Scientia Horticulturae 183 (2015) 31–38

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Expression levels of ethylene biosynthetic genes and senescence-related genes in carnation (*Dianthus caryophyllus* L.) with ultra-long-life flowers

Koji Tanase[a,*], Sawako Otsu[b], Shigeru Satoh[b], Takashi Onozaki[a]

[a] NARO Institute of Floricultural Science (NIFS), National Agriculture and Food Research Organization (NARO), Tsukuba 305-8519, Japan
[b] Laboratory of Genetic Engineering, Faculty of Agriculture, Kyoto Prefectural University, Kyoto 606-8522, Japan

ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form
18 November 2014
Accepted 27 November 2014
Available online 23 December 2014

Keywords:
Carnation
Ethylene biosynthetic gene
Flower life
Senescence
Senescence-related gene
Ultra-long-life flower

ABSTRACT

Carnation (*Dianthus caryophyllus* L.) line 532-6 has ultra-long flower life (29 days without chemical treatment). We investigated ethylene production and expression of ethylene biosynthetic genes and senescence-related genes in the flowers of 532-6 in comparison with those of a control cultivar, 'Francesco' and a cultivar with long flower life, 'Miracle Rouge' (MR). 'Francesco' flowers, but not those of MR and 532-6, showed typical symptoms of ethylene-dependent senescence. The flowers of MR and 532-6 produced very low levels of ethylene as a result of very low expression levels of two ethylene biosynthetic genes, *DcACS1* and *DcACO1*. The expression of some senescence-related genes (*DcbGal*, *DcGST1* and *DcLip*) increased in 'Francesco' petals by day 6, but remained low in the petals of MR and 532-6 throughout the experiment, whereas there was a small difference between the levels of the *DcCP1* transcript in MR and 532-6. The expression of *DcCPIn* was regulated differently in all three cultivars. In 'Francesco' petals, the *DcCPIn* expression level sharply decreased on day 6 and 7; in MR, the *DcCPIn* level increased on day 7 and decreased by day 16, whereas in 532-6 it increased on day 3 and then gradually decreased until day 25. The results suggest that the extended flower life of MR and 532-6 in comparison with 'Francesco' depends on reduced levels of ethylene production, low levels of ethylene biosynthetic gene expression, and senescence-related gene expression; the difference between MR and 532-6 may be due, in particular, to *DcCPIn* and *DcCP1*.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) flowers are sensitive to ethylene, and their senescence is regulated by ethylene produced autocatalytically several days after anthesis (Woltering and van Doorn, 1988; Woodson and Lawton, 1988). The ethylene biosynthesis pathway is 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 the conversion of ACC to ethylene by ACC oxidase (ACO) (Kende, 1993; Yang and Hoffman, 1984). During carnation flower senescence, *ACS* and *ACO* expression and the autocatalytic ethylene production lead to subsequent petal in-rolling, which is a typical form of wilting. Three genes encoding ACS (*DcACS1*, previously described as CARACC3, *DcACS2* and *DcACS3*) and one gene encoding ACO (*DcACO1*, previously described as pSR120) have been identified and their expression patterns examined (Henskens et al., 1994; Jones and Woodson, 1999; Park et al., 1992). *DcACO1* and *DcACS* genes are expressed in gynoecia and petals; *DcACS1* is the predominant *DcACS* gene expressed in petals and *DcACS2* and *DcACS3* are expressed in gynoecia (Jones and Woodson, 1999; Satoh and Waki, 2006). The expression of *DcACS1* and *DcACO1* is enhanced by exogenous ethylene, which induces ethylene production.

Flowers of some carnation cultivars have a long life, which is associated with low ethylene production and low ethylene sensitivity (Wu et al., 1991). Some cultivars and lines with low ethylene production in flowers are 'Killer' (Serrano et al., 1991), 'Sandra' (Wu et al., 1991), 'Sandrosa' (Mayak and Tirosh, 1993), lines 87-37G-2 and 81-2 (Brandt and Woodson, 1992), 'White Candle' (Nukui

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; ACO, ACC oxidase; ACS, ACC synthase; AdoMet, S-adenosylmethionine; bGal, β-galactosidase; CP, cysteine proteinases; CPIn, cysteine proteinase inhibitor; GST, glutathione-*S*-transferase; HQS, 8-hydroxyquinoline sulfate; Lip, lipase; MR, Miracle Rouge; SR, senescence-related; STS, silver thiosulfate anionic complex.

* Corresponding author. Tel.: +81 29 838 6801.
E-mail address: tanase@affrc.go.jp (K. Tanase).

http://dx.doi.org/10.1016/j.scienta.2014.11.025

Table 1
Flower life of carnation (*Dianthus caryophyllus* L.). STS, 2 mM silver thiosulfate for 20 h, followed by distilled water. Zn, 2 mM $ZnCl_2$ throughout the experiment. STS + Zn, STS treatment as above, followed by 2 mM $ZnCl_2$. Values are means ± SE of nine flowers. Different letters indicate a significant difference with $P < 0.05$ by Tukey's test.

Cultivar or line	Treatment	Flower life (days)
'Francesco' (normal life)	–	$9.1^{a} \pm 1.2$
	STS	$21.2^{b} \pm 3.1$
	Zn	$9.6^{a} \pm 0.8$
	STS + Zn	$22.6^{b} \pm 1.6$
'Miracle Rouge' (long life)	–	$22.7^{b} \pm 0.4$
	STS	$22.5^{b} \pm 1.6$
	Zn	$24.8^{b} \pm 0.9$
	STS + Zn	$24.8^{b} \pm 3.6$
532-6 (ultra-long life)	–	$29.0^{c} \pm 0.8$
	STS	$29.0^{c} \pm 4.1$
	Zn	$28.5^{c} \pm 1.2$
	STS + Zn	$31.3^{c} \pm 3.1$

Fig. 1. Flowers of carnation (*Dianthus caryophyllus* L.) 'Miracle Rouge' and line 532-6 on day 25. The cut flowers were kept in distilled water at 23 °C, 70% relative humidity and 12-h photoperiod under cool-white fluorescent lamps (10 μmol m^{-2} s^{-1}).

et al., 2004), 'Miracle Rouge' (MR), 'Miracle Symphony', and lines 006-13 and 62-2 (Onozaki et al., 2006; Tanase et al., 2008, 2013), which have a life of 10–20 days. The expression of *DcACS1*, but not *DcACO1*, is low throughout the flower life in 'White Candle' (Nukui et al., 2004). Our previous study showed that by comparing cultivar with normal flower life, the expression of *DcACS1*, *DcACS2* and *DcACO1* was suppressed in the flowers of MR and 'Miracle Symphony', which resulted in a very low level of ethylene production (Tanase et al., 2008). In particular, low *DcACO1* expression seems to be linked to the low ethylene production in both cultivars.

Many other factors play a role in the regulation of ethylene biosynthesis and petal wilting in senescing carnation flowers (Hoeberichts et al., 2007; Otsu et al., 2007). Petal wilting may be caused by degradation of cellular components, which induces cell death and is mediated by hydrolytic enzymes and their regulators, such as lipases [Lip (Hong et al., 2000; Kim et al., 1999a)], cysteine proteinases [CPs (Jones et al., 1995)] and a cysteine proteinase (CP) inhibitor [CPIn (Kim et al., 1999b; Sugawara et al., 2002)]. Many senescence-related (SR) genes (including *DcLip*, *DcCP1* and *DcCPIn*) have been cloned from carnation petals and their expression patterns examined. The transcript levels of genes encoding a CP [*DcCP1*; previously described as pDCCP1 (Jones et al., 1995)], β-galactosidase [*DcbGal*; previously described as SR12 (Lawton et al., 1989)], glutathione-*S*-transferase (*DcGST1*) and lipase (*DcLip*) are increased during flower senescence and by ethylene

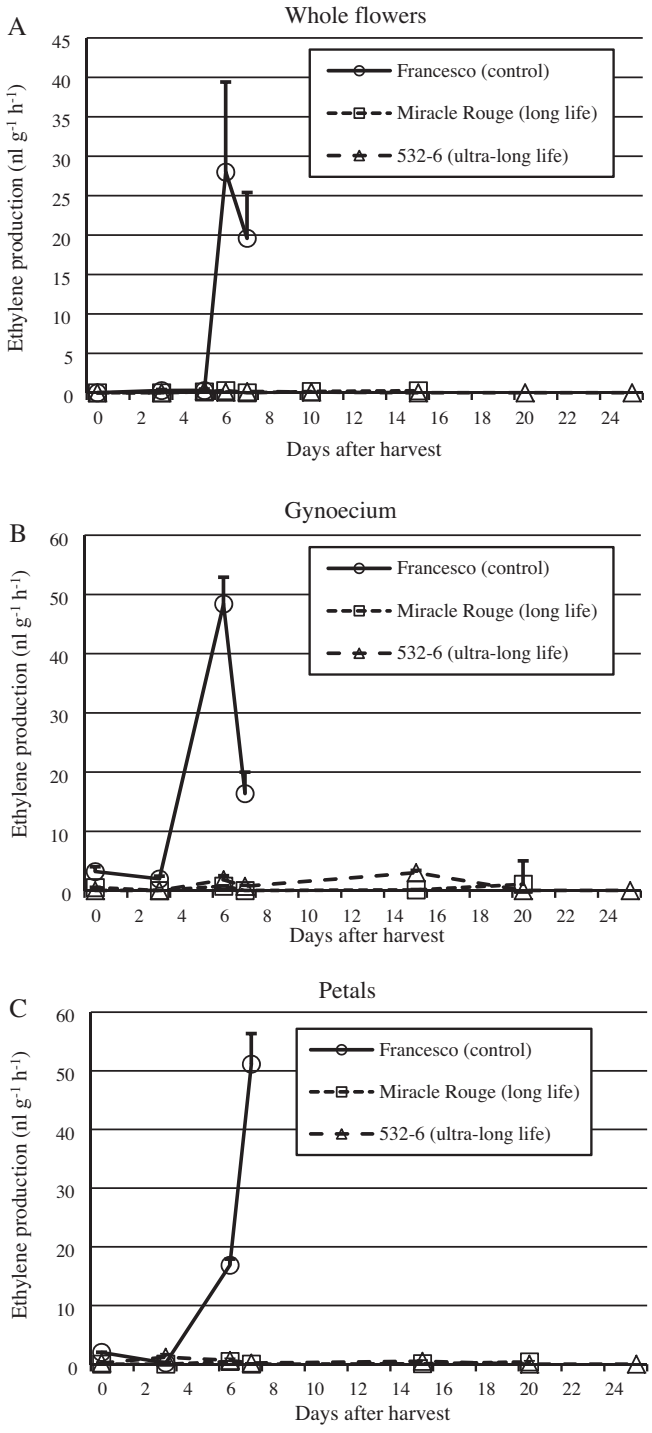

Fig. 2. Ethylene production in carnation flowers during flower senescence. (A) Whole flower. (B) Gynoecium. (C) Petals. Values are means ± SE of three flowers.

treatment (Hong et al., 2000; Kim et al., 1999a; Verlinden et al., 2002), whereas the *DcCPIn* transcript level decreases under these condition (Sugawara et al., 2002; Tanase et al., 2013).

Recently, an ultra-long-life carnation line, 532-6, was reported with a flower life of over 27 days, which is approximately four times that of normal cultivars (Onozaki et al., 2011) and longer than that of any other long-life cultivars studied previously. To obtain clues as to the mechanisms underlying flower longevity in 532-6, we analyzed the expression profiles of ethylene biosynthetic genes and SR genes during flower senescence. We also assessed the effects of