome editing of upstream open reading frames enables translational control in plants. Nature Biotechnology. 2018;36:894.
80. Zhang M, Yuan B, Leng, P. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. Journal of Experimental Botany. 2009;60(6):1579-1588.
81. Zhang Y, *et al.* Anthocyanins double the shelf life of tomatoes by delaying over ripening and reducing susceptibility to gray mold. Current Biology. 2013;23:1094–1100.
82. Zhu Y, Barritt BH. Md-ACS1 and Md-ACO1 genotyping of apple (Malus × Domestica Borkh.) breeding parents and suitability for marker-assisted selection. Tree Genetics and Genomes. 2008;4:555-562.