Unravelling senescence: New opportunities for delaying the inevitable in harvested fruit and vegetables

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Current technology for maintaining the postharvest quality of fresh produce relies on the precise control of storage temperature and/or atmosphere conditions. These technologies have evolved from over 70 years of largely empirical postharvest research. New knowledge about the mechanisms and control of senescence is emerging from biochemical and molecular studies, and is providing exciting opportunities to delay the inevitable loss of quality that accompanies the postharvest senescence of horticultural crops. This knowledge is generating elite plants that have altered postharvest characteristics, and will simplify the future development of effective postharvest technology.

Senescence can be viewed as a developmental change that leads to the death of plant tissue. It occurs naturally in many familiar situations: the ripening of fruit, the shedding of flower petals and the fall of autumn leaves from deciduous trees. Natural senescence is generally accepted as being genetically programmed. Senescence can also be induced by a variety of stresses; these include wounding, nutrient or water deficiency, extremes of temperature, dark treatment and attack by pathogens. Harvesting plant organs for human consumption can precipitate many of these stresses. Harvest stress is particularly severe on organs that are immature and were undergoing rapid preharvest growth, such as asparagus, lettuce, broccoli and cauliflower. These organs do not continue to develop normally after harvest, and senesce rapidly because they are unable to maintain metabolic homeostasis[1].

Whether natural senescence and induced senescence share similar mechanisms and cell signalling processes is unresolved[2]. Although the senescence of different organs can vary in rate and with organ function, several consistent features are found in a range of senescence situations (Box 1). Both natural and induced senescence proceed in a sequential manner in which catabolic processes eventually predominate, leading to cellular breakdown. Furthermore, although some specific differences in the genes expressed during the natural and induced senescence of leaf tissue have been identified[3], other changes in gene expression are similar during the natural or induced senescence of diverse plant organs[4,5]. These latter observations suggest that the regulation of senescence might involve some similar underlying mechanisms in different developmental situations.

The availability of mutant plants that have altered senescence characteristics demonstrates that senescence can be unravelled in different ways. The tomato-ripening inhibitor mutant (*rin*) has mature fruit that remain firm and pale yellow for longer than a year, although the seeds within the fruit may eventually germinate[6]. In *rin* fruit, the whole senescence programme appears to have been slowed down and extensively modified. Thomas[7] identified a non-yellowing mutant of *Festuca* grass that senesces without loss of chlorophyll. A similar 'stay-green' mutant of soybean has also been identified[8]. These non-yellowing mutants demonstrate that specific components of senescence can be separated from the control of the overall senescence programme.

In this article, we focus on three research areas that are making exciting advances in unravelling aspects of both the natural and induced senescence of plant tissue, and examine their potential impact on the future development of postharvest technology. Specifically, we examine the roles of ethylene production and perception in coordinating senescence, the responses of the plant cell wall during senescence, and the senescence responses induced by harvesting immature vegetables.

Ethylene and the coordination of senescence

Ethylene plays an important role during many aspects of normal plant development, for example during early seedling growth, the ripening (early senescence) of many fruit, and leaf senescence. Ethylene also coordinates plant responses to environmental stresses such as wounding and chilling. For these reasons, there have been extensive studies on the effects of ethylene on the postharvest physiology of many fruit and vegetables.

Ethylene is synthesized from the amino acid methionine via the intermediates *S*-adenosyl-L-methionine (SAM) and 1-aminocyclopropane carboxylic acid (ACC; see Fig. 1). The pivotal role that ethylene can play in the ripening of fruit has been most intensively studied in tomatoes. The onset of tomato fruit ripening is characterized by increased ethylene synthesis. This stimulates further ethylene synthesis and regulates the expression of many ripening-related genes (Fig. 1), including those encoding enzymes responsible for the degradation of the cell wall, the production of carotenoids and also sugar

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Box 1. Physical and physiological changes accompanying the senescence of fruit and vegetables

Physical changes:	
Colour:	Loss of green colour Synthesis of new pigments (carotenoids, flavanoids)
Texture:	Softening Wilting Drying
Loss of resistance to pathogens:	Development of infections Lesions
Physiological changes:	
Cellular:	Loss of chlorophyll, disassembly of chloroplast structure Degradation of cell walls Altered membrane composition, loss of fluidity Loss of cellular compartmentation, release of vacuolar contents
Composition:	Altered sugar content, and switch to alternative substrates for respiration Net loss of RNA Increased protease activity, net loss of protein Altered amino acid content

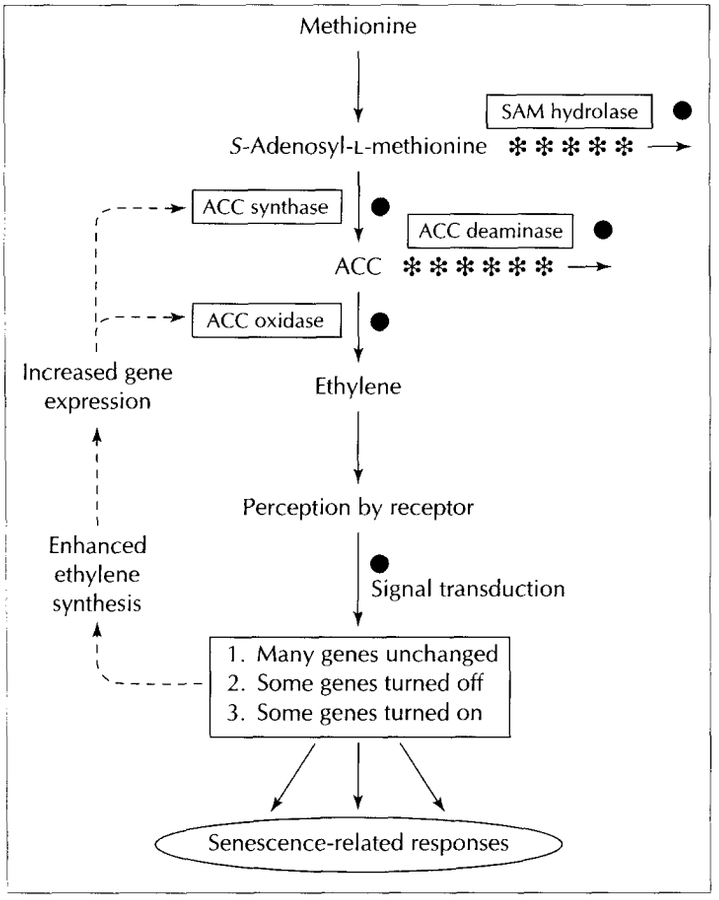

Fig. 1

Interactions between ethylene synthesis and senescence (adapted from Refs 6 and 9). Black circles mark points of ethylene synthesis or perception that have been modified in transgenic plants. The positive feedback effect of ethylene on 1-aminocyclopropane carboxylic acid (ACC) synthase and ACC oxidase is routed by dashed arrows because it occurs only in some plant tissues. ACC deaminase and *S*-adenosyl-L-methionine (SAM) hydrolase are routed by stars because these enzymes are not found naturally in plants[10,11].

metabolism, which contributes to the flavour of the ripened fruit[6]. The application of ethylene to mature green tomato fruit initiates ripening. Conversely, the application of inhibitors of ethylene synthesis delays normal fruit ripening[12].

These observations have now been more critically evaluated using the elegant techniques of molecular biology. The genes encoding the enzymes responsible for ACC formation (ACC synthase; EC 4.4.1.14) or oxidation (ACC oxidase; Fig. 1) have now been cloned from many plants. Transgenic tomato fruit expressing antisense genes (a sequence of DNA inserted in the reverse orientation to the natural gene, and whose transcription inhibits the function of the natural gene) for ACC synthase or ACC oxidase produce little ethylene and ripen more slowly (reviewed in Ref. 13). Supplying ethylene ripens the transgenic fruit. Ripening has also been delayed in transgenic tomatoes overexpressing foreign genes encoding ACC deaminase (EC 4.1.99.4)[10] or SAM hydrolase (EC 3.3.1.2)[11]. These enzymes lower the accumulation of ethylene precursors and reduce ethylene synthesis in the developing fruit (Fig. 1).

The genetic manipulation of enzymes that are involved in ethylene synthesis could provide a relatively simple means to control the ripening of many fruit. Prevention of the increase in ethylene synthesis that accompanies the normal ripening of apples and pears, such that ripening was reliant on treatment with exogenous ethylene, would reduce dependence on refrigeration and controlled-atmosphere storage, and lead to less sophisticated and less energy-intensive postharvest technologies[14].

However, it is not only the concentration of ethylene in a tissue that produces a given effect, but also the ability of the tissue to respond to ethylene. Major advances in the understanding of ethylene perception have recently been achieved following the cloning of several genes involved in ethylene signal transduction from ethylene-insensitive mutants of the small crucifer *Arabidopsis thaliana* (reviewed in Ref. 15). An exciting discovery is that the predicted protein encoded by the first mutant ethylene-response gene to be cloned (namely *etr1*, which may result in a dysfunctional ethylene receptor) shares sequence similarity with bacterial and yeast histidine kinases, proteins known to be involved in cell signalling processes. The *etr1* allele is dominant. When *etr1* is introduced into wild-type *Arabidopsis* by genetic transformation, the transgenic plants are insensitive to ethylene[16]. These results are now having an impact on physiological studies of horticultural crops. The tomato-ripening mutant 'Never ripe' (*Nr*) has fruit that ripen incompletely and petals that have delayed senescence. *Nr* is insensitive to ethylene

and may be homologous to *etr1* (Ref. 17). The emerging picture appears to be that the signalling systems mediating ethylene responses in plants might be evolutionarily highly conserved.

The introduction of dominant genes that confer ethylene insensitivity (such as *etr1*) into crops that are currently sensitive to ethylene following harvest (e.g. lettuce and broccoli) would produce new plants that are insensitive to endogenous and exogenous ethylene[9]. This would have a major impact on postharvest technology because harvest-induced senescence would be delayed in these plant organs, and they could be stored and transported with other ethylene-producing organs without detriment.

Cell-wall structure changes during senescence

The plant cell wall comprises a network of cellulose microfibrils embedded in a matrix of pectic polysaccharides and proteins[18]. The cell wall plays a pivotal role in plant development by defining cell size and shape, acting as a physical barrier to cellular attack by external pathogens, and providing a source of pectic fragments, which may elicit developmental and defence responses in unripe fruit cells. The cell wall is also the source of dietary fibre.

Major changes occur in both cell-wall structure and composition during the natural or induced senescence of many plant organs. The ripening of fruit is accompanied by fragmentation of pectic polymers and hemicelluloses, the solubilization of long-chain pectin, and the loss of specific sugars (e.g. galactose and arabinose) from the cell wall[19]. These modifications reduce cell-wall strength and cell-to-cell adhesion, leading to softening and the characteristic texture of many ripened fruit. Although much less intensively studied, changes in cell-wall structure also accompany the harvest-induced senescence of vegetables. Harvested asparagus spears lose wall-bound galactose from their tips[20] and toughen at their bases owing to the lignification of the cell walls of vascular tissue (reviewed in Ref. 21). These results highlight the dynamic nature of the cell wall during the senescence of plant organs.

The ripening of fruit is accompanied by an increased synthesis of cell-wall hydrolases such as endopolygalacturonase (PG; EC 3.2.1.15), pectinmethylesterase (PME; EC 3.1.1.11), β-1-4-glucanases (EC 3.2.1.4) and glycosidases such as β-galactosidase (EC 3.2.1.23). The genes encoding several of these hydrolases have now been cloned, and the roles of the enzymes encoded by these genes have been assessed during ripening in transgenic tomato fruit.

Historically, PG has been regarded as the key enzyme controlling fruit softening because of its rapid synthesis, high abundance and ability to attack the internal bonds of long-chain pectic polymers during the ripening of tomato fruit. Tomato fruit transformed with an antisense-PG gene were shown to have greatly reduced PG activity, a slightly retarded rate of softening, and enhanced resistance to fungal infections and damage during postharvest handling[22,23]. These findings have recently been commercially exploited with the release onto the market of transgenic antisense-PG FLAVR SAVR™ tomatoes. FLAVR SAVR™ tomatoes can be vine-ripened (for enhanced flavour) and maintain tissue integrity longer (important for transport and marketing) than wild-type fruit[24]. Other sensory and compositional components of fruit quality (taste, pigmentation, nutritional content) are unaltered[24]. However, although PG influences specific aspects of fruit texture, these transgenic experiments have unequivocally demonstrated that PG is not the sole determinant of softening in tomato fruit.

In contrast, PME was originally thought to have a support role in fruit ripening, de-esterifying pectin, and facilitating further hydrolytic action by PG during ripening[19]. Tomato fruit transformed with an antisense-PME gene ripen normally, but disintegrate faster than wild-type fruit when held at room temperature[25]. This suggests that PME may have a more direct role in controlling fruit texture during late senescence, perhaps by regulating the binding of wall-strengthening calcium or by modulating the action of other cell-wall hydrolases[26].

These findings demonstrate that modification of the action of individual cell-wall hydrolases can alter textural changes in ripening fruit. However, none of the enzymes characterized to date acts alone to regulate fruit softening. Softening is the consequence of a series of interdependent responses that is not yet understood.

The ability to clone plant genes and use them to transform plant tissue has led to major advances in our understanding of the role of the plant cell wall during development. Recently, genes encoding endoglucanase and β-galactosidase homologues have been cloned from several horticultural crops[27–29]. Insertion of these genes into transgenic plants should generate new information on the contribution of cell-wall hydrolases to changes in cell-wall structure during senescence. Fruit in which several cell-wall enzymes were suppressed could be produced to better assess their interactive roles during senescence. However, this approach is unlikely to lead to a universal method of regulating cell-wall changes because wall structure can vary with plant species and organ type.

The synthesis of cell-wall polymers during senescence has not received the same degree of research attention that has been directed towards cell-wall breakdown. New knowledge of the contribution of individual polymers to wall strength might suggest strategies for developing plants with improved postharvest characteristics. It might be possible to enhance the synthesis of polymers conferring strength to the wall (improving postharvest handling) without interfering with the development of a desirable texture.

The role of the cell wall in the harvest-induced senescence of immature vegetables is almost completely unexplored. Transcripts encoding β-galactosidase homologues accumulate early after harvesting asparagus and broccoli[29,30]. The potential contribution of cell-wall hydrolases to textural and metabolic changes that occur in harvested vegetable tissue is intriguing and merits

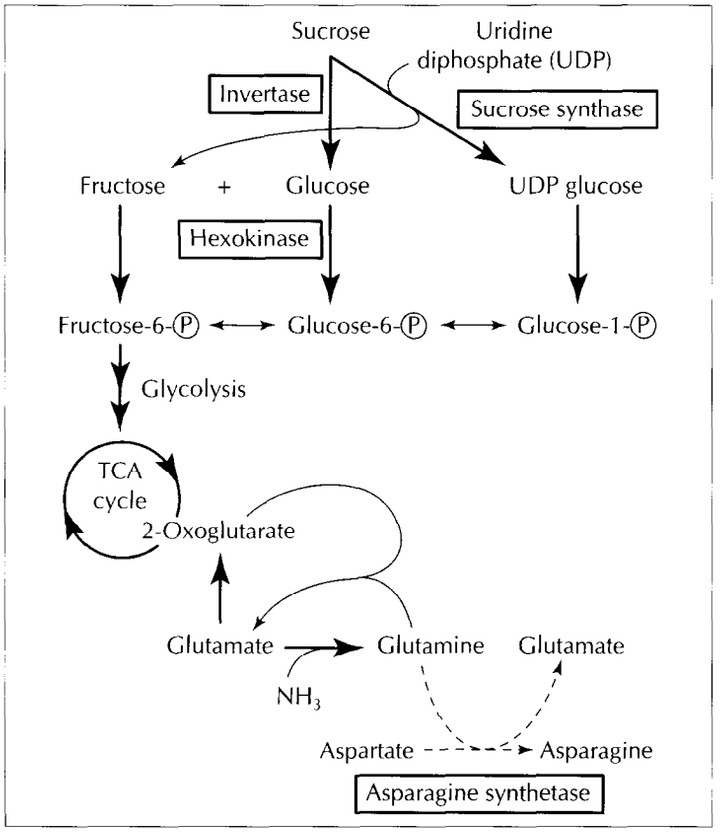

Fig. 2

Interrelationships between primary carbon and nitrogen metabolism in starvation-induced senescence. During normal metabolism, sucrose produced by photosynthesis or from storage reserves is hydrolysed to hexose sugars (glucose and fructose), which are then phosphorylated (encircled P). The phosphorylated sugars then undergo glycolysis and enter the tricarboxylic acid (TCA) cycle, providing energy for respiration and carbon skeletons for biosynthetic reactions. NH_3, either assimilated or produced by tissues, is incorporated into the amino acid glutamine (a C_5 compound) for nitrogen transport and metabolic use. The TCA cycle intermediate 2-oxoglutarate links primary carbon and nitrogen metabolism. During starvation-induced senescence, when the supply of carbon is limiting, asparagine (synthesis routed by dashes; a C_4 compound) is the favoured amino acid for nitrogen transport[35,36]. Key enzymes are indicated in boxes.

further research. This could have a great impact, particularly in the case of fresh cut vegetables, where the maintenance of a desirable texture and the prevention of pathogenic attack are essential for consumer acceptance.

Rapid metabolic changes follow harvest of immature vegetables

In contrast to the extensive studies of the effects of ethylene and of the changes in cell-wall structure during fruit senescence, much less is known about the processes regulating the senescence of immature vegetables such as asparagus, broccoli, cauliflower and lettuce. The early responses of asparagus and broccoli tissues following harvest have been investigated to elucidate the processes regulating the harvest-induced senescence of immature vegetables. Within two to four hours of harvest, the tips of asparagus spears and broccoli florets lose large amounts of sucrose and undergo major changes in gene expression, including the accumulation of transcripts encoding asparagine synthetase (AS; EC 6.3.1.1), the enzyme responsible for asparagine formation (Fig. 2)[31–34]. These cellular responses lead to a markedly altered metabolism: protein and lipid are lost, free amino acids (especially asparagine) and ammonia accumulate, and there is a loss of cellular compartmentation, leading to tissue breakdown. These physiological changes are typical of the starvation responses found in other induced senescence situations such as the dark-induced senescence of leaves[35] and the deprivation of sugars in excised maize root tips[36].

Evidence for the metabolic regulation of plant genes by sugars is now emerging. The gene encoding AS in *Arabidopsis* is induced by extended dark treatment and repressed by exogenous sucrose[37]. Similarly, AS and several other harvest-related genes of asparagus are regulated by sugars (sucrose, glucose and fructose) in asparagus cell cultures (K.M. Davies, pers. commun.). These data strongly suggest that starvation might be a critical stress regulating the expression of genes in immature vegetable tissues.

The genes encoding the enzymes responsible for sucrose degradation [invertase (EC 3.2.1.26) and sucrose synthase (EC 2.1.4.13); Fig. 2] have now been cloned from several plants. Furthermore, hexokinase (EC 2.7.1.1; the enzyme responsible for phosphorylation of fructose and glucose early in the glycolytic pathway; Fig. 2) might be both a key sensor and signal transmitter of sugar repression in plants[38]. Genetic manipulation of enzymes involved in sucrose degradation and sugar perception in harvested immature vegetable tissue would enable direct evaluation of the role of starvation in the regulation of harvest-induced senescence.

This new knowledge about the rapid physiological changes that occur following the harvest of immature vegetables also suggests strategies to maintain quality with the aid of existing technology. The marked benefit of rapid precooling to the postharvest quality of many organs is well known. This is commonly attributed to low temperature reducing the metabolic rate of the tissue and slowing the depletion of substrates (including sugars) that fuel respiration. Controlled or modified atmospheres also markedly influence tissue metabolism, although the physiological mechanisms responsible for the range of effects observed are poorly understood[39]. Recent evidence has shown that the application of an altered atmosphere within an hour after harvest alters sugar metabolism and delays senescence in harvested asparagus spears (P.L. Hurst, pers. commun.).

Producers and retailers are continually striving to improve the consistency and quality of horticultural produce that is presented for sale. Senescence-related biochemical changes can provide endogenous markers of postharvest quality. Ethylene-induced phenylalanine ammonia lyase (PAL; EC 4.3.1.5) activity has been proposed as an index to predict the storage life and quality

of minimally processed lettuce[40]. PAL synthesizes compounds leading to the formation of secondary plant products and undesirable discoloration of the lettuce leaf. Furthermore, the unabated accumulation of asparagine that accompanies the senescence of harvested asparagus spears proceeds at a predictable rate at marketing temperatures[41]. Providing that amenable assay procedures are developed, these endogenous markers have excellent potential to enable retailers to predict shelf life and minimize wastage of harvested produce.

Concluding remarks

The three research areas outlined are but a small sample of the fundamental research that is unravelling senescence, and providing new opportunities to delay the inevitable loss of quality accompanying the postharvest senescence of horticultural crops. The integration of physiological, biochemical and molecular approaches has been vital to the progress achieved. This reflects the complexity of senescence in requiring coordinated interaction between the environment, the plant genome, the cytoplasm and the extracellular space.

The new information generated by these studies has led to the production of plants with altered postharvest characteristics. This information can also be used by traditional plant breeders, enabling them to select breeding lines with improved storage potential. The future success of genetic transformation methods in delivering commercially useful plants will depend on the isolation or development of suitable promoters to control the expression of the inserted genes. Many inserted genes will need to be expressed in the right tissues at the right times to avoid detrimental effects at other stages of development. Ideally, these promoters should be of plant origin to facilitate consumer acceptance, and be both easily and specifically controlled by an external stimulus, such as a gas or the act of harvesting itself. Understanding plant senescence is challenging; controlling plant senescence has exciting potential for improving the postharvest quality of fresh produce that is available to consumers.

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