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# Senescence in cut flowers- A Review

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## INTRODUCTION

The term senescence is derived from a latin word 'Senescere' which means to grow old. It comprises those processes that follow physiological maturity which lead to the event of death of a whole plant, organ or tissue at macroscopic level. At microscopic level the process, however is continuous, since there exists always a turnover of cell organelles at one or other places of the whole body (Voleti *et al*., 2000, Van Doorn and Woltering, 2008 and Yamada *et al*., 2009). It is an integral part of the normal developmental cycle of plants and can be viewed on a cell, tissue, organ or organization level. It is the final event in the life of many plant tissues and is highly regulated process that involves structural, biochemical and molecular changes that in many cases bear the hallmarks of programmed cell death (Makrides and Goldthwaite, 1981; Noh and Amasino, 1999; Buchanan- Wollaston and Morris, 2000; Mahagamasekera and David, 2001; Leverentz *et al*., 2002; Wagstaff *et al*., 2003; Jones *et al*., 2005; Rogers, 2006; Xu *et al*., 2006; Hoeberichts *et al*., 2007; Yamada *et al*., 2009, Shahri and Tahir, 2010a). Senescence though a terminal developmental stage, can be accelerated by an array of both biotic and abiotic factors such as light, temperature, nutrients, ethylene, pathogens and pollination etc. (Taverner *et al*., 1999; Van Doorn and Woltering, 2005; Wagstaff *et al*., 2005; Jones, 2008; Zhou *et al*., 2008; Shahri *et al*., 2009; Shahri and Tahir, 2010b). It is a dynamic and closely regulated developmental process which involves highly coordinated changes in gene expression and requires active gene transcription and protein translation (Shahri and Tahir, 2010c, d). A genetically controlled senescence programme allows for the ordered degradation of organelles and macromolecules with the remobilization of essential nutrients (Hensel *et al*., 1993; Yamada *et al*., 2003; Hoeberichts *et al*., 2005; Jones, 2008; Chapin and Jones, 2007; 2009). It is largely an oxidative process involving a general degradation of cellular structures and the mobilization of the products of degradation to other parts of the plants or organs (Nichols and Ho, 1975; Feller and Keist, 1986; Bieleski, 1995; Fischer *et al*., 1998; Van Doorn and Woltering, 2008).

It is mainly characterized by cessation of photosynthesis, disintegration of organelle structure, intensive loss of chlorophyll and proteins, upregulation of tonoplast localized cytochromes, a drastic increase in lipid peroxidation, proteolytic activity, protease gene expression, poly-galactouronidase activity, nuclease activity, nuclear degradation, vascuolar autophagy, membrane leakage, disruption of cell membranes leading to cellular decompartmentalization and loss of tissue structure (Mahagamasekera and David, 2001; Yamada *et al*., 2003, Hoeberichts *et al*., 2005; Rogers, 2006; Xu *et al*., 2006; Van Doorn and Woltering, 2008; Shahri and Tahir, 2010c, d). The central senescence process seems to begin in the nucleus with the senescence of RNA's which inturn, are used to make certain proteins in the cytoplasm, ultimately resulting in the alteration of plasma membrane and loss of homeostatic ability (Nooden and Leopold, 1988; Hoeberichts *et al*., 2005).

## CHANGES OCCURRING DURING SENESCENCE

**Water relations in cut flowers:** Due to decrease in water absorbtion and increase in water loss through transpiration, the stem develops water deficit conditions results in wilting of flowers. The decrease in water uptake by the stem is mainly due to plugging of xylem vessels caused by growth of microbes mainly bacteria in the vase water or on the dipped portion of stem. Exposure of cut flower to water stress condition, for a short periods lead to the earlier appearance of senescense. Water deficit conditions are reported to cause physiological disorders in the cut stems such

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as stem break in gerbera and bent neck of rose (Halevy and Mayak 1981; Balestra *et al*, 2005). Adverse water relations are also reported to cause changes in hormonal balance. During senescence, the rate of water flux through vessels, tracheids and fibres get reduced and tylose formation was often shown to result in reduced water and oxygen availability for the respiration causes imbalance in water relations of flowers.

The rate of water uptake of a cut flower depends on hydraulic conductance of the water conduits in the stem, water potential difference between the vase water and the cut flower tissue. Water potential of cut flower tissue is affected by water loss due to transpiration – presence of stomata ( Rose, Dianthus, Gerbera, Lilium, Tulip, Orchids) by process leading to cell enlargement, especially growth of the flower petals during flower opening (at later stage sucrose, reducing sugar or starch get reduced - osmotic potential). Water supply to flower at later stages of flower bud opening is inhibited by means of vascular occlusion. It is induced by bacteria, air emboli and tylose formation.

When harvested spikes of gladiolus are kept in vase solution initially there is an increase in the fresh weight of spikes due to increased solution uptake as it required by spikes for opening of flowers. Then decline is attributed to high rate of respiration and also membrane leakage (Ezhilmathi *et al*., 2007). It has been shown that negative water relation would lead to the wilting of flowers.

**Petal senescence and membrane integrity:** A consistent feature of senescence is the loss of differential permeability of cell membranes. The permeability and fluidity of biological membranes is modified by variations in the composition and structure of the lipid bilayer. Membrane deterioration is an early and characteristic feature of plant senescence, which leads to several structural and functional changes. For example the ultra structural changes including the vesiculation of vacuolar and cytosolic compartments have been reported in carnation petals. In daylily flowers, during senescence the degeneration of vacuolar membrane of epidermal cells was reported by Stead and van Doorn (1994).

Leverentz *et al*. (2002) revealed that in ethylene insensitive Alstroemeria flower senescence, the loss of membrane function was not related to lipoxygenase activity. In the ethylene sensitive category, lipoxygenase activity may promote senescence through oxidative membrane damage as seen in rose and carnation. However, in some ethylene sensitive plants such as Phalaenopsis, the lipoxygenase seems not to play any apparent role.

At the biochemical level, senescence is associated with changes in membrane fluidity and leakage of ions in several different flowers. The important changes at the membrane, which include the decrease in all classes of membrane phospholipids and increase in neutral lipids, mainly due to increased action of phospholipases and acyl hydrolases, have been reported. Another important event that leads to loss of membrane permeability is oxidation of membrane lipids (lipid peroxidation) due to lipoxygenases in day lily and carnations.

Since the cell is enclosed within a membrane it is logical to assume that regulation of the cell metabolism is to a large extent by changes in membrane properties. There is deterioration in membrane stability during flower senescence. The first structural change often observed was invagination of tonoplast, resulting in release of various hydrolytic enzymes, followed by autolysis of cell. Often, acid hydrolases such as acid phosphatase, RNase and DNase were released. In those petals, which contain plastids, gradual disappearance of thylakoids followed by tubule presence with invaginations has been observed. Often, the single ribosome and vesiculation of endoplasmic reticulum were the changes at cell organelles level noticed during the early or middle part of senescence.

**Changes in vascular morphology:** In cut flowers, increase in restriction of water movement through stem segments predominantly located in the lower most one centimeter of the stem was noticed (De Stigter and Broekhuysen, 1986). Only a fraction of the microbial cells enters through the vascular system from the vase water and the rest remains attached to the submerged cut surface of the stem, thus blocking the uptake of water (Put *et al*, 1992). Vascular blockage in the cut roses is due to the presence of bacteria and these bacteria cause xylem cell wall degradation.

Bacteria-induced xylem blockage, particularly at the stem-end region, is recognised as a major cause of premature wilting in many species of cut flowers and foliage (Put and Klop, 1990; De Witte and Van Doorn, 1992). Many species of microorganisms isolated from cut flower stems and vase water can produce extracellular polysaccharides (EPS) that manifest as capsules attached to the outer cell walls, as slime released into the environment, or as both (Sutherland, 1977; Put and Klop, 1990).

**Changes in pigmentation:** Colour fading and discoloration is an important factor in determining

display quality of cut flowers, and in many cases is the major reason for the termination of vase life. For example, it was found that all the cultivars of statice (blue, pink, purple and lavender) contain a similar amount of the same anthocyanin (Asen *et al*., 1973). Therefore, measuring pigment content is not always indicative of colour or colour changes. Only in a few studies have colorimetric methods been used for objective definition of the colour changes in cut flowers (Biran *et al*., 1974a, b, c; Mattson and Widmer, 1971; Mayak and Dilley 1976; Parups, 1975a, b). The major types of pigments contributing to the colour of the flowers are carotenoids and anthocyanins. Only a few studies have been carried out on the changes in carotenoids in aging flowers. Flowers are hardly mentioned in a review on pigment changes in senescent and stored tissues (Chichester and Nakayama, 1965).

The changes in carotenoid composition were followed in *Strelitzia reginae* flowers during the various developmental stages of the plastids from the small colourless leucoplasts, through the green chloroplast to increasingly large spindle shaped chromoplast (Simpson *et al*. 1975). A decrease in total carotenoid content was observed in senescing chrysanthemum flowers (Stickland, 1972). An increase in oxygenated carotenoids with age was found in strelitzia (Simpson *et al*., 1975) and rose (Valadon and Mummery, 1969) flowers. This was considered (Goodwin, 1966; Valadon and Mummery, 1969) as a sign of a degenerative and uncontrolled oxidative process. However, the increase in oxygenated carotenoids was observed even in maturing sepals showing no sign of degeneration in their ultrastructure (Simpson *et al*. 1975).

The pigment level stays stable in some flowers and declines drastically in others, while in some flowers a dramatic synthesis of anthocyanins is evident. Little or no changes were found in flowers of Lathyrus (Packet, 1966 a; Sakata and Uemoto, 1976) and Digitalis (Stead and Moore, 1977). A decrease in the anthocyanins content with age was found in chrysanthemums (Stickland, 1972).

Some flowers fade and even turn white upon aging. This was found to be due to decolorization by an enzyme system with the properties of catecholase. In the Masquerade rose the petals are orange- yellow when freshly opened and turn deep red upon senescing. More than a ten fold increase in anthocyanin level was measured during that period (Shisa and Takano, 1964). An increase in anthocyanin formation with wilting is one of the most typical post pollination phenomenon in Cymbidium orchids (Arditti and Flick, 1976 and Arditti and Knauft, 1969). The most important factor determining the colour change in senescing petals seems to be a change in the pH of the vacuole (Stewart *et al*., 1975). However, only in a very few cases is the colour caused by a very low (<3.0) or a very high (> 7.0) pH affecting the anthocyanin.

In most flowers the decisive factor determining the intensity of the colour and its blueing is the copigmentation which is influenced to a great extent by even slight changes in pH. The blueing of red flowers with aging is a well- known phenomenon. A concomitant increase in pH has been demonstrated in Lathyrus (Packet, 1966a). The increase in pH was attributed to the breakdown of proteins and the release of free ammonia. Indeed, treatment of cut flowers with solutions containing sugars, which delayed proteolysis, delayed also the increase of the pH and blueing.

It is interesting to note that the color and the pH changes associated with aging may proceed at very different rates in adjacent cells (Stewart *et al*., 1975). This may indicate that contiguous cells may differ from each other in the rate of proteolysis and aging. In some flowers aging of petals is marked by browning and blackening of the petals, which are caused by oxidation of flavones, leucoanthocyanins, other phenols and the accumulation of tannins (Singleton, 1972).

**Carbohydrate and other macromolecules:** Petal senescence is generally accompanied by a loss of dry matter due to hydrolysis of macromolecules such as starch, protein and nucleic acids and redistribution of carbon and nitrogen compounds to other parts of the flower. Carbohydrate status of the flower petals is one of the factors, which ultimately determines their longevity. There is a sharp decline in the carbohydrate content during the final stage of flower development. This drop in level of macromolecules (starch and cell polysaccharides) occurred with the onset of senescence.

Carbohydrate status of petals related to flower opening, longevity of flowers (Coorts, 1973). Sugars are an important energy source and structural components. Sugar accumulation is a mechanism to reduce petal water potential -promoting water influx - cell enlargement and flower opening. Petal senescence is accompanied by a loss of dry matter. The opening of gladiolus florets was accompanied by a substantial increase in fresh and dry weight and carbohydrate concentration of the perianth. Loss of

total cellular RNA resulted, throughout the aging process (Woodson, 1987).

Sugars or carbohydrates increased the vase life of cut flowers by reducing the sensitivity to ethylene (Mayak and Dilley, 1976; Paulin, 1986). It has also been suggested that maintenance of osmotic pressure might be the reason for the delay in senescence. Paulin (1986) reported that exogenous application of sugars increase vase life by delaying proteolysis, promoting protein and amide synthesis, maintaining osmotic potential, delaying membrane integrity and maintaining mitochondrial structure and function (Halvey and Mayak, 1979).

Decrease in reducing sugars with senescence was reported in carnation (Halvey and Mayak, 1979) and daylily (Bieleski and Reid, 1992). Often, invertase was shown to decrease with increasing age, which has been linked with de novo synthesis of invertase inhibitor (Halaba and Rudnicki, 1986), which makes the oxidation products of sucrose available for transport in carnation and *Ipomea* during wilting.

**Protein metabolism:** Protein synthesis and degradation are the events of central importance during petal senescence. Treatment of flowers with compounds that inhibit protein synthesis, have been found to delay the visible symptoms of petal senescence, revealing that active protein synthesis is required for the execution of cell death in plants. Several genes related to protein synthesis have been found to be differentially expressed during petal senescence. The degradation of proteins and the remobilization of aminoacids to developing tissues is a prominent process during senescence. Protein breakdown occurs in proteosomes, vacuoles, mitochondria, nucleus and plastids but bulk degradation mainly occurs in vacuoles.

Protein degradation during petal senescence has been characterized in several plants, including the monocotyledonous Alstroemeria (Wagstaff *et al*., 2002) and Gladiolus (Azeez *et al*., 2007), and dicotyledons such as carnation (Sugawara *et al*., 2002). The data generally show a decrease in protein levels, but in some species this decrease was much smaller than in others.

The senescence of both climatric and non-climatric flowers have been associated with a loss of protein (Woodson, 1987).The protein content is reduced due to little de novo synthesis and considerable protein degradation. It is understood that free radicals attack amino acid residues of proteins causing conformational changes in proteins causing them to be recognized by specific proteases for degradation. Decrease in proteins which are involved in the synthesis process has been reduced (Woodson, 1987), clearly indicate that mRNA levels increased during the process. Treatment with ethylene resulted in an early increase in the transcript abundance of a senescence-associated cysteine peptidases in the petals, in an early rise in peptidase activity, and later decrease in water-insoluble protein levels which promote senescense of *Dendrobium cv. Khao Sanan* (Ladawan Lerslerwonga *et al.,* 2009).

**Respiration:** The rate of respiration in many flowers rises to a maximum as flowers start to open, followed by a gradual decline as flowers mature. Then it increase dramatically over a relatively short period and finally declines. This second peak in respiration drift was considered to indicate the final senescence stage. It was assumed to be analogous to the climatric rise in respiration of many fruits (Larsen and Frolich, 1969). Respiration is usually taken as a good indicator of the metabolic rate in fruits, vegetables and flowers. The high respiration rates that prevail in most flowers release large amounts of heat, consumption of carbohydrate reserves and elevated transpiration rates (Van Doorn, 2001). Indeed, it was found that chemicals which delay the occurrence of the second peak also extend longevity (Larsen and Frolich, 1969; Mayak *et al*., 1978). A unique respiration pattern was demonstrated in tropical orchid flowers (Hew *et al*., 1978). A circadian rhythm in $CO_2$ production started as soon as flowers opened. The period between amplitudes was about 24 hours and was not affected by continuous darkness. The rhythm was observed also in cut flowers but the amplitude of the rhythm was dampened by the detachment and partially boosted by external supply of sucrose.

The gradual decline in respiration in aging flowers may be caused by short supply of readily respirable substrates, mainly sugars. It was suggested that the content of these substrates may indicate the potential life of the flower at a specific temperature (Nichols, 1973). This is supported by observation of relationship between potential keeping life and dry matter content of the cut flower at the time of the harvest. The respirable substrate pool is composed mainly of sugars. The size of the pool is affected by the rate of hydrolysis of starch and other polysaccharides (Ho and Nichols, 1977) and translocation to the petals (Nichols and Ho, 1975b) from one side, respiration and translocation out of the flowers to other plant parts from the other. This transport is promoted by pollination and ethylene. Supplying cut flowers with exogenous sugar maintains the pool of dry matter and

respirable substrates, especially in petals, thus promoting respiration and extending longevity (Rogers, 1973).

Respiration rate is negatively correlated with organ longevity in plant postharvest physiology (Kader, 1985; Reid, 1985). In general, low respiration rate has been related to increased flower longevity in cut flowers (Kuc and Workman, 1964). Some postharvest treatments increase cut flower longevity as well as flower respiration. Exogenous sugar in vase solutions increases flower respiration but extends longevity in cut roses (*Rosa* L. sp.) (Marousky, 1969), cut carnations (*Dianthus caryophyllus* L.) (Nichols, 1973) and cut gladiolus (*Gladiolus xhortulanus* Bailey) (van der Merwe *et al*., 1986). Comparing spring to summer production, Celikel and Karacali (1991) showed that cut carnation flower longevity was best for plants produced during the summer when flower respiration rates were higher. Thus, flower respiration is not always negatively correlated with flower longevity or it is a specificity of the cut flower system, where a substrate limitation may occur due to detachment from the source organs.

**Environmental stress and petal senescence:** Petal senescence rate in cut flowers is strongly dependent on temperature and on environmental water stress parameters (Halevy and Mayak, 1981). Ethylene is involved in the response of flowers to different stresses leading to a significant rise of the production. In carnations water stress is accompained by accumulation of ACC. Induced increase in the level of endogenous ethylene has been observed to be caused by stressogenic factors *viz*., mechanical wounding, extreme temperature, water loss, drought, diseases and pollination. Reactive oxygen species such as superoxide radical, hydrogen peroxide and hydroxyl radical have a role in lipid peroxidation, membrane damage and consequently in leaf senescence. Free radicals have been involved in programmed cell death, both in animal and in plant cells (Dhindsa *et al*. 1981). Plants possess a well defined antioxidant defence mechanisms which eliminate hazardous free radicals (Larson 1988). Antioxidant protection involves compounds such as carotenoids, ascorbic acid, *a*-tocopherol, glutathione, phenolics and flavonoids (Schoner and Krause 1990) and a battery of enzymatic systems including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidise (GPX), (glutathione-S-transferase) GST and the Halliwell-Asada Pathway (or the ascorbateglutathione cycle). The ascorbate-glutathione cycle involves four enzymes: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) enzymes (Bowler *et al*. 1992; Halliwell 1987). It has been shown that during leaf senescence, proteins, phospholipids and pigments may be degraded by free radicals as free radical scavenging declines (Prochazkova *et al*. 2001).

Florets/petals are the organs which primarily determine the commercial longevity of flowers and as a consequence it is beneficial to study the physiological, biochemical, and genetic processes that occur during floret senescence. Most of the early work on flower senescence focused on ethylene sensitive plants. Chrysanthemum is an ethylene insensitive plant where lipid peroxidation and membrane damage are involved in flower deterioration (*Chrysanthemum morifolium*). Senescence can lead to the loss of membrane stability due to the oxidation of existing membrane components, lipid peroxidation increases during senescence. It may directly, or indirectly, via the formation of free radicals, be involved in the last stages of senescence and cell death in chrysanthemum florets. (Debasis Chakrabarty *et al*., 2007).

**Pollination regulation of flower senescence:** The development of orchid flowers is strictly regulated by pollination that initiates alterations at various organization levels eventually leading to floral senescence. Pollination induces rapid senescence of orchid flowers, thereby reducing their commercial value (Ketsa and Rugkong, 1999). Hence, it becomes imperative to trace out the events associated with floral senescence and their possible manipulation in order to prolong the life span of the orchid flowers. The mechanisms underlying floral senescence are still enigmatic. Senescence, when it does occur, may be imperceptible for weeks, even after becoming apparent, symptoms develop slowly. In orchids, little is known as how various floral organs respond to pollination at biochemical level and how these organs relate to each other in pollinated flowers. Previous studies in other plant species report an efflux of some cellular constituents like vacuolar pigments and electrolytes (Suttle and Kende, 1978; Celikel and Van Doorn,1995), related to the loss of turgor and visible wilting as a result of flower senescence. One of the vital changes in senescing tissues involves the appearance of oxidative damage (Thompson *et al*., 1998) that has detrimental effects on tissues.

Pollination of flowers has been shown to induce senescence (Lovell *et al*., 1987). It was observed in

carnation that petal senescence occur between 2-3 days after pollination, whereas in unpollinated ones it occurs on 6-7 days after pollination. The other parts of the flowers such as style, ovaries, receptacles, etc., have been shown to induce the synthesis of ethylene (Pech *et al*., 1987; Porat *et al*., 1994) however, not necessarily due to fertilization since, flowers, which have not pollinated with pollen tube still growing with in the style, have shown increased ethylene concentration suggesting that a transmissible factor is involved in senescence.

Pollination regulates a syndrome of developmental responses that contributes to successful sexual reproduction in higher plants. Pollination- regulated developmental events collectively prepare the flower for fertilization and embryogenesis while bringing about the loss of floral organs that have completed their function in pollen dispersal and reception. Components of this process include changes in flower pigmentation, senescence and abscission of floral organs, growth and development of ovary and in certain cases, pollination triggers ovule and female gametophyte development in anticipation of fertilization.

Pollination regulated development is initiated by the primary pollination event at the stigma surface, but because developmental processes occur in distal floral organs, the activity of inter organ signals that amplify and transmit the primary pollination signal to floral organs is implicated. Inter organ signalling and signal amplification involves the regulation of ethylene biosynthetic gene expression and inter organ transport of hormones and their precursors. The coordination of pollination- regulated flower development including gametophyte, embryo and ovary development, pollination signalling, the molecular regulation of ethylene biosynthesis and inter organ communication as reported by O'Neill., 1997.

Pollination of orchid flower induces a dramatic increase in ethylene production, which subsequently causes a rapid petal wilting, whereas the longevity of intact unpollinated flowers may reach as long as several months. (O'Neill., 1993). After pollination, wilting began in 2 to 3 days in *Aerides multiflora* and 3 to 4 days in *Rhyncostylis retusa.* There was a higher electrolyte leakage accompanied by a concomitant increase in the levels of malondialdehyde (MDA), indicators of oxidative damage in all the organs after pollination. The flowers of *Aerides multiflora* showed a greater electrolyte leakage, MDA and $H_2O_2$ contents as compared to those of *Rhyncostylis retusa* (Attri *et al*., 2008).

**Plant growth regulators:** Plant Growth Regulators can delay and also accelerate the senescence. Ethylene, Cytokinin and ABA- affect the vase life of cut flower. Petal senescence in cut flowers is delayed or inhibited by cytokinin or gibberellic acid. Cytokinins can also delay petal senescence by maintaining cellular integrity and proteins. In climatric or ethylene sensitive flowers, senescence is accompained by sudden, transient increase in ethylene production and respiration of Ex - Carnations, Petunia, Gypsophila and Orchids. But in non-climatric flowers, Gladiolus, Tulip and Iris generally no increase in ethylene production and respiration during senescense were reported.

**Role of cytokinins in petal senescence:** Cytokinins are known to defer leaf senescence (Richmond and Lang, 1957) and improve the keeping qualities of cut carnation (Heide and Oydvin, 1969; Maclsan and Dedolph, 1962) and rose flowers. MacLean and Dedolph (Mayak and Halevy, 1970) reported a decrease in the respiration rate of cytokinin-treated flowers and proposed that cytokinins increase flower longevity as a result of this reduction in respiration. However, Heide and Oydvin (1969) found only small and inconsistent effects of cytokinins on the respiration rate of cut carnation flowers. They concluded that processes other than respiration mediate the cytokinin retardation of senescence. Mayak and Halevy, 1974 have shown that kinetin increases net water uptake of expanding rose petals and delays wilting of petals especially when flowers are subjected to heat (28 C) and low relative humidity (40-50%). Kinetin had no effect on protein content of rose petals under these stressful conditions, but kinetin retarded the increase in RNase activity normally seen in rose flowers at the onset of senescence. Kinetin is proposed to increase rose flower longevity by improving water balance and delaying senescence processes.

Cytokinins delay senescence in vegetative and floral tissues (Van Staden *et al*., 1988). An inverse relationship between cytokinin content and senescence occurs in some flowers (Van Staden *et al*., 1988). Cytokinin content in roses (Mayak *et al.,* 1972), carnation (Van Staden and Dimalla, 1980), and *Cosmos sulfureus* (Saha *et al.,* 1985) is greatest in young flowers and decreases during corolla opening and development. Rose varieties with longer vase lives have been reported to contain more cytokinins than those with shorter vase lives (Mayak and Halevy, 1970). Results from exogenous application of cytokinins in vase solutions have been variable

(Weaver, 1972; Halevy and Mayak, 1981; Baker, 1983). Cytokinin application delayed senescence in carnations (Mac- Lean and Dedolph, 1962; Heide and Oydvin, 1969; Mayak and Dilley, 1976; Mayak and Kofranek, 1976; Upfold and Van Staden, 1990), roses (Mayak and Halevy, 1970, 1974), *Gerbera* sp. (Van Meeteren, 1979) but the response depended on the type and concentration of cytokinin and the stage of flower development. Interactions between cytokinins and other hormones during senescence have been less studied. Cytokinin applications to carnation flowers delay senescence and are associated with reduced ethylene biosynthesis and decreased sensitivity to ethylene (Eisinger, 1977; Mor *et al.,* 1983; Cook *et al.,* 1985). Study suggested that ethylene production during petunia senescence promotes cytokinin degradation and inactivation by *O*-glucosylation (Taverner *et al.,* 1999). The sensitivity of flowers to ethylene increases as they mature, and this sensitivity change also has a role in the initiation of senescence (Nichols, 1968; Barden and Hanan, 1972; Mayak *et al.,* 1977; Halevy and Mayak, 1981; Woodson and Lawton, 1988).

Recently, thidiazuron (N'-phenyl-N'-1, 2, 3-thiadiazol-5-ylurea, TDZ), a phenylurea derivative, has been characterized as a highly efficacious type of non-purine cytokinin with strong morphogenic potency in a wide range of plant species (Murthy *et al.,* 1998; Mok *et al*., 2000). Like purine cytokinins, TDZ has also been shown to be a potent inhibitor of leaf senescence and flower abscission (Ferrante *et al.,* 2002; Sankhla *et al.,* 2003). In addition, inclusion of 5–45_M TDZ in vase water was found to reduce ethylene-mediated flower abscission and senescence on phlox and lupin stems, respectively (Sankhla *et al.,* 2003, 2005). This treatment also stimulated opening of additional flowerbuds on cut lupin and tuberose stems (Sankhla *et al*., 2003; Uthairatanakij *et al.,* 2007). The mode by which TDZ treatment extends flower longevity has not been determined, although it may act by regulat- ing cytokinin and/or auxin activity (Murthy *et al*., 1998; Mok *et al*., 2000). It is an inexpensive and non-metabolized phenyl-urea compound, has been shown to have a potent cytokinin-like activity at 50-100 times lower concentrations than BAP (Genkov and Iordanka, 1995).

Ferrante *et al.* (2002, 2003) have demonstrated that TDZ dramatically retards chlorophyll degradation in leaves of cut flowers of alstroemeria, tulips and chrysanthemums. TDZ also was reported to reduce flower abscission and the senescence of leaves and flowers in cut inflorescences of phlox and lupins (Sankhla *et al*., 2003, 2005). Although the exact mode of action of TDZ is not well known, evidence suggests that TDZ can modulate cytokinin biosynthesis and/or metabolism, and may mimic the activity of auxin (Murthy *et al.,* 1998; Mok *et al*., 2000). It has been hypothesized that the long-lived cytokinin effect provided by TDZ treatment not only prevents leaf yellowing, but also reduces ethylene sensitivity (Ferrante *et al.,* 2002; Sankhla *et al*., 2005).

Until recently, no genes involved in cytokinin biosynthesis had been identified from plants (Kakimoto, 2001; Takei *et al.,* 2001; Zubko *et al*., 2002; Sun *et al.,* 2003). Plants with altered cytokinin content have been generated by transformation with the *Agrobacterium tumefaciens* cytokinin biosynthetic gene, *ipt* (Medford *et al*., 1989). The *ipt* gene encodes isopentenyl transferase, an enzyme that catalyzes the condensation of dimethylallylpyrophosphate and 5'-AMP to isopentenyladenosine (iPA) 5'-phosphate. This is assumed to represent a rate-limiting step in cytokinin biosynthesis because the introduction of the *ipt* gene into plants results in increased accumulation of many forms of cytokinins (Akiyoshi *et al.,* 1984; Barry *et al.,* 1984; Morris, 1995). Very low increases in endogenous cytokinin content of transgenic plants have been associated with pleiotropic effects including inhibition of root growth, stunted shoots, reduced apical dominance, increased stem diameter, and retarded leaf senescence (Schmulling *et al.,* 1999). An approach to target the expression of *ipt* to senescing tissues with the promoter from SAG12, a senescence-associated gene from Arabidopsis, demonstrated a direct effect of cytokinins on plant senescence (Gan and Amasino, 1995). Numerous plants transformed with SAG12-IPT have significant delays in leaf senescence (Gan and Amasino, 1995; Jordi *et al.,* 2000; Zhang *et al*., 2000; McCabe *et al.*, 2001).

**Role of Ethylene in petal senescence:** Ethylene is a primary plant hormone involved in the senescence of cut carnation flowers (Abeles *et al*., 1992; Borochov and Woodson, 1989; Reid and Wu, 1992). A large amount of ethylene is synthesized several days after full opening of the flower during natural senescence (Manning, 1985; Peiser, 1986; Woodson *et al*., 1992), or several hours after compatible pollination (Nichols, 1977; Larsen *et al*., 1995) or treatment with exogenous ethylene (Borochov and Woodson, 1989; Wang and Woodson, 1989). The increased ethylene production accelerates in-rolling of petals resulting in wilting of the flower. Ethylene is synthesized through the following pathway: L-methionine-S-adenosyl-L-

methionine , 1-aminocyclopropane- 1-carboxylate (ACC) ethylene. ACC synthase and ACC oxidase catalyse the last two reactions. So far, three genes encoding ACC synthase (DC-ACS1, DC-ACS2, and DC-ACS3) and one gene encoding ACC oxidase (DCACO1) have been identified from carnation (Park *et al*., 1992; Henskens *et al*., 1994; Jones and Woodson, 1999; Wang and Woodson, 1991). These genes are regulated in a tissue-specic manner during flower senescence; DCACO1 is expressed in both the gynoecium and petals of carnation flowers that are undergoing senescence, and DCACS1 is also expressed in both the gynoecium and petals, but mainly in the latter, whereas DC-ACS2 and DC-ACS3 occur in the gynoecium (Henskens *et al*., 1994; ten Have and Woltering, 1997; Jones and Woodson, 1999). In carnation flowers, it has been revealed that ethylene is first produced from the pistil and the evolved ethylene induces autocatalytic ethylene production in petals, resulting in wilting of the petals, during the natural senescence of carnation flowers (Ten Have and Woltering, 1997; Shibuya *et al*., 2000). This observation suggests the role of the gynoecium in controlling the senescence of petals in the flowers. In the carnation flowers, if the gynoecium could not produce a sufficient amount of ethylene to induce ethylene production in petals, the whole flower would not suffer from ethylene-dependent wilting in their petals and have a prolonged vase-life.

Carnation plants with such characteristics may be present among cultivars or variants that have been shown to have flowers with a prolonged vase-life. The characterization of ethylene production in those flowers should help to determine the role of the gynoecium in the senescence of carnation flowers, and to elucidate the regulation of genes for ethylene biosynthesis in the gynoecium and petals during senescence of the flower. In the early 1990s, carnation cultivars and strains with unusual ethylene-related behaviour were reported: cvs Killer (Serrano *et al*., 1991), Sandra (Wu *et al*., 1991), Chinera (Reid and Wu, 1992), and Sandorosa (Mayak and Tirosoh, 1993), and strains 87-37G-2, 81-2, and 799 (Brandt and Woodson, 1992).

**Role of ABA in plant senescence:** The plant hormone abscisic acid (ABA) influences numerous aspects of plant growth and development including embryo maturation, seed dormancy, fruit ripening, and water balance in response to environmental stresses (Pandey *et al*. 2003/4, Purty *et al*. 2005). Exogenous applications of ABA accelerate the symptoms of flower senescence in carnation, rose and daylily flowers (Mayak and Halevy 1972, Mayak and Dilley 1976, Panavas *et al.* 1998). Endogenous content of ABA increased during senescence in several flowers (Panavas *et al*. 1998, Hunter *et al*. 2004) and this may be due to water soaking or conversion of carotenoids to ABA (Eze *et al.* 1986, Milborrow 2001). In some flowers, ABA causes senescence through ethylene as inhibitors of ethylene production or action, preventing the response (Mayak and Dilley 1976, Nowak and Veen 1982). In other flowers, *e.g*., daylilies, ABA presumably induces senescence independently of ethylene action, as the senescence of the flower is known to be ethylene independent (Panavas *et al*. 1998). The interrelationship between ABA and other flower components such as carotenoids and pigments has been recently studied. In many organs, the interaction between ABA and anthocyanins has been clearly demonstrated. Exogenous applications of ABA affect pigments biosynthesis in many plants, inducing anthocyanins accumulation in strawberry fruits (Jiang and Joyce 2003), in cut snapdragon flowers and grapes (Sang *et al*. 1992, Jeong *et al.* 2004). The ABA content during flower development has a well defined trend that is common in many plant species such as squash flowers, four o'clock flowers, daylily and daffodil (Panavas *et al*. 1998, Hunter *et al*. 2004). The applications of ABA increased anthocyanins accumulation in flowers, fruits and seeds (Sang *et al*. 1992, Jiang and Joyce 2003, Jeong *et al*. 2004).

ABA may play an important role in the regulation of flower senescence. In ethylene-dependant flowers like carnation, exogenous ABA triggered endogenous ABA production and flower senescence; however, the effects of ABA might be mediated through an increase in ethylene production resulted from ABA application or through an activation of ethylene action (Onoue *et al., 2000)*. Thidiazuron treatment doubled the ABA content but did not affect flower life, confirming the secondary role of ABA during flower senescence of petunia, another ethylene-dependant flower (Ferrante, 2006). In contrast to its suspected secondary role in ethylene-dependant flower senescence, ABA might have a direct effect on the senescence of ethylene-independent flowers. In cocoa (Aneja, 1999) and daylily (Panavas, 1998), exogenous ABA, not ethylene, accelerated flower senescence. Endogenous ABA increased dramatically before any visible signs of senescence, and continued to increase during petal senescence in both these taxa. Treatment of fluridone, an inhibitor of ABA biosynthesis, decreased ABA levels and extended the longevity of cocoa flowers. However, in daffodil, a flower that could respond to

exogenous ethylene but whose natural senescence is ethylene independent, exogenous ABA accelerated flower senescence but such an effect is considered to be mediated through stimulated ethylene production as in ethylene-dependant flowers; ABA was not the primary regulator of daffodil flower senescence since the increase in senescence-associated genes commenced before the rise in ABA content (Hunter, 2004).

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