Chemical and Molecular Genetic Strategies to Block Ethylene Perception for Increased Flower Life

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Keywords: 1-MCP, 1-methylcyclopropene, 1-CPs, 1-substituted cyclopropenes, ethylene, ethylene receptor, *etr1-1*, flower longevity, postharvest quality, receptor mutant gene

Abstract

Ethylene has been known to cause many undesirable effects in a range of ornamental species. Blocking ethylene responses has been proved as an efficient strategy to enhance the longevity of the flowers. The most effective ways to conduct such interference are using chemical compounds or genetic manipulation.

In the last 15 years a large number of volatile chemical compounds have been evaluated for their effects on ethylene production and perception. This has resulted in the discovery that cyclopropenes effectively block ethylene responses at the receptor level. The most promising among them are 1-methylcyclopropene (1-MCP) and a number of other substituted cyclopropenes. A lot of testing remains to be done to uncover the full potential of these compounds, but they do offer promising new ways to improve the postharvest quality and longevity of ornamentals.

Another very effective way for controlling ethylene synthesis and perception is genetic modification. The most promising strategy seems to be the use of the mutant ethylene receptor gene, *etr1-1*, from *Arabidopsis thaliana,* especially when it is expressed under the control of a flower specific promoter.

INTRODUCTION

It has been documented in many studies that ethylene enhances senescence and shortens the display life of many cut flowers and potted plants (Woltering and van Doorn, 1988; Woltering, 1987). Presently a wide range of methods is known for preventing the deteriorative effect of ethylene on postharvest characteristics of ornamentals i.e. through inhibition of ethylene biosynthesis or by blocking ethylene perception. Ethylene perception can be inhibited by chemical compounds, by genetic modification, or by manipulation of environmental factors (controlled atmosphere storage, CA). CA is a commonly used technique for storage of many types of fruit, but is not very effective for ornamental crops (Reid and Serek, 1999). A large group of ornamentals is very sensitive to ethylene and blocking ethylene biosynthesis is not sufficient strategy, since they can still respond to exogenous ethylene, which will cause undesirable effects.

This paper focuses on the most effective ways for preventing ethylene effects: 1) application of chemicals that prevent the binding of ethylene to its receptor; 2) breeding plants that express a dominant mutant receptor which does not bind ethylene.

INHIBITION OF ETHYLENE PERCEPTION BY CHEMICALS

2,5-Norbornadiene

Already in 1973 Sisler and Pian reported alkenes as effective ethylene receptor blockers. 2,5-Norbornadiene (2,5 bicyclohepta-2,5-diene; NBD) was the most stabile among alkenes and since it easily vaporizes at room temperature, it was easy to treat plant material in gas tight chambers. NBD's effectiveness in preventing ethylene effects was proved in carnation flowers (Sisler et al., 1983, 1986). However, NBD is a very

offensively smelling compound and, additionally, NBD is blocking the receptor competitively, which limits NBD's potential for commercial use.

Silver Thiosulphate

For more than 20 years STS was the only effective compound used commercially to prevent ethylene effects in flowers at the receptor level. In many studies, starting already in 1975 by experiments with $AgNO_3$ (Kofranek and Paul, 1975), silver ion has been proved as an effective ethylene antagonist. Silver ion in form of silver thiosulphate (STS) has been commercially used for decades because of easy uptake and mobility in plant tissues (Veen, 1983). In 1986 Sisler et al. have proved by using radio-labeled ethylene, that STS treatment blocks the ethylene binding sites. The action of STS may be the result of the exchange between silver ions and copper in the receptor protein ETR1, which has been shown to contain a copper ion that co-ordinates the binding of ethylene (Rodrigez et al., 1999).

The beneficial effects of STS have been reported for a large range of cut flowers and potted plants (Veen, 1983; Woltering and van Doorn, 1988). However, STS is an environmental hazard and it has already been prohibited in several countries. The use of heavy-metal salts in horticulture will always be a matter of concern, therefore, searching for alternatives to the use of STS was necessary.

Diazocyclopentadiene

A decade after the discovery of NBD and STS few new compounds have been tested. Diazocyclopentadiene (DACP) has been tested on different flowering ornamentals, like carnations, sweet pea flowers and miniature roses (Sisler et al., 1993; Serek et al., 1994a).

DACP, when exposed to fluorescent light, decomposes to unknown compound(s) and becomes a very effective blocker of ethylene binding sites. Even though the active product of decomposition of DACP has not been identified, this volatile compound has been documented as a very strong ethylene action inhibitor. However, the compound is very unstable and additionally can be explosive, which makes it an unlikely candidate for commercial use.

1-Methylcyclopropene

In 1994, a new effective ethylene receptor blocker, discovered by E.C. Sisler, has been reported (Serek et al., 1994b). The incredibly high activity of 1-methylcyclopropene (1-MCP) has been very well documented in a range of ornamental species. References on application of 1-MCP reporting its beneficial effect against ethylene treatment and/or on shelf life of non-ethylene treated flowers/plants have been summarized by C.B. Watkins and W.B. Miller (www.hort.cornell.edu/department/faculty/watkins /ethylene/index.htm).

As already reported in several publications, 1-MCP is a cyclic olefine, which seems to bind, irreversibly, to ethylene receptors and prevents ethylene from inducing something similar to a conformational change. It is a non-toxic, odorless compound, stabile at room temperature. Many studies have shown its protective effects against ethylene in a large range of cut flowers, potted plants and also other horticultural commodities. More information about synthesis and mode of action of 1-MCP, as well as about its physical and chemical properties has been already reviewed by Sisler and Serek (1997, 2003) and Serek et al. (2006).

Two commercial products, based on 1-MCP, have been developed by private companies FloraLife Inc. and AgroFresh Inc. (Rohm and Haas). EthylBloc® is especially developed for ornamental crops, while SmartFresh™ is for edible products. Both products are powders including 1-MCP complexed with γ-cyclodextrin. The powder, when mixed with water or buffer solution releases 1-MCP gas. Treatment of plant material must take place in enclosed areas as much as possible gas-tight for preventing gas leakage, which will reduce effectiveness of 1-MCP.

EthylBloc® was registered for ornamentals in 1999 for use in the USA, and later

also in Canada. In the Netherlands the product has been recently registered for tulip bulbs. Unfortunately, the progress with registration for ornamental crops is very slow, which is regrettable, especially since the use of STS has been limited in several countries. Especially in Europe, where production and consumption of ornamentals is very large, the registration of 1-MCP for use on ornamentals is urgently needed.

The information about product development, practical applications and registration was summarised in several reviews (Watkins and Miller, 2003; Serek et al., 2006) and also additional information can be found on www.rohmhaas.com/ethylbloc.

Other Cyclopropenes

In order to find the most effective ethylene antagonists a number of 1-MCP related compounds have been tested, among several horticultural crops, on ornamentals. Cyclopropene (CP), 3,3-dimethylcyclopropene (3,3-DMCP) and 3-methylcyclopropene (3-MCP) have been tested in carnation, *Campanula* and *Kalanchoë* (Sisler et al., 1996, 1999). All tested compounds were found to be beneficial in protecting plant tissue from the effect of ethylene, but none of these compounds performed better that 1-MCP.

Few years later, 1-substituted cyclopropenes (like 1-MCP) with various carbon chains at the 1-position (linear saturated side chains from methyl (CH_3) to decyl ($(CH_2)_9CH_3$), have been synthesized and tested (Sisler et al., 2001, 2003). This has resulted in achievement of a number of ethylene receptor blockers which were more effective than 1-MCP. General conclusion from this study was that increasing the lengths of side chain gives the better performance of the compounds. 1-decylcyclopropene (1-DCP), the compound with the longest side chain used in this study, showed the best performance. The test was done on bananas, where 1-DCP prevented chlorophyll degradation for 36 days, three fold longer than 1-MCP. The long lasting effect is probably due to anchoring of the compound to the cell membrane by the hydrophobic side chain. This prevents the CP molecule from diffusion from the cell surface, allows a larger amount of compound to bind to the tissue, and consequently bind to ethylene receptor molecules, synthesized long after the initial treatment, thereby prolonging the effect.

1-HCP, 1-OCP and 1-DCP were tested on flowers. *Kalanchoë blossfeldiana* (Kebenei et al., 2003a) and *Lathyrus odorathus* (Kebenei et al., 2003b) were treated with 1-OCP and in general this compound was equally or slightly more efficient than 1-MCP in blocking ethylene action in flowers. Also 1-hexylcyclopropene (1-HCP) and 1-decylcyclopropane (1-DCP) has been shown to be effective in *Kalanchoë* though in somewhat higher concentrations (Kebenei et al., 2003a; Buanong et al., 2007).

Novel Liquid Derivatives of 1-MCP

The commercial application of 1-MCP in the open field, since it is a gaseous compound, is quite difficult, and in many cases simply not possible.

Recently few new compounds, that block ethylene receptors and can be applied either as a gas or as a salt by spraying or dipping, have been prepared and tested. The very first experiments made on bananas demonstrated a very strong inhibition of chlorophyll degradation caused by exposure to ethylene (Sisler et al., unpublished). Testing of *Kalanchoë* flowers with the new compounds is in progress (Sisler et al., unpublished).

BLOCKING ETHYLENE ACTION BY GENETIC MANIPULATION

For many ornamentals, breeding programs focus on better postharvest performance and longer vase life, which in many cases means lower ethylene sensitivity. So far there was no success with breeding of plants that are ethylene insensitive in fully developed flowers (Onozaki et al., 2001).

The very first attempts in producing longer lasting flowers by use of molecular techniques were done on carnations (Savin et al., 1995). Biosynthesis of ethylene was blocked by transformation with an antisense sequence of the carnation-ACO gene under control of a constitutive promoter, which resulted in dramatically reduced ethylene production during flower senescence, and a prolonged cut flower life (8-9 days for

transgenic flowers compared to 5 days for the non-transformed flowers).

A better flower longevity was achived in *Torenia fournieri* by successful transformation with both sense and anti-sense ACC oxidase gene constructs (Aida et al., 1998). A commercial breeding company Florigene produced carnations with reduced ACC synthase activity using a co-suppression technique, which resulted in flowers with much longer vase life (Michael et al., 1993; patent no. WO9635792).

In 1995 Meyerowitz group (Hua et al., 1995) succeeded for the very first time in affecting ethylene sensitivity. They blocked the function of the ethylene receptor by using a mutated *Arabidopsis thaliana "ers"* gene. Obtained transgenic *A. thalianas* plants showed high tolerance to endogenous ethylene.

Further work concentrated mostly on using the mutated dominant ethylene resistance gene *etr1-1* from *Arabidopsis*. The very first experiments with *etr1-1* gene were done with *Petunia* by Wilkinson et al. (1997), Clark et al. (1999), Gubrium et al. (2000) and later by Celvenger et al. (2004). In all mentioned studies *etr1-1* gene was expressed in *Petunia* under control of a *CaMV35S* promoter, resulting in constitutive expression of the *etr1-1* gene. Obtained transgenic *Petunia* plants showed that *etr1-1* from *Arabidopsis* conferred ethylene insensitivity, which resulted in strongly delayed flower senescence in pollinated and non-polinated flowers, in both presence and absence of ethylene. However, constitutive expression of the gene resulted in several additional effects, which are unacceptable for producers, like: strong inhibition of rooting of in cuttings, less efficient seed germination, less efficient rooting and delayed growth of seedlings.

In another study, ethylene insensitive *Petunia* plants with long lasting flowers were produced by using a mutated *ers* homologue from *Brassica oleracea* (Shaw et al., 2002). Unfortunately the mortality of the transgenic plants was high, due to a higher susceptibility to fungal diseases.

A less popular ornamental plant *Nemesia strumosa* has been transformed with a mutated *etr1*-homologue from melon under control of a constitutive promoter (Cui et al., 2004). As in previous investigations the ethylene perception has been controlled and flower longevity extended compared to the wild type.

Bovy et al. (1999) transformed carnation with *etr1-1* under control of a flower-specific promoter from *Petunia* (*fbp1*) to avoid the unintended side effects due to the use of the constitutive *CaMV35S* promoter. Transgenic carnations achieved in this study were highly ethylene insensitive and without the unwanted side effects known from the earlier experiments.

The same gene construct has later been used in transformation of commercially important potted plants: *Campanula carpatica* and *Kalanchoë blossfeldiana* (Fig. 1). The effective methods for regeneration and transformation have been developed for both plant species (Sriskandarajah et al., 2001; Frello et al., 2002; Sanikhani et al., 2006), and transgenic adult plants were achieved and tested (Sriskandarajah et al., 2004, 2007). More than one hundred transgenic lines T0 of *Campanula* were produced and tested for ethylene sensitivity. The tolerance to ethylene differed among the lines. When non-transgenic (control) *Campanula* was exposed to 2 ppm ethylene the flowers wilted within 2-3 days. The best transgenic lines flowered in presence of ethylene up to 27 days. No morphological differences were observed between control and transgenic lines. *Etr1-1* presence did not affect rooting ability of transgenic plants (Sriskandarajah et al., 2007). Also T1 progeny, which was achieved by crossing transgenic lines, was highly ethylene insensitive. The registration process for commercial production and sale of both species is in progress.

Interesting studies on *Petunia* were performed by using a construct containing a promoter from a senescence-associated gene (SAG12) coupled to a cytokinin biosynthetic gene (IPT) from *Agrobacterium tumefaciens*. The aim of this study was to control the cytokinin production in senescing flowers (Chang et al., 2003). The results showed a spectacular increased of flower longevity (450%) due to increased cytokinin level, which delayed ethylene production and improved ethylene tolerance in transgenic plants.

CONCLUSION

Many flowers and potted plants are sensitive to ethylene. Conditions that stimulate endogenous ethylene production (dark- or cold-stress) and the presence of ethylene from other sources (ripening fruit, air pollution) may cause early flower wilting or premature flower and bud abscission. Currently there are several strategies available to increase the performance of flowers and potted plants. A range of effective CP derived ethylene receptor blockers has been developed and their application properties have been improved. In addition, several experiments have shown that flower-targeted expression of a mutated ethylene receptor can prolong flower life without affecting other features of the plant. Broader application of these strategies in ornamentals is currently hampered by slow moving registration procedures.

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Figures

Fig. 1. Differences in flower longevity between transgenic lines of *Campanula carpatica* (right photo) and *Kalanchoë blossfeldiana* (left photo) exposed to 2 ppm ethylene for 10 days.