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Differential Expressions of Expansin and Xyloglucan Endotransglucosylase Genes by Adenosine Triphosphate of Cut Carnation Flowers During Senescence

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Abstract: This study was conducted with a view to explaining this observation in terms of expressions of expansin and xyloglucan endotransglucosylase (XET) genes in relation to senescence progress of cut carnation flower. T wo cDNAs fragments (*DcExp 1* and *DcExp 2*) encoding expansins and two cDNAs fragments (*DcXet 1* and *DcXet 2*) encoding XETs were cloned from petals at the floral opening and three various aging stages of cut carnation flowers, respectively. *DcExp 2* expression appeared initially, increased slowly, reached to the most intensity at 5 days and then decreased at 8 days of vase holding of cut carnation flowers but *DcExp 1* did not express. As compared to the non-ATP-treated cut flowers, *DcExp 2* expression was hardly detected at the final stage of vase holding in the ATP-treated cut flowers, which suggested that *DcExp 2* might be involved in senescence progress of the cut carnation flowers. For XET expression, no accumulation of *DcXet 1* transcript was detected in either non-ATP-treated or ATP-treated cut carnation flowers during vase holding, which indicated that *DcXet 1* was not related to senescence of the cut carnation flowers. *DcXet 2* expressed only within the first 5 days of vase holding in non-ATP-treated flowers. Exogenous ATP supply inhibited the expression of *DcXet 2* at a later stage of vase holding. Thus, ATP treatment appeared to inhibit the expressions of *DcExp 2* and *DcXet 2* at the later stage of vase holding of cut carnation flowers and then extend their vase life.

Key words: Adenosine triphosphate, expansins, xyloglucan endotransglucosylase, senescence, carnation, cut flower

INTRODUCTION

Senescence of cut carnation flowers is limitation for their successful marketing due to harvested petal inrolling and discolouration (Serrano *et al*., 2001; Thompson *et al*., 1982). Trippi and Paulin (1984) reported that cut carnation flower senescence was associated with low ATP production. Lack of energy in plant tissues may lead to the senescence of harvested fresh produce including cut flowers (Duan *et al*., 2004; Rawyler, 1999; Saquet *et al*., 2000; Veltman *et al*., 2003). However, cut flower senescence is relatively complex. Elanchezhian and Srivastava (2001) suggested that the disassembly of the primary cellular walls is an important process in the senescence of chrysanthemum flower.

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Expansins are cell wall-located proteins which act in plant walls by disrupting non-covalent binding between matrix glycan cellulose microfibrils and are, therefore, considered primary regulators of plant cell enlargement (Cosgrove, 1999a, 1999b, 2000; McQueen-Mason and Cosgrove, 1994). XET as one of cell wall degradation-related enzymes is responsible for the reorganization of the cellulose-xyloglucan framework in plants (Fry *et al*., 1992; Nishitani and Tominaga, 1992). Recent many investigations showed that either expansin or XET is involved in fruit ripening, softening and floral opening and senescence and plays a major role in cell wall disassembly event in non-growing tissues (Brummell *et al*., 2002; Gookin *et al*., 2003; Lu *et al*., 2004; Maclachlan and Brady, 1994; McQueen-Mason *et al*., 1992; Redgwell and Fry, 1993; Rose and Bennett, 1999; Rose *et al*., 1997; Sane *et al*., 2005).

In the previous investigation, it was found that exogenous ATP supply could extend the vase life of cut carnation flower (Song *et al*., 2006), but its effect on expressions of expansin and XET genes is unknown. The objective of this study was to investigate effects of ATP on expressions of expansin and XET in relation to senescence of cut carnation flowers.

MATERIALS AND METHODS

Plant Materials

Cut flowers of carnation (*Dianthus caryophyllus* L.) cv. Master in 2005 were obtained from a commercial market in Guangzhou. Flowers were transported by car to the laboratory of South China Botanical Garden (Guangzhou) within 6 h and selected for uniformity at the pre-opening stage of maturity described by Droillard *et al*. (1989). The stem ends were re-cut under water, with about 30 cm in length and stood into 300 mL conical flasks containing 150 mL distilled water. ATP was added to distilled water at a final concentration of 0.1 mmol L^{-1} while control treatment was distilled water. Petals were collected at the bud, opening, maximum expansion and wilting stages described by Trippi and Paulin (1984), i.e., 0, 3, 5 and 8 day during vase holding, then frozen in liquid nitrogen and finally stored at -80°C. There were 4 flowers per flask and 3 flasks (replications) per treatment. Stems were assigned at random to treatments and the flasks arranged in a completely randomized design. Throughout the experiment period, cut flowers were held at 25°C and 70-80% relative humidity with a 12 h light period per day at an irradiance of 12 Wm^{-2} at flower level from fluorescent light tubes.

RNA Extraction

Frozen petals (ca 7 g) were ground to a fine powder in a mortar using a pestle in the presence of liquid nitrogen. Total RNA was extracted using the hot borate method of Wan and Wilkins (1994).

Cloning and Sequences of Expansin and XET Genes

Total RNA (5 μg) prepared from petals was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen, Groningen, The Netherlands) and the product (the first strand cDNA) was subjected to PCR amplification. Degenerate primers were, respectively, designed with reference to the conserved amino acid sequences of expansins and XETs [forward primers: 5'-GSNCAYGCNACNTTYTAYGGNG-3' for expansins and 5'-GAYGARMTNGAYWTHGARTTYMTNGG-3' for XETs while reverse primers: 5'-YTGCCARTTYTGNCCCARTT-3' for expansins and 5'-YGNGTNGCCCANKMRTCNSCRTYCC-3' for XETs]; S = G/C, N = A/C/G/T, Y = T/C and R = A/G). Reactions for the RT-PCR were subjected to one cycle of 94°C for 3 min, 35 cycles (94°C for 1 min, 50°C for 2 min and 72°C for 2 min) and one cycle of 72°C for 10 min. PCR of products were purified and cloned into pMD-18T vector (Takara, Shiga, Japan). The nucleotide sequences of

cDNAs were established for both strands using the ThermalSequenase dye terminator cycle sequencing kit and a 377 DNA sequencer (Applied Biosystems, Foster, Calif).

Syntheses of DIG-Labeled Specific Probes

Two expansin and XET genes were cloned from cut carnation flowers and then named *DcExp1 and DcExp 2* and *DcXet 1* and *DcXet 2*, respectively. DIG-labeled specific probes were synthesized using a PCR DIG probe synthesis Kit (Roche, Mannheim, Germany) and primers for *DcExp1* (forward primers: 5'-AACGGACACTCGTACTTCAAC-3' and reverse primers: 5'-GATAATATAGTCACCGCCTCG-3') and *DcExp 2* (forward primers: 5'-CTTTCCTTCAGGGTCACAGC-3' and reverse primers: 5'-CCATCATTCAATTAGGCAGTCC-3') and *DcXet 1* (forward primers: 5'-AGTCCCATTTCCAAAGAGCCAA-3' and reverse primers: 5'-CCCATCTCAATCTCCTTCTACC-3') and *DcXet 2* (forward primers: 5'-CGTACCATTTCCAAAGAACCAG-3' and reverse primers: 5'-TTACACCCACCCATTGAGATAG-3').

Northern Blot Analysis

Total RNA (10 μg) was separated on a 1.2% agarose-formadehyde gel and capillary blotted onto PVDF membrane (BIODYNE B, 0.45 μm; PALL, Tokyo). The membranes were blot-dried, then cross-linked at 280 nm and finally hybridized with DIG-labeled probes for 16 h at 45°C in high-SDS buffer solution (7% SDS, 5×SSC, 50 mmol L^{-1} sodium phosphate, pH 7.0, 2% blocking reagent and 0.1% N-laurylsarcosine) containing 50% deionized formamide (v/v) (Roche). Blots were washed twice at 37°C in 2×SSC and 0.1% SDS for 10 min, followed by washing twice at 62°C in 0.1×SSC and 0.1% SDS for 30 min. All blots were exposed to X-ray for 30 min at 37°C. The membranes were then subjected to immunological detection using CDP-Star, according to manufacturer's instruction (Roche Applied Science, Mannheim, Germany).

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of Expansin and XET cDNAs

In this study, *DcExp 1* and *DcExp 2* (ca 530 bp) and *DcXet 1* and *DcXet 2* (ca 300 bp) were cloned from cut carnation flowers, respectively (Fig. 1). BLAST search of GenBank revealed that *DcExp 1* shared 76% identity with *DcExp 2* which had 86% identity with *LeExp 18* (LES270960) from tomato while *DcXet 1* shared 78% identity with *DcXet 2* which showed 84% identity with *LeXTH 3* (AY497476.1) from tomato. Thus, *DcExp 1* and *DcExp 2* and *DcXet 1* and *DcXet 2* can be considered to be cDNA of expansins and XETs, respectively.

Effects of ATP Supply on Expressions of DcExp 1 and DcExp 2

There was a dramatically differential expression between *DcExp 1* and *DcExp 2* during floral opening and senescence of cut carnation flowers (Fig. 2). *DcExp 2* expressed initially, increased slowly, reached to the most intensity at 5 day of vase holding and then decreased at the wilting stage of non-ATP-treated flowers but *DcExp 1* did not express. The expression profile of *DcExp 2* was similar to the report of Gookin *et al*. (2003) who found a low level in buds and at the aging stage but a high level of á-expansin expression during floral maximal elongation of *Mirabilis jalapa*. In the ATP-treated cut flowers, the mRNA accumulation of *DcExp 2* was detected within the first 5 days but it hardly expressed at the later stage of vase holding (Fig. 2). The previous study had shown that expansins were involved in plant senescence by regulating hemicellulosic compounds and the development of plant tissue senescence can be delayed when expansin expression was inhibited (Brummell *et al*., 2002; Hayma *et al*., 2001; Mbéguié *et al*., 2002). This present study showed firstly

DcExp 1:
GGGCATGCGACTTTTTATGGAGGGAGTGACGCCTCTGGGACAATGGGTGGAGCGT
GTGGATACGGAAACTTATACAGCCAAGGGTACGGGACTAGCACAGCAGCGTTAAG
CACTGCGCTATTTAACAGTGGATTGAGCTGTGGAGCGTGCTTCGAACTCAAATGTA
ACGATGATCCTAGATGGTGCAATCAAGGAAGCATTATCGTCACGGCTACTAATTTT
TGCCCTCCTAACTATGCCTTGGCCAATGACAATGGTGGGTGGTGCAACCCTCCCCTC
CAACATTTTGACTTGGCCGAACCTTCCTACTTAAAAATTGCCCAATATCGTGCTGG
AATCGTCCCTGTCGCCTTTAGAAGGGTACCCTGTCTGAGGAAAGGTGGAATAAGG
TTCACAATTAACGGACGCTCGTACTTCAACTTGGTTCTAATTAGCAACGTCGCTGG
TGCTGGTGACGTCCACGCCGTGTCAATCAAGGGTTCGAAAACCGGTTGGCAAGCCA
TGTCAAGGAATTGGGGACAGAACTGGCAA

DcExp 2:
GGGCACGCGACATTTTACGGTGGTTCCGACGCCTCTGGTACCATGGGGGGCGCGTG
TGGTTACGGAAACTTATACAGCCAAGGGTACGGGGTTAAAACAGCCGCGTTCAGC
AAAGGCCTGGTTAAAAACCGGCTGAGCTGTGGCGCGTGTTTCGAGCTCAAATGTG
CGGATGACCCGAGATGGTGTCACCCGGGCAGCCCGTCTATCCTCATAACCGCTACC
AATTTCTGCCCTCCTAACTTTGCTCAGCCCAGCGACAACGGCGGTTGGTGCAACCCT
CCTCGCCCTCATTTCGACTTGGCCATGCCCATGTTCCTTAAAATCGCCGAGTACCGT
GCTGGTATCGTCCCCGTCGCTTTCCGCCGGGTTCCATGCCGCAAGCAAGGAGGCAT
AAGGTTCACAATAAACGGTCATTCGTACTTCAACTTGGTCCTAGTCACTAATGTCG
GTGGTGCTGGTGACGTCCATGCCGTGTCAATTAAGGGTTCCAGGACCGGGTGGCA
ACCCATGTCACGGAACTGGGGCCAAAACTGGCAA

DcXet 1:
GATGAGCTTGACTTAGAGTTTATGGGCAATGTGAGTGGCCAACCTTACACTCTACA
CACCAATGTGTTTAGTCAAGGCAAAGGCAATAGAGAGCAACAATTTCACCTTTGG
TTTGACCCAACAAAGAACTTTCATACTTATTCCATTGTTTGGAGCCCAAAACTTAT
CATGTTCATGGTGGATGAGACACCAATAAGGGTATTTAGGAACCAAGAGAAGGAC
ATAGGAGTCCCATTTCCAAAGAGCCAACCTATGAAAATCTACTCAAGCATATGGA
ATGCAGATTACTGGGCGACACA

DcXet 2:
GACGAGCTCGACTTTGAGTTCATTGGGAATGTAAGCGGACAACCTTACACAGTCCA
TACCAATGTGTTTAGCCAAGGAAAAGGTGACCGTGAACAACAATTTCACTTGTGG
TTTGACCCTACTCTCAACTTTCACACTTACTCCATTGTTTGGAACACTAGGCTCATC
ATGTTTTTGGTGGACAATACACCAATAAGGGTGTTTAGGAGTAATGAGAACATAG
GCGTACCATTTCCAAAGAACCAGCCTATGAAGATCCAATGCAGCCTATGGGACGCA
GACGCATGGGCAACCCG

Fig. 1: cDNA fragments of *DcExp 1, DcExp 2, DcXet 1* and *DcXet 2* from petals of cut carnation flowers during vase holding. The underlines indicated the positions of degenerate primers for RT-PCR

that *DcExp 2* could participate in senescence progress of cut carnation flowers and exogenous ATP treatment inhibited the expression of *DcExp 2* of the cut flowers at the later stage of vase holding.

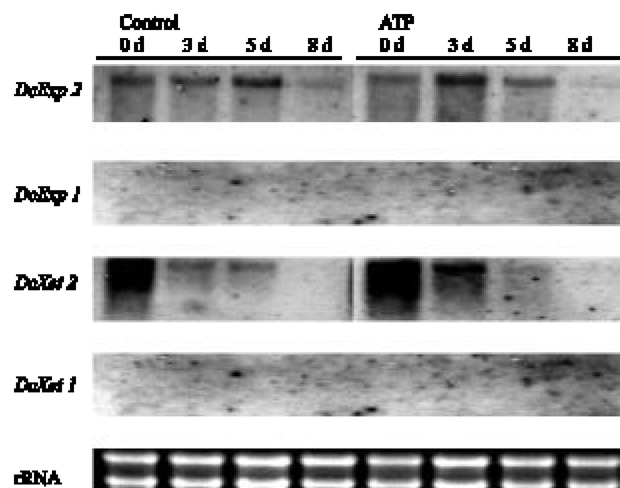

Fig. 2: Changes in mRNA accumulation of *DcExp 1, DcExp 2, DcXet 1* and *DcXet 2* in petals of non-ATP-treated and ATP-treated cut carnation flowers during vase holding

Effects of ATP Supply on Expression of DcXet 1 and DcXet 2

Percy *et al*. (1997) and Ishimaru and Kobayashi (2002) reported that XET gene involved in plant senescence and its expression can be induced during softening and ripening of grape berry and apple fruit. Lu *et al*. (2004) found that an accelerated senescence of harvested banana fruit occurred when XET accumulation enhanced. To examine XET expression in senescence of cut carnation flower, the mRNA accumulation was detected. In this study, no mRNA accumulation of *DcXet 1* was detected either in non-ATP-treated or ATP-treated cut carnation flowers during vase holding (Fig. 2), which suggested that *DcXet 1* was not related to the opening and senescence of the cut flowers. As compared with *DcXet 1*, *DcXet 2* expressed only within the first 5 days in non-ATP-treated cut flowers but within the first 3 days in ATP-treated cut flowers, which suggested that the additional ATP treatment inhibited *DcXet 2* expression at the later stage of vase holding.

In conclusion, in terms of the previous investigation (Song *et al*., 2006) and the apparent senescence characteristic of cut flowers which appeared at a later period of vase holding, this study suggested that the vase life extension of cut carnation flowers by additional ATP treatment was due to the inhibition of expressions of *DcExp 2* and *DcXet 2* at the later stage of vase holding of the cut flowers. Further investigation is required to further understand the differential expressions at protein level of these two genes caused by ATP treatment.

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