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Differential expression levels of ethylene biosynthetic pathway genes during senescence of long-lived carnation cultivars

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Abstract

Ethylene production and the expression of ethylene biosynthetic pathway genes of some carnation cultivars with a long vase life ('Miracle Rouge', 'Miracle Symphony' and 'Sandrosa') and a cultivar with a normal vase life ('White Sim') were investigated. None of the long-life cultivars exhibited normal climacteric-like ethylene production, or petal in-rolling during senescence, but they did exhibit slow petal desiccation. Ethylene production rates from senescing flowers were very low in all long-life cultivars, although slightly higher in 'Sandrosa' than in the two other long-life cultivars. Aminoethoxyvinylglycine (AVG) treatment prolonged flower life in 'Sandrosa', but did not affect flower life in 'Miracle Rouge' and 'Miracle Symphony', which indicated a role of 1-aminocyclopropane-1-carboxylate synthase (ACS) only in 'Sandrosa'. 1-Aminocyclopropane-1-carboxylate (ACC) treatment markedly accelerated senescence of 'Sandrosa' but had only a small effect on 'Miracle Rouge' and 'Miracle Symphony', suggesting that ACC oxidase (ACO) was inhibited or downregulated in the two 'Miracle' cultivars. The levels of *DC-ACS1*, *DC-ACS2* and *DC-ACO1* transcripts in the gynoecium and petals were high at 5 and 6 days after harvest in 'White Sim' but were below the detection levels in 'Miracle Rouge' and 'Miracle Symphony'. In 'Sandrosa', *DC-ACS1* was below the detection limit, whereas the expression of *DC-ACS2* and *DC-ACO1* was very low. The results show that long vase life in these carnation cultivars, 'Miracle Rouge', 'Miracle Symphony' and 'Sandrosa', is correlated with low *DC-ACS1*, *DC-ACS2* and *DC-ACO1* expression in the gynoecium and petals.

Keywords: Carnation; *Dianthus caryophyllus*; Ethylene; Ethylene biosynthesis; Flower senescence; Long-lived flowers

1. Introduction

Ethylene regulates many important growth and development processes, such as fruit ripening and flower senescence, in numerous plant species (Woltering and van Doorn, 1988; Abeles et al., 1992). The biosynthetic pathway of ethylene can be summarized as follows: methionine → *S*-adenosylmethionine (AdoMet) → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene (Kende, 1993). The conversion of AdoMet to ACC is catalyzed by ACC synthase (ACS), and ethylene is produced from ACC by ACC oxidase (ACO) (Yang and Hoffman, 1984; Kende, 1993). ACS activity is inhibited by aminoethoxyvinylglycine (AVG) (Baker et al., 1977). These steps involving ACS and ACO are generally considered to be rate-limiting for ethylene biosynthesis. Both enzymes are well characterized and several genes encoding them have been cloned in plant species, including carnation.

Carnation flowers are studied as model systems of ethylene-induced flower senescence. They show a climacteric-like rise in ethylene production, which is accompanied by petal in-rolling (inward rolling), a typical senescence symptom in carnation (Halevy and Mayak, 1981; Borochov and Woodson, 1989; Wang and Woodson, 1989; Abeles et al., 1992; Reid and Wu, 1992). The rise in ethylene production is associated with an increase in the expression of genes encoding ACS and ACO (Wang and Woodson, 1991; Park et al., 1992; Woodson et al., 1992; Henskens et al., 1994; Jones and Woodson, 1999). Three ACC

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synthase genes (*DC-ACS1*, *DC-ACS2* and *DC-ACS3*) and one ACC oxidase gene (*DC-ACO1*) have been identified in carnation (Woodson et al., 1992; Jones and Woodson, 1999).

In general, cut carnation flowers senesce within about 7 days in normal Sim-type cultivars. Their vase life can be extended by treatment with postharvest chemicals such as silver thiosulfate (STS) (Veen, 1979), which inhibits ethylene action. STS is used widely but there has been concern about potential environmental contamination from waste STS solution. Genetic improvement of carnation flowers has been studied in order to produce cultivars that would require no STS treatment to extend their vase life (Onozaki et al., 2006b).

Carnation lines that vary in ethylene biosynthesis and sensitivity are of great value in the study of ethylene-related processes (Wu et al., 1991). Flowers of some carnation cultivars have a long-life span, which is associated with low ethylene production and/or low ethylene sensitivity. Some flowers with low ethylene production are 'Killer' (Serrano et al., 1991), 'Sandra' (Wu et al., 1991), 'Sandrosa' (Mayak and Tirosh, 1993), line '87-37G-2' and line '81-2' (Brandt and Woodson, 1992), and 'White Candle' (Nukui et al., 2004). Flower senescence in 'Sandrosa' is probably the same as or very similar to that of 'Sandra' (Wu et al., 1991). Onozaki et al. (2006a,b) recently bred two long vase life carnation cultivars, 'Miracle Rouge' and 'Miracle Symphony'. The vase life of these two cultivars seems to be longer than that of any other cultivars reported in the literature. Ethylene production of their cut flowers is low throughout the vase life.

In contrast to cultivars with low ethylene production, the flowers of some other long vase life cultivars such as 'Chinera' and 'Epomeo' seem to have a relatively low sensitivity to ethylene (Reid and Wu, 1992; Woltering et al., 1993), whereas line '799' seems to have both low ethylene production and low ethylene sensitivity (Brandt and Woodson, 1992).

Study of *DC-ACS* and *DC-ACO1* genes expression in 'Miracle Rouge', 'Miracle Symphony', 'Sandrosa' and 'White Sim' might help our understanding of the senescence mechanisms of ethylene-sensitive flowers and enhance the flower life of carnations through genetic improvement. Only one paper has been published on the regulation of expression of some genes involved in ethylene synthesis in a long vase life carnation cultivar, 'White Candle'. In 'White Candle', *DC-ACS2* and *DC-ACO1* transcript abundance was not correlated with long vase life, but *DC-ACS1* gene expression was (Nukui et al., 2004).

Here we report on the expression of four ethylene biosynthesis genes (*DC-ACS1*, *DC-ACS2*, *DC-ACS3* and *DC-ACO1*) in flowers of three long vase life carnation cultivars, in comparison with 'White Sim', a cultivar with a normal, short vase life.

2. Materials and methods

2.1. Plant material

Three long vase life carnation (*Dianthus caryophyllus* L.) cultivars, 'Miracle Rouge', 'Miracle Symphony' (Onozaki et al., 2006a,b) and 'Sandrosa', and a control cultivar, 'White Sim', were grown under natural daylight conditions in a greenhouse (Onozaki et al., 2001). 'Miracle Rouge' and 'Miracle Symphony' were selected to improve the vase life of flowers by cross breeding from 1992 to 2003 in a breeding research program of the National Institute of Floricultural Science, Japan (Onozaki et al., 2006a). 'Sandrosa' is a Mediterranean type of carnation. 'White Sim' is an American Sim-type carnation and has been used as a standard in studies of senescence of carnation petals.

2.2. Flower vase life

Flowers were harvested when the outer petals were horizontal. On day 0, the stems of harvested flowers were cut to 30 cm and held in distilled water under standard conditions with a constant air temperature of 23 °C, RH of 70% and a photoperiod of 12 h (08:00–20:00) under cool fluorescent white lamps (10 μmol/(m^2 s)). The vase life of each flower was determined from harvest; the end of life was defined as when at least two petals showed wilting with in-rolling, browning or desiccation of the petal margins. Fresh weight (FW) of the flowers was measured daily.

2.3. Ethylene measurement

Flowers were placed in a 433 mL glass bottle which was closed and kept at 23 °C for 1 or 2 h. Gynoecia and petals were placed in a 15 mL glass vial which was closed and kept at 23 °C for 1 h. Gas samples (1 mL) were taken from the headspace and injected into a gas chromatograph GC-7A (Shimadzu, Kyoto, Japan) equipped with an alumina column and a flame ionization detector.

2.4. Ethylene sensitivity

At harvest (day 0), flowers were placed in a 70 L chamber with 2 μL/L ethylene under the same conditions as above but under continuous light conditions. They were recorded every 1 h by a digital camera to determine the onset of senescence symptoms. Stems of these flowers were placed into distilled water containing 2 mM AVG (Sigma), 3 h before ethylene treatment and were kept soaking in the same solution during the experimental period.

2.5. ACC and AVG treatment

At harvest (day 0), flower stems were cut to 30 cm. The stem end of tested flowers was placed individually in a test tube containing 1 mM ACC solution for 3 h. After pretreatment, they were transferred to another test tube containing 20 mL distilled water and kept at 23 °C under continuous light conditions. They were recorded every 1 h by a digital camera to determine the onset of wilting or browning symptoms.

For continuous treatment with AVG, the stem end of these flowers was placed in a test tube containing 2 mM AVG solution. Fresh weight and vase life of these flowers was evaluated daily.