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Down-regulation of ethylene production in carnation (*Dianthus Caryphyllus* L.) by an apple derived ACC-cDNA

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Summary: Transgenic carnations were produced with an apple derived antisense ACC-synthase cDNA. Transgenic carnation regenerants were potted in glasshouse. All transformed plants showed normal growth and were true-to-type. Ethylene production – measured at full opening stage – lowered by 30–60 %, no plant with 100 % decrease was identified. The vase-life has been observed for 5 years. 38 % of the transformant carnations showed a higher a relative value in days by more than 2 days to 6 days. Twenty six plants were found exhibiting the most marked alterations in the tested trait. In these plants ethylene production decreased by 37–67 %, they have longer vase-life (by 4 days or more). Since the fragrance variety 'Bibor' was the plant material for genetic modification of vase-life, this trait has been conserved after transformation in spite of the fact that the position of transgene integration cannot be directed.

Key words: ACC-synthase, *MdACS2*, transgenic carnation, vase-life

Introduction

Carnation is one of the leading species in the global flower trade, therefore genetic improvement in the respect of several ethylene dependent traits such as flowering time, petal senescence characteristics or vase-life can have beneficial economic effect in addition to scientific significance. Carnations are typical ethylene-sensitive flowers (*Woltering & Van Doorn* 1988). Our aim was to inhibit ethylene production in flowers. Several methods are available to modify the vase-life of cut flowers for example treatment with chemicals, such as aminooxyacetic acid (*Fujino* et al. 1980), 1 methylcyclopropene (*Hassan & Gerzson* 2002, *Ichimura* et al. 2002) or silver thiosulfate (*Veen* 1979). There are several ways of genetic improvement for instance crossing and selection (*Onozaki* et al. 2001) or genetic modification of carnation with antisense ACC oxidase- (*Savin* et al. 1995) and ACC synthase- (Florigene Pty Ltd. 1995) gene or transformation with *Arabidopsis etr* 1–1 allele (*Bovy* et al. 1999). In our transformation experiments an apple-derived cDNA clone was used in antisense orientation (*Kiss* et al. 1995, *Rosenfield* et al. 1996) to down-regulate ethylene biosynthesis (*Veres* et al. 2002) in carnation, in order to study how the reduced ethylene production influences the flowering characteristics. Carnation ACC synthases: *DcACS3*, *DcACS2*, *DcACS1* show 66%, 68%, and 65% homology to the apple *MdACS2*, respectively.

There is a negative correlation between long vase life and the fragrance of flowers (*Priel* 1999) but long shelf life flowers sale well in the market. For transformation, a variety with intensive fragrance was chosen to produce flowers with long shelf life accompanied with intensive fragrance.

Material and method

Plant material

The fragrance varieties Bíbor'/Purple (Óbuda Horticultural Laboratory Budapest, Hungary) were transformed by *Agrobacterium tumefaciens* LBA 4404, harbouring an antisense apple derived ACC synthase gene construct (*Kiss* et al. 1995, 2000). Carnations (160) transformed with antisense apple ACC synthase gene are named as *CCA*. While those (90) that were transformed with pBI121 containing the GUS gene are labelled as *GUS*. The non-transformant plants (90) handled in the same way as transgenics except *Agrobacterium* infection. The control plants (65) were grown in the greenhouse (Óbuda Horticultural Laboratory, Budapest).

In vivo growth conditions

Wild type and transgenic carnation plants (*Dianthus caryophyllus* L. cv. 'Bíbor') were potted in glasshouse and grown under the same normal greenhouse conditions as the commercial plants.

Measurement of ethylene production

Transgenic and non-transgenic flowers at the full-opening stage were cut to 10 cm and put into special glass with a rubber septum. After a 24 h incubation period with 1% exogenous ethylene they were kept in fresh air for 24 h, and put back to the glass for 24 h.

Ethylene was measured with a gas chromatograph GC 6000 equipped with an activated alumina column and a flame-ionization detector.

Estimation of vase life

Transgenic and non-transgenic flowers at the full-opening stage (their outermost petals were at right angles to the stem of the flowers) were cut to 50 cm. The flowers were put into 2 l test-tube containing 1 l distilled water, and were stored in a room with controlled air temperature of 22 °C, 70% RH.

The vase-life period was estimated by the number of days, until 20 percent of the petals showed in-rolling *(Figure 3)*. The vase-life has been evaluated since 1999 until 2004. For comparison, a relative value was calculated. The value means that in each actual observation period of vase life, the number of days belonging to the control plants (both non-transformed and „transgenic/ *GUS* controls) was subtracted from the number of days determined for the *CCA* plants.

Results

All transformed plants showed normal growth and were true-to-type. Transformed plants flowered by 2 weeks earlier than non-transformed plants.

Ethylene production was measured at full opening stage. The ethylene production of non-transformant plants was compared to *CCA* carnations; the value of controls regarded one hundred percent. Ethylene production of the non-transformant carnation was 100 ±13% and the production of *CCA* plants was lowered by 28-85 ±15% (*Figure 1*), no plant with 100% decrease was identified. There is a correlation between lower ethylene production and longer vase-life (*Figure 2*).

Vase life has been measured for 5 years. Flower longevity is profoundly influenced by the season of blooming. Onset of flowering occurred in different times during the years depending on temperature and light intensity: e.g., in 1999 the first flowering cycle started on 21 of February, while more than a month later in 2002, on 24 of March. Therefore, for sake of comparison a relative value was calculated in days, this means that the vase life was calculated within one flowering cycle. The relative value was obtained by comparing the vase life of „experimental" plants to that of controls: (average of 65 plants). Controls are glasshouse grown normal plants, while „experimental" ones are either transformed or non transformed but handled in the same way as transgenics except *Agrobacterium* infection.

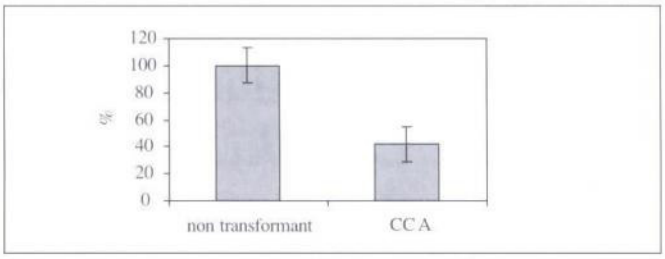


Figure 1. Ethylene production (%) of transformant (CCA), Control and non-transformant carnation in 2003.

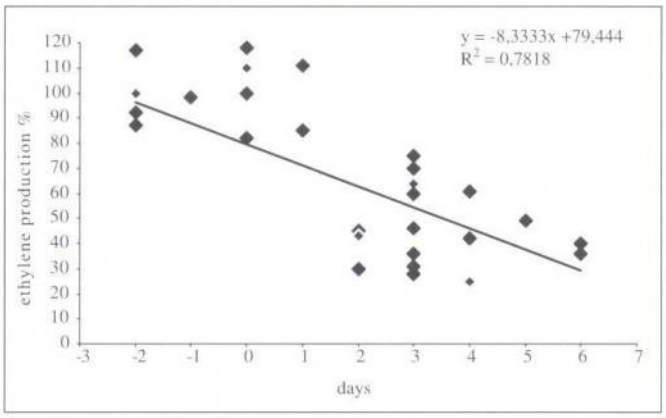


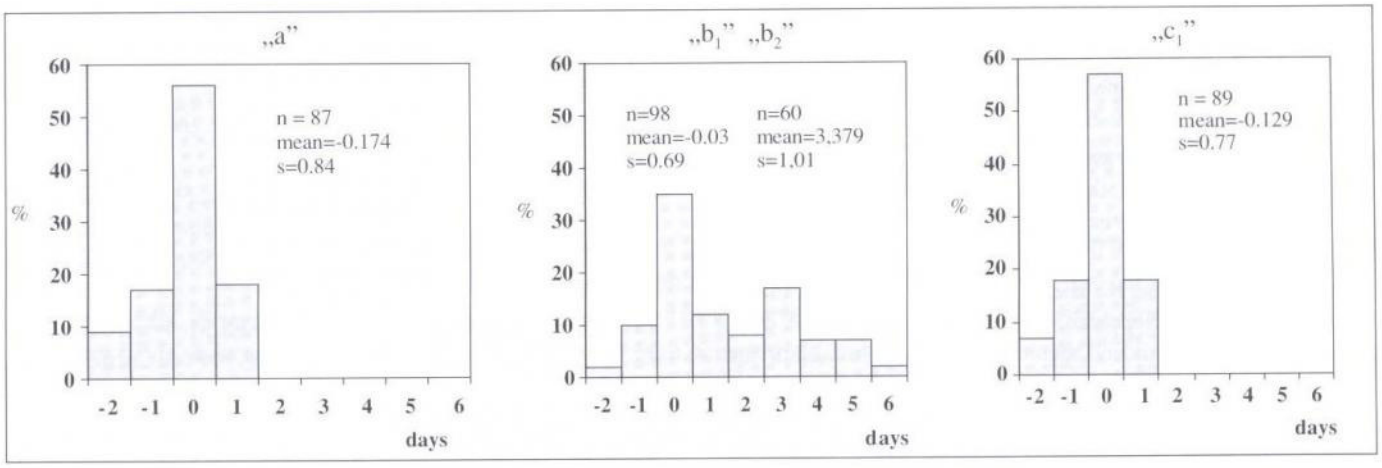
Figure 2. Correlation between the ethylene production (%) and the vase-life (relative value in days) of plants from „b1" and „b2" groups in 2003.

Figure 3 „a" shows the percentage of these non-transformed plants according to the relative value in days, while in *Figure 3 „c"* the percentage of *GUS* transformants can be seen. Statistical t-test proved that *„a"* and *„c"* data, that is non-transformed controls and GUS transformants, do not differ from each other in vase life. The plants in *Figure 3 „b"* can be divided into two parts *„b_1"*, *„b_2"*. The *„b_1"* group consists of plants exhibiting the same vase life as the members of *„a"* and *„c"* groups; at least the prolongation of flower longevity is not significant. The plants belonging to the *„b_2"* group showed significantly altered vase life compared to the relative values of *„a"*, *„b_1"* and *„c"* groups. In spite of the fact that the presence of the transgene was proven in the individuals of *„b_1"* group its expression was low so no effect on vase life could be detected.

Plants, picked up from the groups *„b_1"* and *„b_2"* sharply stand apart from each other on the basis of ethylene production, too (*Figure 2*). The prolonged flower longevity in *„b_2"* plants is accompanied by lower ethylene production (*Figure 1*), so the increase of vase life can be regarded as a consequence of lowered ethylene production due to the antisense ACC-cDNA transformation.

Discussion

As for the consequence of various chemical treatments, the following results have been published about the vase-life expanding effect of aminooxyacetic acid: 3.3 days (*Bovy* et al. 1999); silver thiosulphate: 7.2 days (*Bovy* et al. 1999);



t*=2.326; t(a,c)=1.98 ; t(a,b1)=0.927; t(a,b2)=23.08

Figure 3. The percentage of the plants according to the relative value in days (average of 5 years). Non-transformant („a"); transformants: CCA („b") and GUS („c"). The transformants can be divided into two groups. The first group „b1" (62 % of the plants) does not show any change in vase-life, while the other group „b2" (38 % of the plants) exhibits longer-life phenotype.

1-MCP: 5 days (*Hassan & Gerzson* 2002). The improvement by crossing and selection (*Onozaki* et al. 2001) improved the flower longevity by 13 days. The genetic modification with antisense ACC-oxidase (*Savin* et al. 1995) or ACC-synthase (Florigene Pty Ltd. 1995) prolonged the vase-life of carnation flowers by 3–4 or 12 relative value in days, respectively (at low temperature). Other gene involved in ethylene biosynthesis (*Arabidopsis etr*1-1allele) resulted in 16 days longer vase life. For correct comparison, the effect of temperature should also be taken into consideration: the lower the temperature the longer vase-life could be observed (*Table 1*).

From these results, we can conclude that the transformation with an apple-derived ACC cDNA in antisense orientation resulted longer vase-life in carnation than chemical treatments, or transformation with carnation ACC oxidase gene.

Table 1. Effect of different treatments or genetic modifications on vase-life of carnation calculated as „relative value in days"

Treatment/modification	Relative value in days		References
	22 °C	4 °C	
1-MCP	5		Hassan & Gerzson (2002)
Aminooxyacetic acid		3.3	Bovy et al. (1999)
Silver thiosulfate		7.2	Bovy et al. 1999)
Crossing and selection		13	Onozaki et al. (2001)
Transformation with carnation antisense ACC oxidase gene	3-4		Savin et al. (1995)
Transformation with carnation antisense ACC synthase gene		12	Florigene Pty Ltd. (1995)
Transformation with *Arabidopsis etr 1*-1 allele		16	Bovy et al. (1999)
Transformation with apple antisense ACC synthase gene	6		present study

Figure 4. Carnation flowers 'Bíbor' at room temperature 10 days after harvest. Control, non-transformed, *GUS:* GUS transformant plants, *CCA:* antisense ACC-synthase transformants

Since the fragrance variety 'Bibor' was the plant material for genetic modification of vase-life, this trait has been conserved after transformation in spite of the fact that the position of transgene integration cannot be directed, so a fragrance carnation variety has been obtained with a longer shelf life due to the decreased ethylene production.

Acknowledgements

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