

Postharvest Physiology of Cut Carnation Flowers

Asghar Ebrahimzadeh^{1,2} • Silvia Jiménez¹ • Jaime A. Teixeira da Silva³ • Shigeru Satoh^{4,5} •
María Teresa Lao^{1*}

¹ Departamento de Producción Vegetal, Escuela Politécnica Superior, Universidad de Almería, Almería, Spain

² Department of Horticulture, Maragheh Higher Education Complex, University of Tabriz, Tabriz, Iran

³ Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

⁴ Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, 606-0842, Japan

⁵ Kyoto Institute of Agricultural Biotechnology, Seika-cho, 619-0244, Kyoto Prefecture, Japan

Corresponding author: * mtlao@ual.es

ABSTRACT

The most important challenge for postharvest researchers is to slow the processes controlling flower death to enable cut flowers with longest vase life and best quality to reach distant markets. Senescence of carnation is normally characterized by a climacteric-like pattern of ethylene production in which a surge in ethylene production is followed by a decline. Therefore, ethylene sensitivity is an important determinant in flower longevity of carnations. Nevertheless, postharvest losses in this flower result mainly from exposure to unfavourable conditions that accelerate ethylene production or render the flower more sensitive to ethylene, therefore careful postharvest handling is essential to maximise vase life and maintain flower quality. Pre-treatment of carnations with sugars and anti-ethylene agents such silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) result in a desirable increase in postharvest longevity. Floral preservatives that contain a proper amount of sucrose and different anti-ethylene products (inhibitors of ethylene biosynthesis or action) not only delay petal senescence and decrease tissues' sensitivity to ethylene, but also significantly improve qualitative or aesthetic characteristics of cut carnations. As cut flowers are often exposed to ethylene in the postharvest shipping and marketing environment, it would be useful to develop cultivars that are insensitive or less sensitive to ethylene. Carnations have now been genetically modified through the addition of a mutation of the ethylene binding site which makes them insensitive to ethylene. The breeding of cultivars with genetically superior vase life appears to be a very efficient approach for satisfying the consumer's quality expectations.

Keywords: ACC, anti-ethylene, cut flower, ethylene, flower senescence, vase life

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, aminooxyacetic acid; ATA, aminotriazole; AVG, aminoethoxyvinylglycine; 8-HQS, 8-hydroxyquinoline sulfate; 8-HQC, 8-hydroxyquinoline citrate; 1-MCP, 1-methylcyclopropene; NBD, 2,5-norbomadiene; NO, nitric oxide; PA, polyamines; PCD, programmed cell death; SAM, S-adenosylmethionine; STS, silver thiosulfate; TBZ, thiabendazole

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INTRODUCTION

Importance of postharvest

The goals of postharvest research and extension are to maintain quality and safety and minimize losses of horticultural crops and their products between production and consumption. Strategies to prevent loss include the use of genotypes that have a longer postharvest life, use of an integrated crop management system that results in good keeping quality, and the use of proper postharvest handling systems that maintain quality and safety of the products. Thus, most horticulturists are involved to some extent in some aspects of postharvest horticulture, at least as consumers desire ornamentals with attractive appearance and long post-production life (Kader 2003).

Owing to its excellent keeping quality, wide range of forms, ability to withstand long-distance transport and remarkable ability to rehydrate after shipping, carnation is preferred, by growers of exporting countries, to other flowers (Nowak and Rudnicki 1990). Carnation is used to refer to *Dianthus caryophyllus* and its cultivars, and to hybrids of *D. caryophyllus* with other species of *Dianthus*, which are commonly referred to in trade, botanical and horticultural literature as carnations. For example, Border carnation is one kind of carnation also known as wild carnation or clove pink that has been used extensively by breeders for centuries. As a result many cultivated varieties and hybrids exist. Also the newest form of carnation is the result of a series of natural crosses between *D. caryophyllus* and *D. sinensis* (Office of Gene Technology Regulation 2006).

Carnation: use and markets

Although carnations are sold all-year round, they are in particular demand for Valentine's day, Easter, Mother's day and Christmas; while standard carnations are in greater demand, the miniature types have gained fast popularity for their potential use in floral arrangements and also as a cut flower at a comparatively low price (Nowak and Rudnicki 1990).

In many countries, carnation is one of the most popular cut flowers and of highest economic importance in the floriculture industry. Cut flowers of carnation are used in two forms or categories, i.e., the standard type in which carnations have one flower on a stem and the spray type in which carnations have multiple flowers on a stem. In recent years, spray type carnation flowers have become popular because they can be grown with less labour and meet modern consumer's demand. Modern cut-flower varieties of carnation have been selected for flower size, petal number, and stem length, postharvest longevity and disease resistance (Satoh *et al.* 2005a).

In 2004 global trade in cut flowers was valued at around US\$5.5 billion and this was predicted to steadily increase. Nearly 70% of this trade was with the EU or with Japan, the top cut flower species being sold at Dutch auctions. Total world import of carnations amounted to €190 million, corresponding to €97 million (649 million stalks) imported from European countries (AIPH 2005; reviewed by Xia *et al.* 2006). The major suppliers of carnations to Europe are Colombia, the Netherlands and Spain, while the major consumers are the United Kingdom, the Netherlands and

Germany.

Outside of Europe, the US and Japan are also major cut flower markets. In the US, domestic production of carnations, along with the other 'everyday' species such as roses, chrysanthemums, alstroemeria and gladioli, has decreased. However, imports from countries such as Colombia, Ecuador and the Netherlands have replaced local production. During 2004, about 36% of US cut-flower imports were fresh roses, followed by chrysanthemums (9.5%) and carnations (9.4%). The major cut flower varieties in the Japanese market are chrysanthemums (32%), carnations (8.2%), roses (7.2%), gerbera (3.4%), lilies (3%) and orchids (1.7%) (Office of Gene Technology Regulation 2006). Although recently, carnation's main production area has moved to the high tropics: Colombia and Kenya, however, there is still substantial production around the Mediterranean: Spain, Turkey, Italy, Morocco and Israel (Hassan 2005).

QUALITY CHARACTERISTICS IN CUT CARNATION FLOWERS AND VARIETIES

Stem length, physical conditions of the flower and flower longevity are the three major postharvest considerations in handling cut carnations. The grades and standards have mainly been developed by the Society of American Florists (SAF) and the European Community (EC). In these methods, the vase life and ornamental (aesthetic) value of carnation are determined by observing senescence profiles, i.e., in-rolling of petal margin and wilting of whole petals as well as ethylene production (Nowak and Rudnicki 1990; Satoh *et al.* 2005a).

Standard procedures for the determination of vase life in different cut flowers have been proposed by Reid and Kofranek (1980). The relative importance of several quality attributes have been studied in cut carnation. The condition of the flower is by far the most important consideration (25 points out of 100) in respect of consumer acceptance and is followed by form (20 points), colour and flower size (15 points). Freedom of infection and price and condition of foliage also influence the overall quality and consumer acceptability in carnation (Salunkhe *et al.* 1990).

There are many flower varieties of carnation. Varieties are divided into groups based on plant form, flower size and colour, disease resistance and flower type: standards, sprays (minis or miniatures), and midis (*chinensis*). Standard flowers have a single large flower per stem, whereas sprays have a larger number of smaller flowers. The flowers of midis are smaller and the stem is shorter than the standard type, and there are twice as many flowers (per plant per annum) as standards. Midis can produce either a single flower per stem, or have multiple side branches with flowers. Normally, one plant may produce 10 to 20 flowering stalks per year.

The choice of cultivar changes rather rapidly depending on consumer preference, technological progress in respect of production and market outlets. In the case of carnation, the time of onset of ethylene production and the amount of ethylene produced in the flowers vary with the carnation cultivar, and thus influence their vase life and subsequent acceptance by consumers (Nukui *et al.* 2004). White and pink standard carnation are in greatest demand, followed by red, yellow and bicoloured (Salunkhe *et al.* 1990).

DYNAMICS OF QUALITY LOSSES

Postproduction losses of floriculture crops have been conservatively estimated at 20%, a massive sum when we consider that the wholesale value of floriculture crops in 2004 was estimated at \$5.5 billion (AIPH 2005; reviewed by Xia *et al.* 2006). Decreasing postproduction losses can put dollars back into the pockets of floriculture professionals at all stages of the production and marketing chain.

Decreased plant quality and sale ability following production are generally the result of plant senescence. While senescence is a natural part of plant development, environmental stresses during production, transport, in retail environments, and in the home can accelerate senescence (reviewed in Teixeira da Silva 2006). The extent of ethylene damage depends on how sensitive the plant is to ethylene, the amount of ethylene it is exposed to, and how long it is exposed. Some plant species like geranium, petunia, carnation, orchids and snapdragons are very sensitive to ethylene. Others, including anthurium, gerbera, tulip, chrysanthemum and cyclamen are relatively insensitive to ethylene. Wounding plants causes them to produce ethylene and ethylene in turn causes a decrease of water uptake in cut carnation flowers and subsequently caused them to senesce (Mayak *et al.* 1977) and lose weight (Brandt and Woodson 1992); therefore packaging and handling that minimizes mechanical damage will extend longevity of cut flower. In flowers sensitive to ethylene, any stress can cause an increase in the ethylene production that will dramatically shorten the vase life of the flower. Particularly in carnation losses result mainly from exposure of flower to high temperatures, water stress and other conditions that accelerate ethylene synthesis or render the flower more sensitive to ethylene (Salunkhe *et al.* 1990; Reid 2004; Yangkhamman *et al.* 2005; Fukai *et al.* 2007; Yangkhamman *et al.* 2007).

PREHARVEST FACTORS AFFECTING POSTHARVEST QUALITY

Preharvest conditions have a considerable effect not only on quality and longevity of cut carnations right after harvest, but also on the response to postharvest treatments (Hewett 2006). Growing factors such as temperature, light, nutrition and also other preharvest factors such as greenhouse management, age of the plant and covering materials affected the quality and appearance of fresh cut flowers (Celikel and Kraracaly 1995).

The appearance of fresh flowers is a primary criterion in making purchasing decisions. Product appearance is characterized by size, shape, form, colour, condition and absence of defects. A wide range of preharvest factors can modulate the appearance of the harvested product. These include: (1) biological factors (pathological, entomological), (2) physiological factors (physiological disorders, nutritional imbalances, maturity), (3) environmental/cultural factors (e.g. temperature, light, humidity, water relations, etc.), (4) mechanical damage, (5) extraneous matter (growing medium, vegetable matter, chemical residues); and (6) genetic variation. Creating and/or maintaining production conditions that minimize undesirable product appearance are essential (Celikel and Kraracaly 1995; Kays 1999). These factors are related to the level of carbohydrate reserves of the plant and also with the vase life.

Genotype

The genotypic differences of the vase life of cut carnation flowers result from the variation in ethylene biosynthesis ability under mild temperature conditions in each genotype. Different levels of 1-aminocyclopropane-1-carboxylic acid (ACC) content and ACC oxidase activity in petals or the limited conversion of ACC to ethylene caused various amounts of ethylene production, resulting in various lengths of vase life and different senescence symptoms in cut carnation flowers (Wu *et al.* 1991a; Brandt and Woodson 1992).

Since the ability of cut carnation flowers to produce ethylene is determined genetically it is possible to select a low ethylene producing carnation (Onozaki *et al.* 2001, 2004; reviewed in Onozaki 2008).

Cultural practices

Cultural practices and greenhouse management can affect the quality of produced cut flowers. Disbudding is one of important practices that affect flower quality as well as quantity in carnation. Arévalo *et al.* (2007) showed that disbudding in carnation production at different developmental stages of the main floral bud affected mainly the number of bent flowering stems. It is also suggested that good cultural practices reduce injury and losses during harvesting and postharvest handling procedures. Celikel and Kraracaly (1995) pointed out that differences in greenhouse management and covering materials affected the physical and chemical characteristics and also the vase life of cut carnation flowers. They noticed, in glass covered greenhouse, that flowers in cultivars 'Astor', 'Aurigo', 'Pink', 'Calypso', 'Scania' and spray 'Red Deby' had a longer vase life than those grown in greenhouse covered with plastic. However; plastic covering material increased the fresh weight of flowers (Salunkhe *et al.* 1990; Celikel and Kraracaly 1995).

Environmental factors

Environmental conditions (such as light intensity and duration, temperature, water availability and CO₂ concentration) modify crop quality and are important in determining final product quality at the consumer level. Temperature and light are two major determinants of the success of the carnation industry. The colour, grade and quality of cut carnation are influenced by the growing temperatures. Flowers that are grown in areas where the weather during flower bud development is warm are more susceptible to adverse conditions during storage or shipment (Salunkhe *et al.* 1990; In-Byung *et al.* 2007). The optimum range of night temperatures for carnation during winter is 10-12°C and spring and summer is 12°C while that during the day in winter is 15-18°C and in summer is 21°C. Optimum temperatures will depend upon available solar radiation. Higher day and night temperatures, especially during flowering, result in abnormal flower opening and calyx splitting. Best quality carnations are produced in an area having high light intensities during winter, when at the same time the temperatures during summer months are mild. Due to high respiration rates in summer, high temperatures have an adverse effect on carnation flower longevity. Furthermore, it has been reported that the sugar content of petals was higher in the flowers cut in autumn and decreased slightly towards summer (Celikel and Kraracaly 1995).

Carnations are considered to be relatively insensitive to photoperiod and need around 40,000 lux for growth. Generally, long photoperiods promote flowering in carnations while short days delay it. Although the early stages of flowering development are favoured by long days, the later stages of bud development are controlled by light intensity. However, flower quality is adversely affected when the plants are maintained continuously under long photoperiods, which causes excessive elongation of internodes. Shoots only with 4-7 pairs of leaves are more sensitive to light intensity and photoperiods than other stages. Therefore, flower quality can be improved by providing long days for only a short period (4-6 weeks) during this stage (Salunkhe *et al.* 1990).

Prolongation of the vase life of cut flowers at high light intensities during the growth period is associated with an increase in the carbohydrate content of flowers and a decrease in ethylene production (Nowak and Rudnicki 1990). Some investigations on cut flowers showed that in flowers grown at a higher light intensity, longevity was extended in carnation (Pun and Ichimura 2003). The vase life is also influenced by the period of production throughout the year and

also the time from the beginning of production (Nowak and Rudnicki 1990; Celikel and Kraracaly 1995).

Nutritional factors

A good and balanced nutritional regime results in the best quality cut flowers with an extended postharvest life (reviewed in Balas *et al.* 2006). Nutrient deficiencies may result in postharvest losses due to reduced quality of the products. Nutrient deficiencies, especially of Ca and K, significantly reduce the postharvest quality of cut carnations. It is established that carnations need a high Ca supply. The optimum ratio of K: Ca: Mg in the nutrient solution for carnation is 55: 35: 10 (Sonneveld and Voogt 1986).

Therefore, an adequate supply of nutrients during the growth of plant is necessary in order to ensure quality flowers. At the initial period of growth, 'Sim' carnation was very susceptible to N alongside with a high consumption of K. In a total proportion of all other elements N exceeds K. Optimum ratio of nutrients at this period N: P₂O₅: K₂O = 43.4: 17.5: 40.1 (Mantrova 1977; Salunkhe *et al.* 1990). K deficiency has been shown to reduce water uptake, tolerance to ethylene and the potential life of flowers. Adequate amounts of inorganic salts like potassium chloride, ammonium and potassium nitrate delayed senescence by maintaining the osmotic potential in petal tissues and substituted metabolic sugars in extending vase life of carnation (Salunkhe *et al.* 1990).

HARVESTING MANAGEMENT

Flowers for direct sale to final consumers, such as in farmers' markets, should be harvested slightly more mature than flowers sold to retailers for resale; selling to wholesalers requires a slightly less mature flowers than a retailer would require. The ideal stage of maturity will also vary with the intended use. The rate at which the flowering stem declines is dependent on the tissue temperature and water status of the flower, stem and foliage. High temperatures accelerate the rate of decline.

Flowers should be harvested at the proper stage of development for maximum vase life. The optimum stage varies with the species grown and the time of the year. The maturity at which carnations are harvested depends on the proposed marketing procedure. Buds at the 'paint-brush' stage, with upright petals, will open quickly. Flowers for immediate use are normally harvested with the outer petals between the vertical and the horizontal. Spray carnations normally are harvested with at least one opened flower on the inflorescence (Salunkhe *et al.* 1990; Reid 2004; Office of Gene Technology Regulation 2006).

Morning harvest is often advantageous over afternoon harvest because the temperature is lowest during the morning, plant water content is high, and the rest of the day is available for packing and flower distribution (Nowak and Rudnicki 1990). The other important factor in maintaining quality of the harvested flowering stem is to preserve the water status of the plant tissue. Harvested stems, left dry until brought into the grading/packing shed, may wilt to a point beyond their ability to recover.

POSTHARVEST FACTORS

Postharvest systems should be designed to provide proper treatment of each plant species to prolong quality of the flowers and to make the most effective use of labour possible.

Genotype

There are few studies that actually deal with this topic directly. However, Onozaki *et al.* (2001) claimed that the production of ethylene by cut carnation flowers is determined genetically as it is possible to select a low ethylene producing carnation. To test this theory in detail, Pranom Yang-

khamman *et al.* (pers. comm.) kept cut carnation flowers of 10 cultivars ('Moutarde', 'Pink Exerea', 'Mambo', 'Marlo', 'Corsa', 'Peachy Mambo', 'Paha', 'Michelle', 'Zebah', and 'Magny Cours') at 24 or 32°C. Differences in vase life resulted into clustering into three groups: those for which the vase life at 32°C was longer than at 24°C ('Moutarde' and 'Zebah'); those with equivalent vase life at 24 and 32°C ('Pink Exerea', 'Marlo', 'Peachy Mambo', 'Michelle' and 'Magny Cours'); and those for which the vase life at 32°C was shorter than at 24°C ('Mambo', 'Corsa', and 'Paha').

Storage, grading, packing and transport

Both standard and miniature carnations are graded by stem strength, stem length, bloom diameter, and freedom from defects. Stem strength is determined by holding the stem horizontally at a point one inch above the minimum length for the grade. If the deviation of the flower head is more than 30° from the horizontal (with the natural curvature down), the flower is considered defective. Other defects include slab sides, bullheads, blown heads, singles, sleepy appearance, splits, discoloration, and damage from insects and diseases. Standard carnations are bunched, and tied at the base and at least one other place below the flower heads. Instead of different colour labels, some growers indicate different grades by the colour and/or number of rubber bands on each bunch. Standards for miniature carnation bunches vary; a bunch normally contains a minimum of 30 buds total, at least 7 of which are open. With standard carnations, flower heads may be alternated (5 high, 5 low) at the top of the bunch to produce a neat and compact bunch and reduce the risk of neck breakage (Reid 2004).

Correct postharvest handling is essential to maximise vase life and maintain flower quality. Dehydration is the major factor leading to deterioration of flowers and foliage. It can be minimised by controlling temperature and relative humidity during postharvest storage.

Temperature is the most important postharvest factor. Most studies on flower longevity have been carried out under moderate temperatures while senescence of cut carnation flowers under high temperature conditions had remained unclear until recent studies by Yangkhamman *et al.* (2007) elucidated this process. Until now, only short-term exposure to high temperatures has been studied from the viewpoint of pest control and temperature fluctuation during transportation (Maxie *et al.* 1973; Verlinden and Woodson 1998). By lowering flower temperature as soon as possible after harvest, respiration rate and water loss are reduced, ethylene production is suppressed and sensitivity to ethylene is reduced while microbial activity is slowed. On the other hand, ethylene production in cut carnation flowers was delayed after exposure to high temperature (Verlinden and Woodson 1998). The blocking of ethylene production at high temperatures can be caused by inhibition of ethylene biosynthesis enzymes activities in cut carnation flowers (Brandt and Woodson 1992; Yangkhamman *et al.* 2005). Yangkhamman *et al.* (2007) showed that high-temperature depression of ethylene production in cut carnation flowers cv. 'Excerea' can occur because of inhibition of ACC synthase and ACC oxidase activities in flowers. Substantial ACC oxidase activity in styles in preclimacteric carnation flowers had also been reported by Manning (1985) and Woodson *et al.* (1992). Large differences were apparent between ACC synthase activity in petals at 24 and 32°C (Yangkhamman *et al.* 2007). These ACC-accumulation-related activities were markedly decreased in petals at 32°C, indicating that a low ACC synthase activity and ACC accumulation in petals are a factor of ethylene biosynthesis inhibition under high-temperature conditions. In addition, the respective expressions of *DC-ACO1* and *DC-ACS1* were low in both gynoecia and petals of flowers kept at 32°C. In naturally senescent carnation flowers, expression of ACC synthase and ACC oxidase genes occurs first in the ovary, and then in the style and petals (ten Have and Woltering 1997). Satoh *et al.* (2005c) and Shibuya *et al.* (2000) claimed that

gynoecia produced ethylene first; then the ethylene diffused to petals where the autocatalytic ethylene production started. Yangkhamman *et al.* (2007) showed that low ACC synthase and ACC oxidase activities under high-temperature conditions were transcriptionally regulated. The carnation ACC synthase gene is known to show differential expression in a tissue-specific manner: *DC-ACS1* is expressed in petals, although *DC-ACS2* and *DC-ACS3* are expressed in styles (Jones and Woodson 1999a); ACC synthase and ACC oxidase gene expression in petals is regulated by ethylene from gynoecia (Jones and Woodson 1999b; Shibuya *et al.* 2000; Jones 2003). Verlinden *et al.* (2002) showed that carnation flowers exposed to 44°C for 24 h showed delayed transcription of both ACC synthase and ACC oxidase genes associated with delayed ethylene production. Yangkhamman *et al.* (2007) showed that ACC synthase and ACC oxidase gene expression in flowers was retained at both 24 and 32°C, consistent with the activities, except for that of ACO oxidase in gynoecia of flowers kept at 32°C. The high ACC oxidase activity with low *DC-ACO1* expression in gynoecia of flowers at 32°C indicated the existence of another ACC oxidase gene (*DC-ACO2*) which was associated with ACC oxidase activity in gynoecia of cut carnation flowers (Norikoshi *et al.* 2008). However, some physiological disorders, petal colour fading and browning (Yangkhamman and Fukai 2007) and also high respiration rate occurred under high-temperatures conditions (Teklic *et al.* 2003).

It is important to know the correct storage temperature for each product handled. Once flowers are harvested they should be cooled as quickly as possible after processing.

Maintaining high humidity (95-98%) during the storage period reduces water loss. Dehydration greatly affects quality, causing wilting and shrivelling. High humidity should be used with low temperature storage because humid conditions in combination with warm temperatures favour the growth of fungi and bacteria. For cut carnation flowers the optimum storage temperature are from 0 to 1°C (Stevens 1995; Reid 2004). Based on Nowak and Rudnicki (1990) suitable temperature for carnation long-time dry storage (4-6 months) is 0-1°C while, optimum temperature for wet storage is 4°C.

Flowers are placed at 1°C into a box with a polyethylene film and newspaper. The open flowers can be stored from 2 to 4 weeks, while the buds harvested in developmental early stages can be stored up to 4 months. Freezing injuries may occur at temperatures lower than 0.5°C. The symptoms are leaf and flower collapse and water soaking of floral tissues.

Bruising and breaking flowers destroys their aesthetic and economic value. Wounded plant tissue increases the production of ethylene gas accelerating maturation of flowers and greatly shortening vase life. Good sanitation measures, removing all plant residues from storage areas and not placing any fruit or fruit-type vegetables near stored flowers, will reduce the potential for ethylene damage (Reid 2004).

Carnations are usually packed in standard horizontal fibreboard boxes. Standard carnations ship better and last longer if purchased in the bud stage while miniature carnations should be purchased when at least one flower per stem is open (Reid 2004).

Florist shop management

Cut stems should be placed into either water or a fresh flower food (floral preservative solution). The typical fresh flower food contains water; a simple sugar that serves as a food source; a chemical to prevent or retard the growth of microorganisms that can plug the conductive tissue; and an ingredient to acidify the water, typically citric acid (see Balas *et al.* 2006 for review).

The quality of used water can influence the effectiveness of the fresh flower food solution on prolonging flower appearance. The water to make the solutions should be analyzed for total dissolved salts, content of individual salts, pH and alkalinity. Ideally, the final food solution should

have a pH in the range of 3.0 to 4.5. When a flowering stem is cut from the plant, it is severed from its food supply. The food source must be replaced. Simple sugars are used as the source of nutrition for fresh cut flowers. They provide the energy to complete flower development, open buds and colour stability. Fresh flower food solutions contain 0.5 to 3% sugar are used continuously. On the other hand, food solutions could be used as pulse for some flowers like gladiolus, freesia and carnation (Weerts 2002) and they may contain up to 20% of sugar for only relatively short periods of time. The fresh flower food solution in the storage container should be deep enough to cover the ends of all stems with sufficient margin for error and to allow for uptake without having to constantly refill avoiding stems sucking air. Rinsing stems and leaves under tap water before recutting and placing into containers with solution will aid in keeping storage solutions clean. Leaves should be removed from the stems up to a point just above where they will not become submerged in the solution (Stevens 1995).

Vase life

Vase life of cut flowers is one of the main characteristics determining the commercial value of the ornamental flowers (Nukui *et al.* 2004). Usually the vase life of carnation flower has been determined by observing senescence profiles i.e., in-rolling of petal margin and wilting of whole petals as well as ethylene production. This method has been used successfully for cut carnation flowers of the standard type. However, in spray type carnation flowers, the vase life of the flowers is determined by the sum of the flowering period of each flower. Satoh *et al.* (2005a) proposed an alternative method to estimate the vase life of spray carnation by observing the number of open flowers i.e., the percentage of open flowers and the total number of initial flowers buds. In that study they were defined vase life as the period during which 40% or more flowers were fully open.

FLOWER SENESCENCE

Senescence can widely be defined as the combination of events that lead to the death of cells, tissues or organs (Reid and Wu 1992). It is mediated by a series of highly coordinated physiological and biochemical changes, such as increased activity of hydrolytic enzymes, degradation of macromolecules, loss of cellular compartmentation and increase in respiratory activity. These changes are related to changes in expression of genes and synthesis of proteins (Borochoy and Woodson 1989).

Flower senescence is a common cause of quality loss and reduced vase life of flowering plants and cut flowers (Serek and Reid 2000; Teixeira da Silva 2006). Senescence in many flowers is accompanied by pollination promoting the production of ethylene which ultimately causes petal wilting, abscission and sleepiness (florets failed to re-open) of petals and a climacteric increase in ethylene production. This is induced by several factors, e.g., water stress, carbohydrate depletion, microorganisms, and ethylene effects (Wu *et al.* 1991a, 1991b).

The challenge for postharvest researchers is to slow the processes controlling flower death to enable cut flowers to maintain good quality with a long life. A thorough understanding of the processes that lead to cell death of floral tissues is integral to achieving this goal. Postharvest performance of cut flowers is affected by the developmental stage of a flower at harvest, pro-senescence signals that originate from specific tissues within the flower (e.g. pollination-induced petal senescence), and stress-related metabolism (in response to temperature, wounding, nutrient starvation). Plant hormones, membrane stability, water availability, cellular proteolysis and carbohydrate metabolism act in concert to determine the differential rate of senescence for each floral organ. Currently, flowers can be grouped into several categories based on postharvest technologies that can extend their vase life (e.g., sensitivity to ethylene, chilling

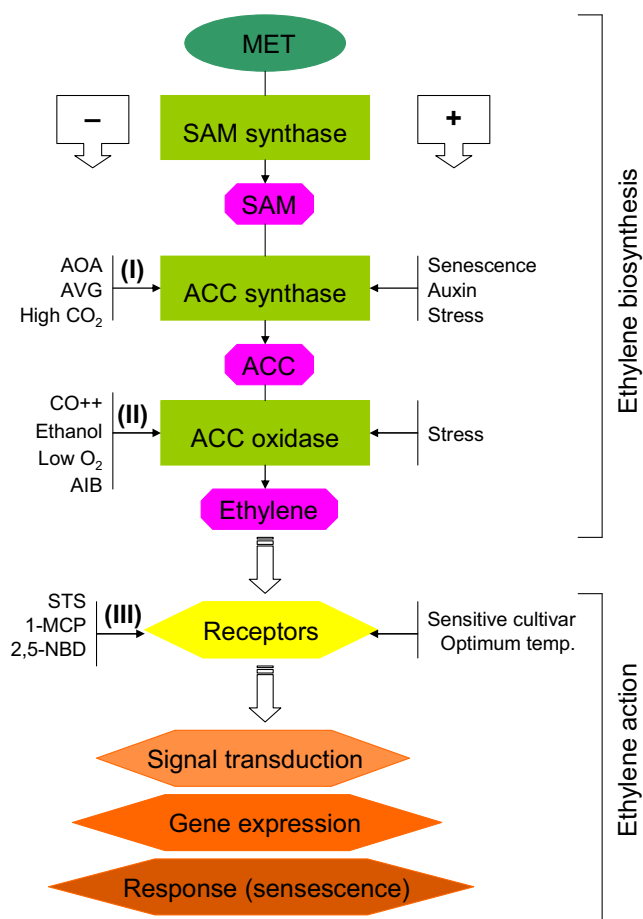


Fig. 1 Factors that affect ethylene biosynthesis and action. Through pathway of ethylene two steps involving ACC synthase (I) (inhibited by AOA and AVG and stimulated by senescence) and ACC oxidase (II) (inhibited by AIB and ethanol and stimulated by Mn²⁺ and stress) are rate-limiting for ethylene production. Chemicals (STS, 1-MCP, etc.) are also effective at the receptor level (III) and prevent the binding of ethylene. 2,5-NBD = 2,5-norbornadiene that blocks ethylene action by competing with ethylene for binding sites.

sensitivity, leafy stems, multiple/single flowers per stem, woody stems) based in delayed process of flower senescence that has been shown to be a genetically programmed event (Eason 2006).

Senescence of climacteric flowers is associated with an increased rate of ethylene production and respiration, concomitant with the onset of petal wilting (Halevy and Mayak 1981). Exposure to exogenous ethylene promotes senescence of climacteric flower petals with activation of ACC synthase and/or ACC oxidase (Fig. 1; Woltering and van Doorn 1988; Borochoy and Woodson 1989).

The phytohormone ethylene plays a vital role in regulation of flower senescence in many species. The best known visible morphological ethylene effect is petal in-rolling and sleepiness. This response is associated with a change in cellular ultrastructure of upper part of petal; in particular there is a loss of cellular integrity – clearly a very late event that leads to death of the tissue. Concomitant with this disruption of petal structure there is growth of the ovary. Clearly there is redistribution of energy between the parts of the flower (van Staden 1995).

In carnation, a large amount of ethylene is synthesized several days after full opening of the flower during natural senescence (Woodson *et al.* 1992), or several hours after compatible pollination (Nichols *et al.* 1983; Larsen *et al.* 1995) or treatment with exogenous ethylene (Buanong 2006).

Woltering and van Doorn (1988) studied petal senescence in mature flowers of 93 species from 22 families.

Most of families (*Geraniaceae*, *Libiatae*, *Ranunculaceae*, *Rosaceae* and *Scrophulariaceae*) showed initial abscission in response to ethylene except for a few families (*Caryophyllaceae*, *Campanulaceae*, *Malvaceae* and *Orchidaceae*), which showed wilting as their primary senescence symptom (Woltering and van Doorn 1988; reviewed by van Doorn 2008; van Doorn and Woltering 2008).

In addition senescence of the petals of many cut flowers from *Compositae*, *Iridaceae*, *Liliaceae* families (e.g. *Iris*, *Tulipa*, *Hemerocallis*, and *Gladiolus*) appears not to be related to ethylene. Flower senescence in these plants is ethylene-independent. The senescence of these flowers is not accelerated by exposure to exogenous ethylene, nor delayed by inhibitors of ethylene biosynthesis or by ethylene antagonists. The major events that occurred in the ethylene-unresponsive daylily are an early decline in phospholipid synthesis, an increase in cell permeability that leads to an increased efflux of sugars and ions, a respiration climacteric, early wilting and then autolysis of petal tissue (Serek *et al.* 1994a; Buanong 2006).

HORMONAL REGULATION OF FLOWER SENESCENCE

Phytohormones play a central role in the regulation of senescence by either stimulating or inhibiting senescence. Flower senescence proceeds by coordinated regulation of plant hormones and response to them. Cytokinins and gibberellins tend to retard flower senescence while ethylene and abscisic acid (ABA) promote it (Halevy and Mayak 1981). In many dicotyledonous flowers whose senescence is ethylene-dependent, ethylene production is associated with onset of flower senescence and chemical or genetic inhibition of ethylene synthesis or action delays it. However, senescence of many monocotyledonous flowers is mostly ethylene-independent and is thought to be primarily regulated by ABA (Kim and Miller 2008).

ABA may increase prior to or during senescence in attached or detached organs. This hormone interacts with other growth regulators as it induces a reduction in the levels of cytokinins, gibberellins and auxins. ABA is less active than ethylene. The interaction between cytokinins and ABA is counteractive as cytokinins inhibit ABA effects and ABA causes a reduction in cytokinin levels. Jasmonic acid (JA) is widely distributed in plants and its derivatives are active at very low concentrations. Treatment with ABA hastens programmed cell death (PCD)-associated events, such as ion leakage, lipid peroxidation, etc. (Zhou *et al.* 2005).

Methyl jasmonate (Me-JA) promotes plant senescence due to the stimulatory effect on ethylene synthesis. Me-JA induces senescence with a stronger action than ABA. Cytokinins can often alleviate or reverse the effects caused by JA in plant tissues (Salunkhe *et al.* 1990; Serek and Reid 2000). Ethylene and cytokinins are prominent senescence-regulating phytohormones. Ethylene is the key phytohormone in promoting senescence in cut flowers. Cytokinins, on the other hand, counteract the effect of ethylene on senescence (Serek and Reid 2000).

Several plant hormones have been shown to influence ethylene metabolism in carnation. Auxins are thought to promote petal senescence through the stimulation of ACC synthase activity. Cytokinins like kinetin, benzyladenine, zeatin and dihydrozeatin have the capacity to extend longevity. There are contradictory results in different studies about cytokinin effects on carnation. In an earlier study, cytokinin stimulated flower senescence in carnation by increasing ethylene production by the gynoecium (Woodson and Brandt 1991), while recently it has been shown that cytokinin applications delayed flower senescence in carnations (Wawrzyńczyk and Goszczyńska 2003).

Concerning gibberellins, it has been shown that GA₃ (gibberellic acid) delayed carnation senescence, when applied to young flowers. In all parts, endogenous levels of ACC are reduced by GA₃ treatment. This is most pro-

nounced in the petal bases, which are important regulatory sites for ethylene production; they may be involved in controlling the onset and degree of petal in-rolling (van Altvorst and Bovy 1995; Zhou *et al.* 2005).

Ethylene

The removal of the flower from the parent plants changes rapidly hormonal balance in particular organs, and these changes affect ethylene production and/or action. There can be little doubt that ethylene is pivotal in carnation flower senescence. This makes it essential that a closer look is taken at the biosynthesis and action of this hormone.

Ethylene has been shown to play a central role in physiological process of senescence in flowers (Sisler *et al.* 1983; Buanong 2006). Biosynthesis of ethylene in flower tissues is under strict metabolic regulation and is subjected to induction by a variety of signals including emasculation, pollination, wounding, auxin, ABA and environment stress (Borochoy and Woodson 1989).

Ethylene production is the most important signal for the onset of the PCD of flowers in ornamentals. Inhibitors of ethylene production may delay PCD and improve the quality of ornamentals after harvest (van Staden 1995; Zhou *et al.* 2005). Flower senescence is controlled by both: an increase in ethylene production and an increase in sensitivity to ethylene (Borochoy and Woodson 1989). Increased ethylene production during senescence of carnation flowers in petals was associated with a concomitant increase in ethylene biosynthesis in styles, ovaries and receptacles (Woodson *et al.* 1992). The ethylene produced initially in gynoceia might induce autocatalytic ethylene production and in-rolling in carnation petals (Satoh *et al.* 2005c). The increased ethylene production is correlated with the increased concentration of ACC, the increased activity of ACC synthase and ACC oxidase (Borochoy and Woodson 1989; Woodson *et al.* 1992) and the expression of both ACC synthase and ACC oxidase genes in senescing petals (Jones 2002; Satoh *et al.* 2005b, 2005c).

Both flowers and fruits have been shown to increase their responsiveness or sensitivity to ethylene as they mature. The treatment of immature carnation petals with exogenous ethylene does not induce autocatalytic ethylene production or enhanced expression of senescence-related genes (Jones 2002).

Physiological, biochemical and molecular aspects of ethylene biosynthesis and action

Ethylene biosynthesis

In higher plants, ethylene is synthesized from methionine via a pathway involving the conversion of *S*-adenosylmethionine (SAM) to ACC and the oxidation of ACC to ethylene (Fig. 1). The enzyme ACC synthase converts SAM to ACC and methylthioadenosine while ACC oxidase catalyzes the conversion of ACC to ethylene, HCN and CO₂. In addition, methionine is regenerated in the Yang cycle (Adams and Yang 1977).

ACC: The immediate precursor of ethylene in higher plants is ACC. The endogenous ACC level in various flower parts increases during senescence. Application of 1 mM ACC stimulates wilting in whole carnation 'Yellow Candy' flowers (Pun *et al.* 2001b). Recently, Tanase *et al.* (2008) reported that ACC treatment markedly accelerated senescence of 'Sandrosa' but had only a small effect on 'Miracle Rouge' and 'Miracle Symphony' long life cultivars.

The stimulation of ethylene production after ACC application was largely responsible for the reduction of the flower vase life. Exposure to ethylene of isolated carnation petals, separated into upper and basal parts, showed that the majority of ethylene production is evolved from the basal part of the petals. Hsieh and Sacalis (1987) suggested that ACC is transported from ovaries to the petals. Drory *et al.* (1993) further indicated that during flower senescence,

ACC is transported from petal bases to their upper parts where ethylene is then released and wilting occurs. Apparently mRNA is spatially regulated within carnation flowers.

Endogenous ACC content in the basal portions of senescing carnation petal is 3 to 5 times higher than in the upper parts. Application of ACC to the upper portion of senescing petals increases their ethylene production (van Altvorst and Bovy 1995).

ACC synthase and ACC oxidase: The conversion of SAM to ACC is catalyzed by the pyridoxal phosphate-requiring enzyme ACC synthase as a cytoplasmic enzyme, which represents the rate-limiting step in ethylene biosynthesis in many plant tissues (Woodson and Jones 2003; Buanong 2006). In carnation flowers, the increase in ethylene synthesis during senescence occurs simultaneously with an increase in ACC synthase activity and an increase in ACC content. Cytoplasmic ACC synthase is found in most plant tissues. This enzyme is highly labile and requires low levels of pyridoxal phosphate as a co-factor for catalytic activity (van Altvorst and Bovy 1995). ACC synthase is inhibited by aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) and its analogues (Fig. 1). ACC synthase action is stimulated by various stress conditions (Yakimova and Woltering 1997). In carnations water stress is accompanied by accumulation of ACC (Borochoy *et al.* 1982).

The final step in the ethylene biosynthetic pathway is catalyzed by ACC oxidase, formerly referred to as ethylene-forming enzyme (EFE). ACC oxidase activity is located in the cytosol, but may also be situated in membranes (Bouzaen *et al.* 1990; Wang and Woodson 1991). ACC oxidase requires Fe²⁺ and its chelated form, as well as the reductant, ascorbate, as cofactors for catalytic activity (Yang 1985).

ACC oxidase is denatured by heat, requires oxygen, and is saturable by ACC (van Altvorst and Bovy 1995). The activity of ACC oxidase is also pH dependent. The optimum pH for activity ranges between 7.5 and 8.0. ACC oxidase is stimulated by Mn²⁺ and inhibited by 2,5-norbornadiene (NBD) (Wang and Woodson 1989; Woodson *et al.* 1992).

Control of ethylene

Ethylene plays a decisive role in petal degradation during senescence of climacteric flowers (van Altvorst and Bovy 1995). It is, therefore, important to inhibit ethylene synthesis and action and to treat the causes of the increase in ethylene sensitivity during senescence. Abeles *et al.* (1992) divided ethylene antagonists into the following two groups:

(A) Those that act at the synthesis process such as high CO₂ levels, ethanol, AVG, AOA, silver ions and various chelators.

(B) Those that act as competitive inhibitors of ethylene by binding to the ethylene receptors such as silver and certain cyclo-alkenes (Sisler and Serek 2003; Buanong 2006). Compounds preventing an ethylene response interact with the receptor and compete with ethylene for binding. A single exposure of plant tissue to these compounds is enough to prevent binding of ethylene because they remain bound for a long period of time, saturating the receptor and even high levels of ethylene not inducing any action (Sisler and Serek 2003).

Treatment with ethylene antagonists is a line of defence primarily taken by the cut flower grower immediately after harvest. However, not all of the compounds mentioned above are suitable for commercial use due to either consumer safety or high costs.

Inhibitors of ethylene biosynthesis

Compounds such as AVG and AOA effectively delay senescence of climacteric flowers by inhibiting the action of ACC synthase. The addition of ethanol to the vase medium can also lengthen vase life by inhibiting the conversion of ACC to ethylene (Wu *et al.* 1992; van Altvorst and Bovy

1995; Podd and van Staden 1999; Pun *et al.* 2001b).

The activity of ACC oxidase can be blocked by Co^{2+} , Triton X-100, α -aminoisobutyrate and allocoronamic acid derivatives (Serrano *et al.* 1990; van Altvorst and Bovy 1995).

High CO_2 levels suppress ethylene synthesis by inhibiting the activity of ACC synthase. Some reports also indicated that hypoxia delayed flower senescence in carnation. A low O_2 concentration (hypoxia) suppressed the onset of the climacteric rise in CO_2 evolution and increased the vase life of flowers two-fold over that of flowers treated with silver thiosulfate (STS), indicating that the retarding effects of hypoxia on the onset of senescence transcend its inhibitory effects on the action of C_2H_4 . It is suggested that the sudden increase in water loss may be the result of a C_2H_4 -induced increase in hydraulic conductivity of the petals (Solomos and Gross 1997). In addition, chelators such as 8-hydroxyquinoline sulfate (8-HQS) or 8-hydroxyquinoline citrate (8-HQC) that are commonly used as antimicrobial agents in preservatives also act by inhibiting ethylene synthesis (Hassan 2005). Ethylene production in cut carnation flowers is inhibited by pulse treatment with STS (Reid *et al.* 1980; Halevy 1981). STS is a superior ethylene inhibitor but it is environmentally unfriendly because it contains heavy metals (Mayers *et al.* 1997). Novel chemicals such as 1-methylcyclopropene (1-MCP) have been studied as an alternative to STS (Mayers *et al.* 1997; Lurie 2007; Reid and Celikel 2008). However, there are some limits to its use and it is rather expensive. Boric acid, ethanol and acetaldehyde have been used as components of vase solution to inhibit ethylene synthesis in cut carnation flowers, they are environmentally friendly and the price is lower than 1-MCP (Heins 1980; Heins and Blakely 1980; Wu *et al.* 1992; Serrano *et al.* 2001; Podd *et al.* 2002). Boric acid depresses ACC oxidase and ACC synthase activities as well as the conversion of ACC to ethylene, resulting in depressed petal in-rolling (Serrano *et al.* 2001). Boric acid and ethanol inhibited ethylene synthesis in cut 'Exerea' carnation flowers under high temperature conditions (Pranom Yangkhamman, pers. comm.).

Although these compounds are effective to varying degrees in inhibiting ethylene synthesis and extending vase life, their commercial use is limited by factors such as the costs involved, practical implications on their application, toxicity and, most importantly, the fact that they do not protect the flower against the presence of exogenous ethylene in the surrounding atmosphere (Sisler and Serek 1997).

Inhibitors of ethylene action

These compounds inhibit ethylene action by binding to the ethylene receptors, thus preventing the binding of ethylene. Such inhibitors are effective in protecting plant tissues from endogenous as well as exogenous ethylene and suppress the autocatalytic activity of ethylene on its own synthesis (Abeles *et al.* 1992).

Silver applied in the form of STS complex, is very effective in inhibiting ethylene action (Reid and Wu 1992; Hassan 2005). The silver ion released from the complex prevents the binding of ethylene to the ethylene receptor protein. Treatment of cut flowers with STS results in a suppression of respiration, the surge in ethylene production and delayed senescence (Hassan 2005).

Cyclic olefins such as NBD, *cis*-butene, *trans*-cyclooctene and 1-MCP also act as inhibitors of ethylene responses by effectively preventing the binding of ethylene to its receptors (Sisler *et al.* 1986; Wang and Woodson 1989; Sisler *et al.* 1996; Hassan 2005). 1-MCP is an effective inhibitor of ethylene action due to its ability to bind irreversibly to the ethylene receptors or, at least, to remain bound for many days (Sisler *et al.* 1996; Sisler and Serek 1997). Treatment of most climacteric cut flowers and fruit results in a marked delay in senescence and ripening (Serek *et al.* 1995).

Other hormones

ABA has for years been thought to play a major role in

the regulation of flower senescence and many studies have attempted to correlate an endogenous increase in ABA with a rise in ethylene production. However, the synchronized behaviour of these two hormones in senescing carnation petals has made it difficult to assess their respective roles in relation to the postharvest process. Serrano and Martínez-Madrid (1999) found that ABA levels increased in amino-triazole-treated carnations during senescence, without any rise in ethylene production, which indicates a separation of relationship between these two hormones (Serrano and Martínez-Madrid 1999).

Exogenously-applied ABA accelerated the senescence of cut carnation flowers through the stimulation of ethylene biosynthesis. In the gynoecium of cut carnation flowers, ABA content began to increase immediately after harvest, reached a maximum 5–7 days after harvest and then declined. A substantial increase in ABA content was observed on the 3rd day after harvest, 2 days before the surge in ethylene production. On the other hand, in the petals of carnation flowers, ABA content steadily increased from the 1st day after flower harvest, but a significant rise in ABA content took place on the 5th day when the surge in ethylene production occurred. These results suggested the involvement of ABA as a crucial factor in the induction of ethylene biosynthesis in the flower tissues, which results in senescence of carnation flowers (Onoue *et al.* 2000).

Natural and artificial cytokinins: In addition to their important roles in controlling and stimulating cell division, the cytokinins, zeatin and its derivatives and analogues also inhibit leaf and flower senescence. Cytokinins are known to reduce the sensitivity of plants to ethylene, and for some years a commercial vase preservative containing 6-benzyladenine (BA) was marketed for use with carnations. Kinetin and BA delay flower senescence and ethylene production in cut carnation flowers (Mor *et al.* 1983; Cook *et al.* 1985; Wawrzyńczak and Goszczyńska 2003).

There is an inverse relationship between cytokinin content and senescence (e.g., roses, carnation, gerbera), and the response of tissues to cytokinin depends on the type and concentration of cytokinin and the developmental stage of the flowers. Raised cytokinin content in plants has also been linked to improved tolerance of stress. Further molecular and genetic analyses are required to fully understand the role of cytokinins in the regulation of flower senescence (Eason 2006).

Cytokinins delay the senescence of cut carnations (Mor *et al.* 1983). Pre-treatment with cytokinins blocked the conversion of applied ACC to ethylene as well as the *in vivo* production of ACC and ethylene. It has been reported that a BA pre-treatment reduced the development of ACC synthase in basal portion of the petal and also reduced ACC oxidase in the petals (Mor *et al.* 1985).

Other growth regulators

In recent years, researchers have studied the properties of a range of new chemicals with plant hormone or growth regulator activity. The anti-senescence effects of the polyamines and the various effects of JA and Me-JA are the most notable of these. Although intriguing findings have been reported, none of these effects has been unequivocally proven to be a natural regulatory process, nor have these compounds proved to be of practical commercial value in the postharvest life of ornamentals.

Lower levels of endogenous gibberellins in carnation flowers lead to flower senescence while exogenously-applied GA_3 delays senescence through the delay and depression of ethylene biosynthesis; GA_3 also brought the delay or reduction of the climacteric ethylene production as well as the reduction of ACC content in cut carnation flowers, these resulted in delayed flower senescence and longer vase life (Saks *et al.* 1992; Saks and van Staden 1993a). GA_3 reduced the rate of fresh weight loss which started during the pre-climacteric of the cut carnation flowers (Saks and van Staden 1993b).

'Exerea' flowers treated with both GA_3 (0.1, 0.5 and 1

mM) and BA (1 mM) maintained higher fresh weight, resulting in longer vase life (Pranom Yangkhamman, pers. comm.) and depressed petal in-rolling. In addition BA pretreatment effectively maintained the water balance in flowers at high temperature (32°C). Flowers pretreated with 1 mM BA combined with or without $Mg(NO_3)_2$ maintained high values of b^* and c^* at this temperature.

Polyamines

By contrast to ethylene, polyamines (PAs) are reported to be effective anti-senescence agents. The major forms of PAs are putrescine (Put), spermine (Spm) and spermidine (Spd) and are found in every plant cell (reviewed in Kuznetsov and Shevyakova 2007; Pang *et al.* 2007).

PAs and ethylene use a common precursor, SAM, for their biosynthesis. But these two molecules show opposite effects in relation to senescence. It was found that PAs inhibited the accumulation of the wound-inducible ACC synthase transcript. It has been suggested that both, salicylic acid and PAs may specifically regulate ethylene biosynthesis at the level of ACC synthase transcript accumulation. Other PAs like Spm and Spd seem to be more active in retarding senescence. Most of the observations indicate that various PAs can delay senescence in a number of plant tissues by inhibiting ACC synthesis (Lee *et al.* 1997). The ethylene biosynthesis could also be modulated by the *in vivo* biosynthesis of PAs since ethylene and PA biosynthetic pathways share SAM as a common intermediate and could compete for the available SAM during senescence (Serrano and Romojaro 1991; Pandey *et al.* 2000).

Lee *et al.* (1997) found that Spm delayed the senescence of cut carnation flowers and reduced ethylene production and ACC content and the activities and transcript amounts of ACC oxidase and ACC synthase in petals. It was suggested that the endogenous PAs possibly suppress ethylene production. Treatment of cut carnation with aminotriazole retarded senescence and increased flower longevity and it also inhibited the climacteric peak of ethylene production but the treatment had no effect on the levels of PAs (Serrano and Martínez-Madrid 1999). Furthermore, Serrano and Romojaro (1991) found that total amount of PAs (Put+Spd) in non-climacteric 'Killer' and climacteric 'Arthur' carnations were similarly high but during senescence period, climacteric cultivar 'Arthur' showed a dramatic decrease in total amount of PAs and 'Killer' that maintained a high amount of PAs showed greater longevity (Serrano and Romojaro 1991).

Pollination

The pollination of many flowers results in rapid wilting or abscission of floret. In ethylene-sensitive flowers, this response is associated with enhanced ethylene production (Nichols *et al.* 1983) and developmental changes such as ovary growth. These processes are induced by a translocated signal that precedes in the growing pollen tube, after compatible pollination, to the ovary and petals. Yangkhamman and Fukai (2007) also confirmed that the ovary and seed development involved ethylene biosynthesis in cut carnation flowers.

In carnation, ACC oxidase expression plays an important role in amplifying the ethylene signal in styles and petals.

Styles of mature carnation flowers contain constitutive levels of ACC oxidase mRNA that lead to high levels of ACC oxidase activity (Woodson *et al.* 1992).

While pollination stimulates the expression of ACC oxidase, the inhibitors of ethylene action prevents this increase. Moreover, the application of AVG, an inhibitor of ACC synthase, to carnation styles has been shown to effectively block pollination-induced ethylene production by the style, indicating that this early ethylene is dependent on ACC synthase activity (Woodson and Jones 2003).

It is clear that ethylene plays a critical role in pollination-induced petal senescence, as inhibitors of ethylene action prevent this response. The expression of ACC oxidase

genes in the ovary and petals of carnation is completely dependent on ethylene (Jones and Woodson 1999a), suggesting that ethylene produced in response to primary pollination signals in the gynoecium is critical to propagate the signal throughout the flower (Jones and Woodson 1999a; Woodson and Jones 2003).

Studies of ethylene production following pollination of carnation showed a progression of ethylene synthesis from the stigma and style to the ovary, the receptacle, and, finally, the petals. This suggest movement of a stimulus for ethylene production from the stigma following pollination, the presence of high level of ACC oxidase activity in stigma, and the rapidity with which ethylene production increases following pollination suggested that ACC in the pollen might be the initial pollination stimulus (Peiser 1986).

Symptoms of pollination such as flower wilting and increased ethylene production in carnations, may occur 2 to 4 days after pollination (Nichols 1980).

BIOLOGICAL, PHYSIOLOGICAL AND MOLECULAR CHANGES OF CUT CARNATION FLOWERS DURING SENESCENCE PROCESS

Each step in the life span of postharvest flowers is associated with a number of coordinated biochemical, physiological, hormonal and structural changes that are strongly modulated by the fluctuations of environmental factors and stressors of biotic and abiotic origin. In general, the senescence of ethylene-sensitive flowers, such as carnations, is associated with a loss of membrane integrity, climacteric rise of respiration and enhanced ethylene synthesis (Yakimova *et al.* 1997).

Understanding the physiology and biochemistry of petal senescence as a result of aging and biotic or abiotic stresses is therefore essential for improving the postharvest quality of ornamentals (Zhou *et al.* 2005).

Biochemical and molecular changes

Main biochemical and molecular changes during senescence are related to macromolecules such carbohydrates, proteins and lipids.

It has been demonstrated that active degradation of starch occurred more intensively in senescing and stressed tissues where an enhanced induction of α -amylase was observed. In stress situations cells require more sugars to fulfil the energy and carbon needed for the defensive response to stresses. Since the cut flowers suffer from an energy deficiency, and are susceptible to different stresses, the demand for hexoses in petals might be satisfied partially by the hydrolysis of starch. Moreover, the activity of α -amylase plays an important role in the mechanism of petal opening and regulates the appearance of senescence syndrome (Yakimova *et al.* 1997).

Proteins play a crucial role in cells to preserve life. Protein synthesis is, therefore, crucial for optimum cell functioning, whether such proteins perform structural, maintenance or regulatory functions. The rate of protein synthesis is directly proportional to the quantity of mRNA present in the cell. Senescence is marked by changes in the activities of certain enzymes.

Carnation senescence is associated with increased polyribosome activity, and major changes in patterns of protein synthesis (Reid and Wu 1992). The way in which changes in mRNA quantities occur is closely related to protein synthesis and implies that the protein synthetic capacity of the cell remains functional during senescence as the requirements for specific proteins increase or decrease. An increase in amino acids levels was noted at the onset of senescence in carnation petals. However, overall cellular mRNA synthesis decreased even though senescence was accompanied by an increase in polysomes (poly (A) +RNA) and polypeptides (Woodson *et al.* 1992).

The increased activity in catabolic enzymes such as ribonuclease is associated with decline in macromolecules

including DNA, RNA, proteins and membrane lipids. Clearly, the onset of senescence is associated with elaboration of catabolic enzymes which presumably play an important role in remobilizing the cell content of the petals and eventual death of cells (Reid 1989).

Regulation of the senescence-associated activity of proteases may be achieved with different molecular strategies. Firstly, the interaction between proteases and their inhibitor proteins have been linked to modulation of cell-death processes in tissues. In certain cut flowers (*Sandersonia* and *Iris*), chemical inhibition of protease action delays the onset of senescence, and the accumulation of cysteine protease mRNAs in senescing carnation flowers is associated with a corresponding decline in protease inhibitor mRNA, indicating inhibitor proteins may play a role in regulating senescence-associated protease activity in flowers (Sugawara *et al.* 2002). Secondly, proteases have been shown to be localised to the plant vacuole, and both post-translational modification and subcellular localisation provide the cell with a means to regulate protease activity. Another avenue for extending the display life of cut flowers through modification of proteolysis is the down-regulation of senescence-associated cysteine proteases (Eason 2006).

On the other hand, it is clear that sugars delayed ethylene production in carnation flowers (Miura *et al.* 2000; Hassan 2005; Pun *et al.* 2005). However, there is a lack of understanding of the mechanism of sugar in this process. Regarding some reports may suggest sugar delay expression of a protease gene and also delay in the cysteine protease activity (Sugawara *et al.* 2002; Pun and Ichimura 2003), and also ethylene responsiveness (Eason 2006).

It has been suggested that sugars have a role not only as an energy source but also in regulating gene expression (Eason 2006). The accumulation of transcripts was induced by treatment with ethylene and delayed after treatment with sucrose (Eason 2006; Hoeberichts *et al.* 2007); therefore, complex interactions occur between sugar and ethylene signalling mechanisms that are tissue-dependent (Iordachescu and Verlinden 2005). This indicates that sugar may be an early regulator of senescence (Zhuo *et al.* 2005).

Inhibition of water loss is likely to be caused by stomatal closure by sucrose. This view is supported by Zhuo *et al.* (2005) who reported that sucrose treatment partially closed stomata.

In addition, a sharp increase in microviscosity of carnation microsomal membrane was observed during aging. The increase in microviscosity corresponded to an increase in the ratio of the sterol to phospholipid. No change in the content of free sterol occurred during senescence, but phospholipid content was reduced. This was attributed both to reduced synthesis and increased hydrolysis by phospholipase A (Halevy 1981).

Physiological changes

Senescence of the whole plant or individual organs is characterized by a decline in the rates of anabolic processes and an increase in the rates of certain catabolic processes (van Altvorst and Bovy 1995).

Senescence in climacteric flowers such as carnation is characterized by a climacteric rise in respiration rate, ethylene synthesis and a gradual increase in ethylene sensitivity during the late stages. Respiration of cut carnations is preceded by a rise in ethylene evolution. The inhibition of both the biosynthesis and action of ethylene eliminates the rise in respiration without preventing eventual senescence (Weerts 2002).

In addition, pollination of these flowers leads to an acceleration of senescence involved in a marked stimulation of ethylene synthesis and a sudden increase in sensitivity of the corolla to ethylene. The possibility that the sensitivity factor is short-chain saturated fatty acids (C_7 - C_{10}) has been postulated. Applications of these acids to the stigmas of carnation flower results in a sudden increase in ethylene sensitivity and a marked acceleration of senescence (White-

head 1994; Buanong 2005).

Other consistent feature of senescence is the loss of differential permeability of cell membranes (Thompson 1988). Deterioration of cellular membranes causes increased membrane permeability, loss of ionic gradients and decreased function of key membrane proteins (e.g., ion pumps) (Borochoy and Woodson 1989). One of the most obvious symptoms of the final stages of senescence in petals is the loss of water even when the flowers are held in water. This may indicate a loss of membrane integrity, causing increased permeability and leakage (Halevy 1981).

Changes in the properties of membranes, such as increases in microviscosity, alterations in saturation/destruction ratios of fatty acids and peroxidation of lipids, are known to occur during petal senescence, with a causal link to reactive oxygen species (ROS), that are often elevated as a result of stress and have been implicated in the progression of petal senescence (Borochoy and Woodson 1989).

Membrane deterioration is commonly associated with progressive decreases in membrane phospholipid content through phospholipase activity. Lipase and lipoxygenase enzymes participate in biochemical transformation reactions of lipids. Raised lipase (lipolytic acyl hydrolase) activity has been linked to the onset of membrane leakiness in carnation (Hong *et al.* 2000).

The onset of senescence-associated wilting of floral organs has been temporally linked with modifications of the cell wall in carnation (de Vetten and Huber 1990). Anatomical changes to flower petal cells suggest that cell walls swell or break down as the internal mesophyll cells become separated from each other and collapse during petal expansion and subsequent senescence. The loss of cell order that occurs during petal senescence is often accompanied by an increase in activity of cell wall hydrolases, depolymerisation of hemicelluloses and loss of neutral sugars, particularly galactose and arabinose (Eason 2006).

GENETIC MODIFICATION OF FLOWER SENESCENCE IN CARNATIONS

The past decade has seen increasingly rapid isolation and identification of senescence-associated genes from cut flowers (Eason 2006).

Use of gene transfer technology to delay flower senescence has highlighted the need for tightly regulated transgene expression to avoid affecting other non-target developmental processes, particularly in the modification of plant hormone levels (e.g., poor rooting and lower disease resistance in ethylene-insensitive plants) (Clark *et al.* 2004). Thus, the need for tissue-specific promoters is paramount for exploiting this avenue of crop development in commercially important cultivars. Alternatively, modifying the expression of metabolic genes may produce satisfactory postharvest improvements without the need to alter hormone biosynthesis or perception, which may have pleiotropic effects (Pun and Ichimura 2003; Eason 2006).

The onset of carnation petal senescence is accompanied by a significant increase in the production of ethylene. This ethylene production was shown to be associated with a concomitant increase in the expression of ACC synthase and ACC oxidase mRNAs (Woodson *et al.* 1992) and enzyme activities, suggesting it was regulated at the levels of both transcription and translation (van Altvorst and Bovy 1995).

De Benedetti *et al.* (2003) reported that molecular markers as a breeding tool have a high efficacy to improve cut flowers longevity in carnation. They indicated also that flower vase life is probably a complex qualitative trait in carnation, involving more than a single gene or mechanism and controlled by gene showing predominantly additive effects. Genes that control ethylene production, ethylene sensitivity and genes that are affected by the presence of ethylene have been identified in cut flowers (Kosugi *et al.* 2000; Iordachescu and Verlinden 2005). Genetic modification of carnation in order to down-regulate ethylene production or

responsiveness to ethylene has resulted in flowers with prolonged vase life (reviewed by Satoh *et al.* 2006). A number of researchers have generated genetically modified (GM) carnation lines that have altered ACC oxidase or ACC synthase expression (Office of Gene Technology Regulation 2006).

In carnation, three unique ACC synthase genes have been identified and characterized (Jones and Woodson 1999a). *DC-ACS1* was shown to be expressed primarily in senescing petals and styles during the final wave of increased ethylene that follows pollination (Woodson *et al.* 1992; Jones and Woodson 1999a). The expression of *DC-ACS1* was shown to be under the regulation of ethylene, as it was blocked by inhibitors of ethylene action such as NBD (Jones and Woodson 1999a). In striking contrast, *DC-ACS2* and *DC-ACS3* were shown to be expressed in pollinated styles at 1 and 6 h after pollination, respectively (Jones and Woodson 1999a). The expression of *DC-ACS3* is independent of ethylene and appears to be related to primary signals associated with the interaction of pollen with the pistil.

The final step in the ethylene biosynthetic pathway is catalyzed by ACC oxidase. In contrast to ACC synthase, this enzyme is often constitutive in plant tissues. In carnation, it was shown that petals did not exhibit significant activity of ACC oxidase until the onset of petal senescence (Woodson *et al.* 1992). This increased ACC oxidase activity was associated with the expression of ACC oxidase mRNA encoded by the *DC-ACO1* gene. The expression of ACC oxidase mRNA in carnation petals is under strict regulation by ethylene. It has been reported that the expression of the *DC-ACO1* gene probably affects that of the *DC-ACS1* gene (Satoh *et al.* 2005c). Inhibitors of ethylene action prevent the expression of the *DC-ACO1* gene in petals and ethylene stimulates expression prior to the onset of senescence (Woodson and Jones 2003).

In senescing carnation flowers, ethylene is produced from the gynoecium, and acts as a diffusible signal received by petals to induce the expression of ACC synthase (*DC-ACS1*) and ACC oxidase (*DC-ACO1*) genes in the petals. This results in autocatalytic ethylene production in the petals (Satoh *et al.* 2005c). Treatment of carnation flowers with chemical inhibitors of ethylene synthesis or action prevents the increase in ethylene production, the expression of senescence-related genes, and the premature onset of senescence (Wang and Woodson 1991; Woodson *et al.* 1992).

Three ethylene receptor genes, *DC-ERS1*, *DC-ERS2* and *DC-ETR1*, have been identified in carnation. Recently, it has been reported that *DC-ERS2* and *DC-ETR1* are ethylene receptor genes responsible for ethylene perception in carnation flower tissues during senescence (Shibuya *et al.* 2002), and has been noted, when *Arabidopsis etr1-1* was over expressed in carnations using a flower-specific promoter, vase life was extended three fold, from 8 days to 24 days (Bovy *et al.* 1999). These flowers did not show the petal in-rolling phenotype typical of ethylene-dependent carnation flower senescence. Instead, petals remained firm and finally started to rot and decolorize. The level of *DC-ERS2* mRNA decreased in the petals and increased slightly in the ovaries, whereas the level of *DC-ETR1* mRNA showed no or little change in any of the tissues from anthesis to senescence (Shibuya *et al.* 2002).

ACTUAL SPECIFIC TREATMENTS

A major cause of quality deterioration in cut flowers is the blockage of xylem. This blockage might be due to air or bacterial growth that accumulates in vase solution or in the vessels (van Ieperen *et al.* 2002). The blockage of vessels led to water stress and it is well known that the limiting factor of vase life is water stress, which is expressed in the form of early wilting of leaves and flowers (Put *et al.* 2001; Zhou *et al.* 2005). This problem can be overcome by regular recutting of stem ends (removing about 2 cm), by acidifying the vase water (pH 3-4) or including a biocide (bactericide) in vase solutions.

Antibiotic agents

The addition of antibiotic agents in the storing solution has been recommended (Hassan 2005). Chemicals like 8-HQS, 8-HQC, silver nitrate (AgNO_3), STS, thiabendazole (TBZ), quarternary ammonium salts (QAS), aluminum sulphate $\text{Al}_2(\text{SO}_4)_3$ are very important germicide in preservatives used in floral industry. These agents act as biocide (bactericide) and also are able to increase water uptake (Ichimura *et al.* 1999; Hassan 2005). The application of 8-HQS significantly increased the vase life as well as the gain of the fresh weight of cut carnations in comparison with untreated control (Knee 2000; Ebrahimzadeh *et al.* 2003; Hassan 2005).

Sugars

Other important factors that can affect cut flower longevity include water quality and availability of respirable substrates. The design of suitable preservatives to extend flower longevity should keep these factors in mind.

Sugar solutions are well known for their ability to improve postharvest quality and extend the vase life of cut flowers, although the hypothesis of a sole sugar starvation or sugar accumulation signal in inducing petal senescence has not been validated (Eason 2006). The integration of sugar containing pulsing solutions into postharvest regimes is effective for maintaining quality and delaying the onset of senescence of many cut flowers.

Beneficial effect of sugars on flower senescence was attributed to the supply of substrates for respiration, structural materials and osmoticum. Exogenous application of sucrose supplies the flower with much needed substrates for respiration and not only prolongs the vase life but also enables cut flowers harvested at the bud stage to open, which otherwise would not occur naturally (Pun and Ichimura 2003).

The roles of sugars have been classified into the following 4 categories:

1) Supply of substrate for respiration

Several types of sugars like sucrose, glucose, mannitol, etc., are beneficial to the prolongation of the vase life of cut flowers. Among the different types of sugars, sucrose has been found to be the most commonly used sugar in prolonging the vase life of cut flowers. Sugars contribute energy through respiration to maintain the flower metabolism.

Sucrose also promoted bud opening of several cut flowers such as *Dianthus*, *Liatris*, *Gypsophila*, *Limonium*, *Gladiolus*, and *Rosa hybrida*. Sugars during bud opening supply energy through respiration and the carbon skeleton required for the floral structure (Brochov and Mayak 1984; Pun and Ichimura 2003; Hassan 2005).

2) Maintenance of adequate water balance

The prolongation of the vase life of cut flowers by the application of sugar has also been attributed to an increase in the uptake of water by the flowers. It is, therefore, suggested that sugars may be effective in the maintenance of an adequate water balance in cut flowers by the reduction in the loss of water and not due to the increase in uptake (Ichimura *et al.* 1999; Miura *et al.* 2000; Pun and Ichimura 2003).

3) Decrease in sensitivity to ethylene

The decrease in the sensitivity to ethylene in carnation flowers treated with sucrose was recorded as early as in the mid-1970's (Mayak *et al.* 1977) although the mode of action of ethylene sensitivity reduction by sugars is unclear. It has, however, been suggested that the reduction in sensitivity to ethylene may be due to the accumulation of carbohydrates and not to the increase in osmolarity alone or suppression of the expression of the genes responsible for the sensitivity to ethylene (Pun and Ichimura 2003). Sucrose reduces the sensitivity to ethylene when ethylene concentrations are still under $0.5 \mu\text{L L}^{-1}$ (Pun *et al.* 2001a). In another study, sucrose treatments delayed flower senescence in car-

Table 1 Common anti-senescence preservatives for cut carnation flowers with their mechanism of action.

Ethylene inhibitors	Agent	Mechanism of action	Reference
Inhibitors of ethylene synthesis	AVG, AOA	Inhibits the biosynthesis of ethylene; (inhibits ACC synthase activity)	Yang 1985; van Doorn and Woltering 1991; Yakimova <i>et al.</i> 1997
	AIB	Competitively inhibits EFE (ACC oxidase)	Serrano <i>et al.</i> 1990; Wawrzyńczak and Goszczyńska 2004
	ATA	Inhibits ACC synthase biosynthesis	Altman and Solomos 1995; Serrano and Martínez-Madrid 1999; Wawrzyńczak and Goszczyńska 2004
	8-HQS	Antimicrobial additive in preservatives; inhibits ethylene synthesis	van Doorn and Pierik 1990; Ichimura <i>et al.</i> 1999; Kneec 2000; Hassan 2005
	8-HQC		
	Chelators		
	Boric acid	Lowers ACC synthase activity; inhibits synthase of ACC oxidase	Serrano <i>et al.</i> 2001
NO	Delays senescence (inhibits the conversion of ACC to ethylene); Reduces transpiration	Leshem <i>et al.</i> 1998; Ku <i>et al.</i> 2000; Bowyer <i>et al.</i> 2003	
Inhibitors of both ethylene synthesis and action	DPSS (1,1-dimethyl-4-(phenylsulfonyl)semicarbazide)	Inhibits ethylene production: *directly (effect on ACC oxidase) *indirectly through the effect on the ABA-related process	Midoh <i>et al.</i> 1996 Onoue <i>et al.</i> 2000
	High CO ₂ level	Inhibits activity of ACC synthase Inhibits activity of ACC oxidase Competitor of ethylene action	Sisler and Yang 1984
	Ethanol and methanol	Inhibits sensitivity to ethylene Inhibits ethylene action Inhibits ethylene biosynthesis (ACC synthase and ACC oxidase)	Pun <i>et al.</i> 1999 Wu <i>et al.</i> 1992 Heins and Blakely 1980; Wu <i>et al.</i> 1992; van Altvorst and Bovy 1995; Podd and van Staden 1999; Pun <i>et al.</i> 2001a
Inhibitors of ethylene action	AgNO ₃	Inhibits ethylene synthesis Inhibits ethylene action Antimicrobial agent	Veen 1979 Beyer 1976 Nowak and Rudnicki 1990
	NBD	Prevents binding of ethylene Decreases ACC synthase and ACC oxidase	Sisler and Yang 1984; Sisler <i>et al.</i> 1986; Peiser 1989 Peiser 1989; Wang and Woodson 1989; Sisler and Serek 1999
	STS	Inhibits ethylene action	Farhoomand <i>et al.</i> 1980; Reid <i>et al.</i> 1980; Menguc and Usta 1994; Altman and Solomos 1995; Hassan 2005
	1-MCP and analogues	Prevents binding of ethylene	Sisler <i>et al.</i> 1990; Serek <i>et al.</i> 1995; Sisler <i>et al.</i> 1996; Sisler and Serek 1997; Serek and Reid 2000; Reid <i>et al.</i> 2001; Hassan 2005; Hashemabadi and Mostofi 2007
	BA	Decreases ethylene sensitivity	Whitehead 1994; Wawrzyńczak and Goszczyńska 2004
	Cytokinins	Suppresses ethylene binding	
	Sucrose	Reduces ethylene sensitivity; delays ethylene production Controls water balance; supplies substrate	van Doorn 2004; Pun and Ichimura 2003; Pun <i>et al.</i> 2005; Eason 2006 Mayak and Dilley 1976; Brochov and Mayak 1984; Ichimura <i>et al.</i> 1999; Pun <i>et al.</i> 2001a, 2001b

nation and decreased ethylene responsiveness of the petals. The reduced ethylene production of the petals treated with sucrose, when exposed to ethylene, correlated with decreased *in vitro* ACC oxidase and ACC synthase activities. Significantly lower levels of the ethylene precursor ACC were observed in sucrose-treated flowers (Verlinden and Garcia 2004).

4) Delay in climacteric ethylene biosynthesis

It is clear that sugars delayed ethylene production in carnation flowers (Pun and Ichimura 2003; reviewed in van Doorn 2004; Hassan 2005). However, there is a lack of understanding of the mechanism of sugar action. A concentration of 5% sucrose was found to be the best for extending the vase life of cut carnation flowers (Hemmati and Ebrahimzadeh, unpublished data), and was associated with a delayed ethylene peak.

As mentioned before, ethylene is one of the most important limiting factors in the longevity of climacteric cut flowers such as carnation, freesia and rose. There are different ways to avoid or decrease undesirable effects of ethylene: low temperature, control of O₂ and CO₂, avoidance of cut flowers exposure to exogenous ethylene, use of preservatives.

Anti-ethylene compounds

In recent years a number of approach using growth regulators to overcome the effects of ethylene have become avail-

able (**Table 1**). The most important commercially-available agent is the anionic STS complex.

STS

Silver ion is a very effective inhibitor of ethylene action, and its thiosulfate complex is very stable and moves easily in tissues, allowing us to use it in commercial flower preservatives. STS is the widely used ethylene binding inhibitors and appear to have farther benefits than as a biocide, which makes it an even popular substance.

STS is in widespread commercial use to inhibit effects of ethylene and prolong vase life in many ornamentals including carnation. However, as silver is a heavy metal, it cannot be used on food and feed, and many countries prohibit its use (Buanong 2006).

It has been reported that STS had a positive effect on the vase life increasing two times more than control and also improved petiole size and bud opening processes in cut carnation flowers (Menguc and Usta 1994; Altman and Solomos 1995). STS is a highly mobile in the xylem of carnation flowers and may become a practical treatment for carnation flowers (Reid *et al.* 1980).

There are some contradictory results in different studies about application method and optimum concentration of STS. In earlier researches cut carnation flowers continuously treated with 0.2 mM STS exhibited no morphological or respiratory responses to any concentration of exogenous ethylene, whereas both a respiratory increase and an irrever-

sible petal wilting were observed in flowers pulsed with 0.5 mM STS. It suggests the interactions between silver ions and ethylene are competitive (Altman and Solomos 1995), while later information from research on carnation supports that pulsing cut carnation flowers with STS and sucrose inhibited the ethylene synthesis, improving postharvest quality (Burzo and Dobrescu 1995; Hassan 2005).

In other study, Serek *et al.* (1995) reported that carnation flowers pulsed with 1 mM STS for 2 h had significantly longer longevity in comparison to the untreated control. This variation could be due to differences in cultivar sensitivity to ethylene.

1-MCP

Some synthetic cyclopropenes have been shown to bind the ethylene receptors and prevent the biological actions of ethylene for extended periods. MCP (methylecyclopropene) and its analogues (1-MCP, 1-OCM, 1-DCP and 3-MCP) exert their effect by blocking the binding site of ethylene in the receptor (Sisler and Serek 1997; Buanong 2006; Lurie 2007). All of these compounds have been found to be effective antagonists of the ethylene response, but 1-MCP is more stable than others (Sisler and Serek 1997, 1999). 1-MCP is a gas in its natural state but marketed under the trade name Ethyl Bloc[®] in powder form, which is added to water to release the gas (Hassan 2005). Ethyl Bloc[®], the only ethylene action inhibitor approved by the EPC (Environment Protection Agency), is a patented break through: 100% environmentally friendly, non-toxic and safe to use (Reid *et al.* 2001). The concentration of 1-MCP needed to protect carnation flowers against ethylene action is much lower than other chemicals. The binding of 1-MCP of carnation seems to be irreversible or at least remains bound for a very long time (Hassan 2005).

1-MCP is a non-toxic inhibitor of ethylene action, which acts as a competitive and irreversible inhibitor of ethylene to its receptor (Sisler *et al.* 1996). The treatment of cut carnations with 1-MCP inhibited subsequent ethylene action to the same extent as an optimal treatment with STS, the only commercial treatment presently available (Serek *et al.* 1994b, 1995; Sisler and Serek 1997, 2001; Hassan 2005).

In carnations loss of response to ethylene is greatly retarded by storage at lower temperatures. For example, the time taken for the carnation flowers to recover 50% of their response to ethylene, which is about 4 days at room temperature, is more than a month at 0°C (Reid *et al.* 2001). In 'White Sim' carnations treated with 1-MCP (50 ppb, 6 h) which followed by exposure to ethylene (1 ppm, 24 h), the in-rolling response was completely inhibited in flowers that were treated at room temperature (20°C) or above. For cut flowers such as carnation, penstemon (*Penstemon* sp.), snapdragon (*Antirrhinum majus*) and stock (*Matthiola incana*), 1-MCP treatments result in display life that is extended more than 200% (Mayers *et al.* 1997). It has been reported that 1-MCP, not only significantly delayed the wilting of the cut flowers of 'Temp' carnation but also had desirable effects on the flower opening index at $P \geq 0.01$ and in dry matter at $P \geq 0.05$ (Hashemabadi and Mostofi 2007).

Nitric oxide

Interest in nitric oxide (NO) to extend the postharvest life of horticultural commodities is of recent origin. Postharvest application of NO has been shown to be effective in extending the postharvest life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment at low concentration (Leshem and Wills 1998; Pandey *et al.* 2000).

It has been reported that postharvest senescence was inhibited when carnations were continuously exposed to the NO-releasing compounds *N-tert-butyl- α -phenylnitron* and 3-morpholinoyl-nonimine (Leshem *et al.* 1998), and a reduction of transpiration about 20% after a 24 h exposure to NO gas (Ku *et al.* 2000). Regarding other study fumiga-

tion with NO has been found to extend postharvest life of carnation flowers by about 30% and the NO donor compound, 2,2'-(hydroxynitrosohydrazino)-bisethanamine (DETA/NO) dissolved in water did so about 50% (Bowyer *et al.* 2003). The effectiveness of NO and particularly donor compound (DETA/NO) on both ethylene-sensitive and insensitive flowers suggests it may have significant commercial application in the future. The mode of action of NO in delaying the onset of flower senescence has yet to be studied at the molecular level.

Aminoxyacetic acid

Experiments showed that the inclusion of AOA in the vase water, together with sugar, had a positive effect on the time to flower senescence (Fujino *et al.* 1980; Rattanawisalanon *et al.* 2003). Indeed, AOA is acid and therefore may inhibit microbial growth in the vase water. Treatments with AOA or AOA+sucrose effectively retarded the longevity of cut spray carnation flowers 'Regina' and 'Naslada'. The same substances affected positively bud growth and allowed the flowers to reach fully open stage (Yakimova *et al.* 1997).

AOA has been found to effectively delay petal wilting of carnations through an inhibition of ethylene biosynthesis on ACC synthase level. There is ample evidence indicating that changes in the activity of several enzymes functioning during plant development play an important role during organ senescence (Salunkhe *et al.* 1990).

Aminotriazole

It has been reported that aminotriazole (ATA) inhibits ethylene biosynthesis by inhibiting ACC synthase biosynthesis and the autostimulatory effect on ethylene, with the latter being temporally mediated. Continuous postharvest treatment of carnations with ATA results in significant extension of vase life (Altman and Solomos 1995; Serrano *et al.* 1999) but because it has been classified as a putative carcinogen, its commercialization as a cut flower preservative is difficult. The continuous application of 10 mM α -aminoisobutyric acid to cut carnations delayed the loss of fresh weight and the peak of ethylene production for 4 days (Serrano *et al.* 1990).

CONCLUSION AND PROSPECTS

Nowadays, the technological development of the flower industry could allow obtaining flowers for the consumer with high quality, including the longer vase life as an important quality index.

Ethylene biosynthesis is an important process during petal senescence and is, therefore, associated with the postharvest quality of ornamentals, especially the vase life of cut flowers. Therefore, our demand is a justified knowledge about dynamic of ethylene synthesis, its receptors and the effects on the carbohydrates, protein and lipids anabolism involved in senescence.

Moreover, the role of sucrose as well as the new agents in delaying senescence in harvested cut carnation should be elucidated further in order to more clarification. And finally, future investigations are required to: exact evaluation of molecular and biochemical roles of organic matters either in biosynthesis and action of ethylene or sensitivity to ethylene and its regulation mechanism which is not yet completely clarified.

Looking into derivatives or analogues of present anti-ethylene compounds or looking over new agents might lead extending the vase life of carnation flowers. These products must be easy to use, not expensive and friendly with the environment.

The study of improvement of new varieties is also an interesting aspect, with an objective of producing flowers with a longer vase life, to preserve their freshness during transport, or to design new vase water additives that extend flower longevity and finally study of petal PCD in molecu-

lar level. Meanwhile genetically improvement of flowers by introducing useful genes into the plants via genetic modification, could present helpful knowledge. The last methods require no chemical treatment to attain longer vase life in carnation flowers. Finally all of these integrated strategies could be regarded as a part of the future of postharvest science.

ACKNOWLEDGEMENTS

We thank Dr. Felix Romojaro (University of Murcia, Spain) for his invaluable advice.

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