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Improving ornamental's vase life through molecular approaches: A review

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Abstract
Many cut flowers deteriorate rapidly after harvest, and short vase-life constitutes a major obstacle in the marketing of flowers. The post-harvest life of many flowers is determined by the onset of petal senescence. Petal senescence is an active process involving many biochemical and physiological changes. Ethylene is a primary plant hormone involved in the senescence of many flowers. Inhibition of the synthesis or action of ethylene delays the onset of senescence symptoms and increases the vase life of the flowers. Generation of transgenic plants with suppressed production or action of ethylene is an excellent way to lengthen the longevity of ornamental plants. This review focused on genetic mechanism of ethylene biosynthesis pathway manipulation and their application on some important ornamental flowers.

Keywords: flower, vase life, senescence, ethylene, biosynthesis pathway

Introduction
Floriculture has emerged as a viable diversification option in the agri-business. It is a rapidly expanding industry recording a growth rate of more than 15 per cent per annum in the last two decades. Flowers are highly perishable unlike other horticultural or agricultural crops. Owing to poor keeping quality, the post-harvest losses in floriculture are significantly higher than any other sector. Although there has been significant increase in the area, production and productivity of flower crops in the last two decades, there is an urgent need to minimize the huge post-harvest losses in terms of the value of the produce which are estimated to be 30-40 per cent of farm value. The post-harvest losses become important especially when dealing with the export of fresh flowers to distant and foreign market.

Retention of postharvest quality is essential in floricultural crops. Postharvest senescence and organ loss are major limitations to cut flower quality and their marketing of many species of cut flowers and considerable effort has been devoted in developing postharvest treatments to improve cut flower quality (Bowyer *et al*., 2003) [5]. As cut flowers must have the capacity to survive several weeks in the distribution chain before they reach consumers' hands, resistance of flowers to senescence promoting factors such as ethylene and bacterial infection is very important (Chandler, 2012) [8]. Loss of vase life of cut flowers during all stages of postharvest handing ranged from 20 to 40% with the mean loss of vase life per day of 6–7% (Hoogerwerf *et al.*, 1994) [21]. Reduction of vase life of cut flowers from bacterial contamination of water varied from 0.2 to 12% (Hoogerwerf *et al.*, 1994) [21].

The post-harvest life of many flowers is determined by the onset of petal senescence. The petals exhibit a characteristic 'in-rolling' behavior during senescence, and also in response to exogenously supplied ethylene. This 'in-rolling' can be delayed, or even prevented, by treatments (such as the application of silver ions or cytokinins) that block the biosynthesis or action of ethylene. The quality and post-harvest life of many plants and flowers are often reduced by the presence of ethylene in the environment and as plant hormone in plant. Generally, cut flowers are treated with different kinds of chemicals for increasing their shelf life (Teixeira da Silva *et al.*, 2013) [35]. We can minimize losses by applying post harvest handling techniques but these are not a permanent tactics for overcoming this problem. It might be possible by applying genetic-engineering approaches to reducing the ethylene-regulated senescence of flowers. The isolation of the genes of the ethylene signal-transduction pathway opens up many new possibilities for engineering longer-life flowers. Enhanced vase life could be obtained by the introduction of resistance to ethylene or by the inhibition of expression of endogenous ethylene biosynthesis genes. Transgenic ornamental plants have potential to enhance leaf and flower longevity. To achieve the target of enhanced vase life, different biotechnological techniques have been used (Matas *et al.*, 2009).

Ornamental horticulture, and particularly floriculture, is well suited to the application of genetic engineering technology. Genetic engineering allows the introduction of genes from outside the gene pool, and is precise, because a gene or genes targeted for a specific trait can be introduced. Biotechnology also shortens the time frame for new variety development. Where phenotypic novelty is very important, as it is in ornamental horticulture, this is critical (Chandler and Lu, 2007) [6]. Underwood and Clarke (2011) [37] have recently reviewed the potential for GM to improve leaf and flower longevity in transgenic ornamental crops.

Concept and mechanism of genetic manipulation for extending flower vase life

The quality and post-harvest life of many plants and flowers are often reduced by the presence of ethylene in the environment. Ethylene has a variety of deleterious physiological effects, such as bud, flower or leaf abscission and senescence (Zacarias & Reid, 1990) [40]. Climacteric flowers have short life due to the sensibility to ethylene (Thompson & Wang, 2002) [36]. Ethylene biosynthesis in higher plants is developmentally and environmentally regulated. The plant hormone ethylene is a small alkene involved in many important processes throughout the life cycle of plants, from germination to senescence. It is unique among hormones in that it is volatile and gaseous at ambient temperature, which may be the reason for the role of this hormone in the control of flower maturation and senescence. However, Vase life of cultivars is greatly influenced by genetic variation. Large differences in vase life of cut rose have been observed among cultivars which were grown and tested under identical condition (Marissen, 2001; Fanourakis *et al.*, 2012) [23, 17].

However, ethylene biosynthesis, perception and signal transduction have become the targets of extensive genetic manipulation in order to extend the shelf life and appearance of ornamental flowers. Flower wilting is caused by the death of cells as a result of increased membrane permeability, activation of reactive oxygen species and the decreased expression of protective enzymes (Rubinstein, 2000) [28]. These changes are triggered in many cases by ethylene and the up regulation of ethylene biosynthesis genes can be seen prior to the senescence of many flower species.

The plant hormone ethylene is involved in senescence in many flowers and vase life can be extended by either blocking ethylene biosynthesis (Savin *et al.*, 1995) [31] or ethylene reception (Bovy *et al*., 1999) [4].

Two main approaches have emerged as a result of numerous efforts to engineer transgenic plants with longer-lived flowers. The first is based on the antisense ACC synthase and oxidase gene strategies, which are involved in the ACC cyclization and subsequent conversion to ethylene (Crozier *et al*., 2000) [14]. It has been possible the modification in the levels of this hormone through the introduction of genes involved in the synthesis of ACC-synthase or ACC-oxidase in anti-sense orientation and the second employs heterologous expression of mutated ethylene receptor genes. Other possibility is the introduction of genes associated to ACC degrading enzymes thus interrupting the ethylene biosynthetic pathway (Thompson & Wang, 2002) [36]. Also, the effects of ethylene may be altered reducing the ability of the plant to perceive it (Stearns & Glick, 2003) [34].

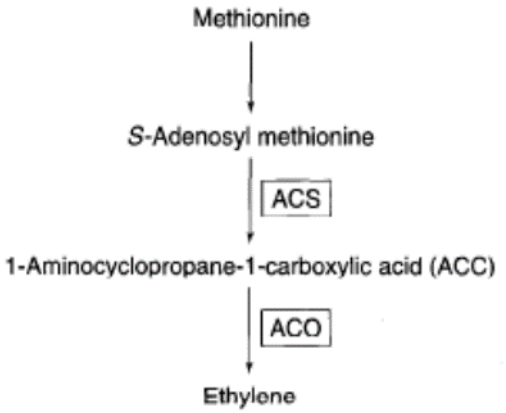

Fig (a): Ethylene Biosynthesis Pathway

The initial substrate on the biosynthetic pathway of ethylene is methionine, from which the compound SAM (*S*-adenosylmethionine) is produced as a result of the action of the enzyme SAM synthase. The conversion of Sadenosyl methionine (SAM) to l-aminocyclopropane- 1-carboxylic acid (ACC) and the conversion of ACC to ethylene are catalysed by ACC synthase (ACS) and ACC oxidase (ACO), respectively (Fig. a).

ACC synthase is a PLP (Pyridoxal Phosphate) Enzyme, which mediates a unique alpha, gamma elimination of the substrate SAM to form ACC. The cloning of ACC synthase and ACC oxidase gene has permitted scientists to use antisense RNA techniques to genetically engineer perishable crops, with suppressed ethylene production rate and thereby to extend the self-life and to reduce spoilage.

Gene for improvement of vase life in flowers

Traits	Candidate Gene and Pathway
ACC synthase	Inhibition with reduced ehtylene production
ACC oxidase	Inhibition with reduced ehtylene production
ACC deaminase	Over expression with reduced ethylene production
Etr 1	Expression of a defective gene with reduced ethylene production
ERS	Expression of a mutated gene with reduced ethylene production

***Source*:** Aswath, C. & V.C. Hanur (2009) [2]. Indian Horticulture, 54(1):30-33

Carnation

Carnation has been used for many years as a model system for studying the physiology of flower senescence. Petal senescence in carnation is an active process involving many biochemical and physiological changes. Carnations with longer vase life have been developed, but were not commercialized. Florigene Pty. Ltd. has developed transgenic carnation varieties which produce flowers with an enhanced vase-life, as a result of alteration of either ethylene biosynthesis or ethylene perception. The flowers from the transgenic plants do not require chemical treatment for maximum vase-life (Chandler, 2007) [6].

Ethylene production during senescence and ethylene sensitivity at a young flower age trigger flower senescence and have been considered in improving vase life in transgenic carnation. Transgenic carnations with delayed petal senescence have been created by silencing the ACO gene to

down regulate ethylene production (Savin *et al*., 1995) [31]. Using antisense and sense suppression technology, several cultivars were transformed with ACC oxidase. The vase life of these transgenes was almost twice that of the original variety, increasing from 5 to 9 days (Savin *et al*., 1995; Chandler, 2007) [31, 6]. Co-suppression using ACC synthase (acs) has been successfully used to delay carnation flower senescence (Chandler, 2007) [6]. To inhibit ethylene synthesis in carnation, Bovy *et al*. (1999) [4] generated transgenic plants containing A. thaliana etr1–1 gene. Vase life of transgenic carnation flowers was increased 3 fold (senescence was delayed by 6–16 days) compared to the control plant. Vase life was even longer than thatmeasured in control flowers treated with chemicals such as with Aminooxyacetic Acid (AOA) or Silver thiosulfate (STS).

In carnation, autocatalytic ethylene production is caused by the expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes (Jones and Woodson, 1999) [18] and wilting is caused by the expression of the cysteine proteinase (CPase) gene (Panavas *et al*., 1999) [26] during senescence after harvest. In order to improve vase life, sACO (sense ACC oxidase) or aACO genes (antisense ACC oxidase) were transferred by Agrobacterium-mediated transformation into potted carnation (Dianthus caryophyllus L. 'Lillipot'). Results showed that *in vivo* ACC oxidase activity in leaflet segments of cultured transgenic shoots was much lower than that in the non-transformed plant. Transgenic cut flowers had a prolonged vase life compared with control plants (Kinouchi *et al*., 2006) [19, 29]. Despite the fact that transgenic carnation exhibiting an improved vase life has not yet been commercialized, in the longer-term the incorporation of specific transgenic lines, most probably carrying a mutated ethylene receptor gene, may be a useful addition to a conventional breeding program (Chandler, 2007) [6]. Genetically engineered flowers produced by Florigene, an Australian-based biotechnology company. (a) So-called 'long-life' carnations have an ACC oxidase gene inserted in an antisense direction, which suppresses ethylene biosynthesis and leads to greatly extended postharvest petal life (left) compared with non-transgenic control flowers (right). The flowers here were picked 9 d before the photograph was taken. Similar delayed senescence can also be achieved by supressing ASS synthase expression or by enhancing cytokinin biosynthesis through expression of an *ipt* transgene.

Rose

Pompelli *et al*., (2007) [25] Studies were realized aiming to investigate the differential responses of flower opening to ethylene in rose cultivars. In these studies were cloned cDNA fragments of three Rh-ACSs (*Rosa hybrida* – ACC Synthase) and one Rh- ACO (*Rosa hybrida* – ACC Oxidase) genes that were designated as Rh-ACS1, Rh-ACS2, Rh-ACS3 and Rh-ACO1 respectively. Additionally, Northern-blotting analysis revealed that among three genes *acs*, ethylene-induced expression patterns of Rh-ACS3 gene corresponded to ACS activity and ethylene production in both cultivars. The results obtained suggest the existence of cultivars more sensitive to ethylene than others; and the changes of Rh-ACS3 expression caused by ethylene might be related to the acceleration of flower opening in the cultivars more sensitive cultivars and the inhibition in the more tolerants ones (*Ma et al*., 2005) [22].

Petunia

Mutated ethylene receptor genes have frequently been used to convey resistance to ethylene in transgenic floral species. Bleecker *et al*. (1988) [3] showed that etr1-1 is a dominant mutation that confers ethylene insensitivity in other plants. Ethylene-insensitive petunia was created by Wilkinson *et al*. (1997) [39] when they introduced etr1-1 under the control of the constitutive CaMV35S promoter. Transfection of petunia with etr1-1 placed under the control of floral-specific promoters FBP1 (floral binding protein) and AP3 (involved in floral organ development) resulted in a vase life of up to five times that of non-transformed flowers (Cobb *et al*., 2002) [13]. The transgenic petunia plants were insensitive to ethylene, and produced flowers that were larger and had a longer vase life than those from non-transformed plants. They found, however, that disease resistance was compromised. Earlier, Clark *et al*. (1999) [10] observed reduced adventitious root formation in ethylene- insensitive transgenic petunia and concluded that ethylene plays an important role in the response of roots to environmental stimuli. More recently, Clevenger *et al*. (2004) [12] reported that transgenic ethylene-insensitive petunias carrying the etr1-1 transgene had a decrease in pollen viability, root mass, seed weight, and seed germination. While seed germination and weight are maternally regulated, the transgene was found to be completely dominant in its effect on flower senescence.

Shibuya *et al*. (2004) [33] have recently isolated PhEIN2, a petunia homolog of the Arabidopsis EIN2 gene, and constructed transgenic petunia plants with reduced PhEIN2 expression, and significantly delayed flower senescence. Some undesirable traits similar to those seen in transgenic etr1-1 plants were observed including inhibition of adventitious and seedling root hair formation, increased hypocotyl length, and premature death. A more dramatic reduction in ethylene sensitivity was achieved with the expression of both the etr1-1 and PhEIN2 transgenes (Shibuya *et al*., 2004) [33].

Chrysanthemum

Normally, leaves of cut flowers show yellowness, even before the start of senescence. On the other hand, exposure to ethylene can also speeds up yellowing. This yellowing of leaves is a feature of senescence. This destroys flower attractiveness, diminishes quality and shortens vase life. Consequently, transgenic plants with reduced ethylene sensitivity i.e., chrysanthemum is anticipated to possess improved vase life (Satoh *et al*., 2006) [29]. In transgenic D. grandiflorum, reduced leaf senescence has been proved very useful (Satoh *et al*., 2008) [30]. Current studies have shown that yellowing of the leaves of some chrysanthemum cultivars is induced by ethylene (Doi *et al*., 2003) [15]. Narumi *et al*. (2005) [24] transferred mDG-ERS1 (etr1-4) into C. morifolium 'Sei-Marine' in order to reduce ethylene sensitivity in chrysanthemum. Their results revealed the usefulness of the mDGERS1 (etr1-4) gene in conferring reduced ethylene sensitivity in chrysanthemum, and gave further support for the action of the DG-ERS1 gene in the perception of ethylene by chrysanthemum leaves.

Arabidopsis thaliana

In a study to investigate the regulation of ACC synthase gene expression, the promoters of *Arabidopsis thaliana* ACS genes, AtACS4, AtACS5, and AtACS7, were fused to a GUS reporter gene, and the recombinant transgenes were introduced into Arabidopsis to produce three groups of AtACS: GUS transgenic plants (*Wang et al*., 2005) [38]. The data on histochemical and fluorometric studies revealed that promoters of AtACS4, AtACS5, and AtACS7 were all active

in dark-germinated seedlings. AtACS5 had the highest promoter activity in leaves of 2-week-old lightgrown seedlings among the three AtACS genes studied. The promoter activities of all these AtACS genes were also found in the reproductive organs. AtACS5 and AtACS7 were highly expressed in petals, sepals, carpels, stamens, cauline leaves, inflorescence stems, and siliques, while AtACS4 expression was undetectable in the petals of open flowers (*Wang et al*., 2005) [38]. Accordingly, each AtACS gene had a unique expression profile during growth and development. It appears that at any developmental stage or any growth period of Arabidopsis, there is always a member of AtACS multigene family that is actively expressed.

- For instance, transgenic carnations produced 90% less ethylene than non-transformed plants (Savin *et al*., 1995) [31], transgenic begonia flowers had longevity similar to non-transformed flowers treated with silver thiosulfate (Einset and Kopperud, 1995) [16], and antisense ACC oxidase transgenic torenia had a vase life up to 3.5 times that of non-transformed plants (Aida *et al*., 1998) [1].
- Studies with clover showed that the suppression of ethylene activity occurred in transformed plants with the gene in anti-sense orientation. Thus flowers showed delayed petals senescence and a prolonged shelf life as compared to the control flowers (*Kosugi et al*., 2002) [20].
- Transgenic torenia with an antisense ACC oxidase gene also produced more flowers per stem. Kosugi *et al*. (2002) [20] reported that cut flowers of a transgenic carnation with an ACC oxidase cDNA in the sense orientation (sACO transgene) had a longer vase life than flowers of non-transformed carnation plants, and produced negligible amount of ethylene. The sACO transgene is thought to inhibit expression of ACO1, probably by co suppression in the gynoecium, which then suppresses ethylene production in all flowers of sACO-1 plants.
- In the case of pot plants, flower longevity on the plant is also important, as is the health of the leaves. Delayed leaf senescence has been observed in transgenic tobacco (*Nicotiana tabacum*) and petunia plants by introduction of genes that affect cytokinin synthesis (Clark *et al.*, 2003) [11].
- In one study, the delay to senesce was the result of expression of a gene for biosynthesis of cytokinins (Chang *et al.*, 2003) [9], the over production or external application of which are known to delay senescence. A strategy using a mutant ethylene receptor gene was also effective in petunia (Shaw *et al.*, 2002) [32]. Insertion of anti-sense ACC oxidase in transgenic torenia resulted in plants with flowers that had an extended vase-life (Aida *et al.*, 1998) [1] and keeping quality has been improved in begonia (Hvoslef-Eide *et al.*, 1995).
- Success has been witnessed in Oncidium and Odontoglossum by mutating ethylene receptor gene (Raffeiner *et al*., 2009) [27].
- The main disadvantage of this strategy is that plants deprived of ethylene perception do not develop normally. Therefore, the use of organ and tissue-specific promoters that are inducible either developmentally or by a selective external stimulus is indicated.

Conclusion

Genetic engineering overcomes almost all the limitations of traditional breeding approaches. Recent developments in plant molecular biology provide opportunities to use techniques of genetic engineering for improvement of flower crops for modify flower colour, improve vase life, floral morphology, scent and disease resistance. In flowering crops with novelty it is also neccessery a longer vase life after harvesting. So that the flowers reach from the producer to the consumer without any obstacle or any addition cast of handling. Studies show that in genetic manipulation for vase life of flower increase 6-9 day vase life, which is difficult by post-harvest handling technique and convectional breeding methods.

Ornamental horticulture, especially floriculture, is well suited to application of genetic engineering technology. One reason is that the end product is not a food. Not only does these remove possible obstacles to commercialization as a result of some consumer attitudes towards consumption of genetically modified organisms, but reduces the need to undergo food safety studies, thereby reducing the cost of commercialization.

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