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ORIGINAL RESEARCH

# Subcellular Localization and Changes in mRNA Abundance of CEBP, a Nuclear-Encoded Chloroplast Protein, During Flower Development and Senescence

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**Abstract** CEBP, a nuclear-encoded chloroplast protein, has been previously cloned as a putative transcription factor involved in ethylene signaling in carnation development and senescence. In order to more clearly define CEBP role and function, we carried out experiments to define its pattern of mRNA abundance and possible subcellular localization. Changes in CEBP mRNA abundance showed a dramatic drop from anthesis to open flower stage of development immediately preceding the ethylene climacteric associated with flower senescence. A similar but less dramatic decrease in CEBP was observed upon ethylene exposure, again before endogenous climacteric ethylene was observed. The pattern of CEBP mRNA abundance suggests a response to or involvement in the initial steps of the petal senescence process. GFP co-localization showed that a GFP–CEBP construct was directed to the nucleus, whereas a CEBP–GFP construct localized to the chloroplast. Nuclear localization was expected as CEBP was cloned as an ethylene-responsive element-binding protein or transcription factor. The role and function of CEBP in the chloroplast, however, remains unclear. Future lines of inquiry based on our results are discussed.

**Keywords** Carnation · GFP localization · Ethylene · Transcription factor

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## Introduction

In many commercially valuable flowering plants, flower senescence is associated with a significant increase in ethylene production. This climacteric rise in ethylene has been shown to play a regulatory role in the events leading to the demise of the flower, although the trigger for the increase in ethylene production has remained elusive (Borochov and Woodson 1989; Rubinstein 2000; Verlinden 2006). Continued perception of ethylene is necessary to sustain climacteric ethylene production and the expression of several senescence-related (SR) genes (Lawton et al. 1990). One of the tightly ethylene-regulated SR genes, SR8, was found to encode a glutathione *S*-transferase (GST1; Meyer et al. 1991). In subsequent *GST1* work, an ethylene-responsive element (ERE) was found in its promoter. ERE shared a nearly conserved octonucleic sequence, ATTTCAAA, with other EREs of genes from tomato and bean, and the *Arabidopsis ERF* gene (Maxson and Woodson 1996). ERF is an integral part of the transcriptional cascade that leads to ethylene responses (Solano et al. 1998). Interestingly, in the *ERF* promoter, the octonucleic sequence ATTTCAAA is part of a region that has been shown to interact with EILs, transcription factors involved in the upregulation of ethylene sensitive genes

(Solano et al. 1998). *EILs* have been cloned from carnation, and the mRNA abundance of at least one *EIL*, *DC-EIL3*, was shown to increase during flower development and suggested to play a role in triggering flower senescence (Iordachescu and Verlinden 2005; Hoeberichts et al. 2007). Surprisingly, additional work with the ERE region of *GST1* promoter did not lead to cloning of an *EIL*, but to the discovery of the carnation ethylene-responsive element-binding protein (CEBP). CEBP is a putative transcription factor with homology to other plant nucleic acid-binding proteins (Itzhaki et al. 1994; Maxson and Woodson 1996), including significant homology to the maize nucleic acid binding protein, *Arabidopsis* figwort mosaic virus protein and a number of closely related nuclear-encoded chloroplast proteins (Li and Sugiura 1990; Schuster and Gruissem 1991; Ohta et al. 1995; Boyle and Brisson 2001; Haerizadeh et al. 2006).

The predicted CEBP protein is 32 kDa in size and contains two highly conserved RNA-binding motifs, RNP-1 and RNP-2, as well as an acidic region and a putative nuclear localization signal towards the protein C-terminus (Maxson and Woodson 1996). Messenger RNA abundance of *CEBP* was found to be greater at anthesis than in senescent petal tissue, an unexpected result since CEBP was thought to activate *GST1* gene expression during senescence. Although CEBP has a putative chloroplast transit peptide at the N-terminus, the focus of past research and the circumstantial evidence obtained so far indicates nuclear localization of the CEBP protein (Maxson and Woodson 1996). However, most proteins similar to CEBP are nuclear-encoded chloroplast proteins and are believed to have a role in splicing and/or processing of chloroplast RNAs (Li and Sugiura 1990). Additional support for the possible chloroplast localization of CEBP is the fact that petals and corollas of a number of species do indeed contain chloroplasts (Weiss et al. 1988; Vainstein and Sharon 1993; Pyke and Page 1998).

Based on previously described work and observations, we hypothesized that CEBP can locate both to the nucleus and chloroplast. In order to gain insight into the possible function of CEBP in flower development and senescence, we decided to gather some basic information about CEBP and carnation petals in general, including CEBP expression patterns. Therefore, we first set out to characterize changes in *CEBP* mRNA accumulation. Second, we decided to test the possibility that a fusion protein containing both CEBP and green fluorescence protein (GFP) can locate to the chloroplast and/or nucleus. Regulation of CEBP at the transcript level as well as its possible localization to both chloroplast and nucleus could help define future work in studying the role and function of CEBP in petal development and senescence.

## Materials and Methods

### Plant Materials

Carnation plants (*Dianthus caryophyllus* L. cv. Improved White Sim) were grown at the West Virginia University Plant Sciences greenhouses. Plants were grown under standard greenhouse conditions: 21°C (day/night) and 60% to 80% relative humidity. The plants were fertilized with a solution containing 400 mg $L^{-1}$ nitrogen from a 20N/10P/20K complete fertilizer applied once weekly (Peters Fertilizer Inc., Allentown, PA, USA). Flowers from these plants were used for all mRNA abundance studies. For the transient transformation, carnation flowers *D. caryophyllus* L. cv. Yukon White were used.

### Treatments and Ethylene Production

*D. caryophyllus* L. flower life span can be chronologically divided into eight distinct physiological stages: bud, open bud, paintbrush, anthesis, open flower, late open flower, early senescent flower, mid-senescent flower, and late senescent flower. Ethylene production in all floral developmental stages was assessed in order to correlate it with the mRNA abundance (Iordachescu and Verlinden 2005). Petals, ovaries, and styles of eight to 12 flowers from each developmental stage were collected, analyzed for ethylene production, immediately frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction and quantitative PCR (qPCR) analysis.

In order to obtain the tissue of flowers exposed to ethylene, *D. caryophyllus* L. cv. Improved White Sim flowering stems (eight to 12) were collected at anthesis stage of floral development and cut to 25 cm in length. Cut flowers were held in distilled water and placed in a closed 20-L glass jar. Ethylene was injected to a concentration of 10 μL $L^{-1}$ using a hypodermic needle and syringe through the rubber septum located in the lid of the jar. Flowers were exposed to ethylene in the glass jar for 0, 1, 2, 4, 6, 12, and 24 h. Petals, ovaries, and styles from flowers exposed to ethylene were collected separately at each time point and frozen for subsequent RNA extraction and quantitative PCR (qPCR) analysis.

### Quantitative Polymerase Chain Reaction

Total RNA was extracted by previously described protocol (Iordachescu and Verlinden 2005). Total RNA obtained from tissues and organs during floral development and exogenous ethylene treatment was reverse-transcribed according to the protocol provided by Promega Corp (Promega, Madison, WI, USA) for the ImProm-II™ Reverse Transcription System. Quantitative real-time PCR

was performed on an iCycler iQ™ Multi-Color Real Time PCR Detection System (BioRad, Hercules, CA, USA) using "Platinum® SYBR® Green qPCR SuperMix UDG" (Invitrogen, Carlsbad, CA, USA). Fluorescein reference dye added to the master mix was used to determine well factors in the experiments. Well factors were collected at the beginning of each experiment and were used to compensate for system or pipetting non-uniformity in order to optimize fluorescent data quality and analysis.

Actin (internal control) and CEBP primers were designed using PREMIER Biosoft software "Beacon Designer 3" (Premier Biosoft International, Palo Alto, CA, USA). The actin primers consisted of the sequence 5′-GATCTGGCATCATACCTTCTAC-3′ (sense primer) and 5′-ACATACATAGCAGGAGCATTG-3′ (anti-sense primer). The predicted actin PCR product amplified from these primers was 150 bp in length. The CEBP primers consisted of the sequence 5′-TGGCTTCGGCTTTGTCAC-3′ (sense primer) and 5′-CGTCGTGGTCTCTCTTCAG-3′ (anti-sense primer). The predicted *CEBP* PCR product was 126 bp in length. Actin and CEBP primers were tested to ensure their specificity and accuracy. For each reaction, 18 μL of master mix, together with 2 μL of cDNA (corresponding to 1 pg of original total RNA), was vortexed and added to the 96-well plate creating a final reaction volume of 20 μL. The PCR plate was then inserted in the iCycler and run with a three-step cycling program. The first step was a 2-min hold at 50°C followed by a second step 2-min hold at 95°C. The third step consisted of 45 cycles at the following conditions: 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. All experiments were performed three times. All actin and CEBP qPCR reactions were replicated three times.

### Amino Acid Alignment

In order to identify sequences similar to CEBP and generate a comparative alignment, we searched GenBank databases using the NCBI blast feature (http://blast.ncbi.nlm.nih.gov). An amino acid alignment was generated using the ClustalW multiple sequence program (http://www.ebi.ac.uk, Thompson et al. 1994) with any complete sequence we obtained that was associated with a peer-refereed article.

### Statistical Analysis

Data were analyzed by analysis of variance using the SAS software (Statistical Analysis System, Cary, NC, USA). Tukey's studentized range (HSD) multiple comparison procedure was utilized when appropriate. Effects were considered significant if the *P* value was less than 0.05.

### CEBP Subcellular Localization Assay

In order to elucidate the possible cellular localization of CEBP, CEBP was tagged with GFP. A plasmid (pAVA319) containing GFP was purchased from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, Columbus, OH, USA). The vector contains a dual 35S promoter from the cauliflower mosaic virus (CaMV), a translational leader (TL) sequence from tobacco etch virus (TEV), a GFP coding sequence, and a 35S transcriptional terminator from CaMV (von Arnim et al. 1998). The vector also carries an ampicillin resistance gene. Two constructs were generated in the pAVA319 vector. Since the nuclear localization signal and chloroplast targeting peptide are present at the C-terminus and N-terminus of the CEBP protein, respectively, two chimeric genes were constructed in the eventuality that GFP would interfere with subcellular GFP targeting. The first construct was created by inserting *CEBP* in frame and downstream of *GFP* into the *Bgl*II–*Xba*I restriction site, resulting in a *GFP–CEBP* construct. The second construct was created by inserting *CEBP* in frame and upstream of *GFP* into the *Nco*I restriction site, resulting in a *CEBP–GFP* construct. The constructs were completely sequenced in both directions to determine integrity and correct reading frame (Davis Sequencing Inc., Davis, CA, USA). Particle bombardment with the two previously described constructs was performed on plants representing three stages of flower development: open bud, anthesis, and mid-senescent flower (after the petals entered the climacteric stage), and for both the lower and upper parts of the petals.

Plasmid DNA containing the GFP fusion proteins was bound to 1.0 μm gold particles according to the manufacturer's instruction (BioRad Laboratories). The DNA-covered gold particles were bombarded into carnation petals with a PDS-1000 biolistic delivery system (BioRad Laboratories) with a rupture setting of 1,100 psi. Bombarded petals were maintained in the dark for 16 h at 25°C and subsequently placed on glass slides and analyzed for the presence of the GFP green fluorescence with an FW4000 fluorescence imaging workstation (Leica, Cambridge, UK).

## Results

### CEBP mRNA Accumulation

Carnation flowers used in our experiments showed a normal climacteric pattern of ethylene production and helped us to define more clearly the flower stages of development (Fig. 1). *CEBP* transcript levels in petal tissue (Fig. 2) increased significantly from bud to anthesis stage

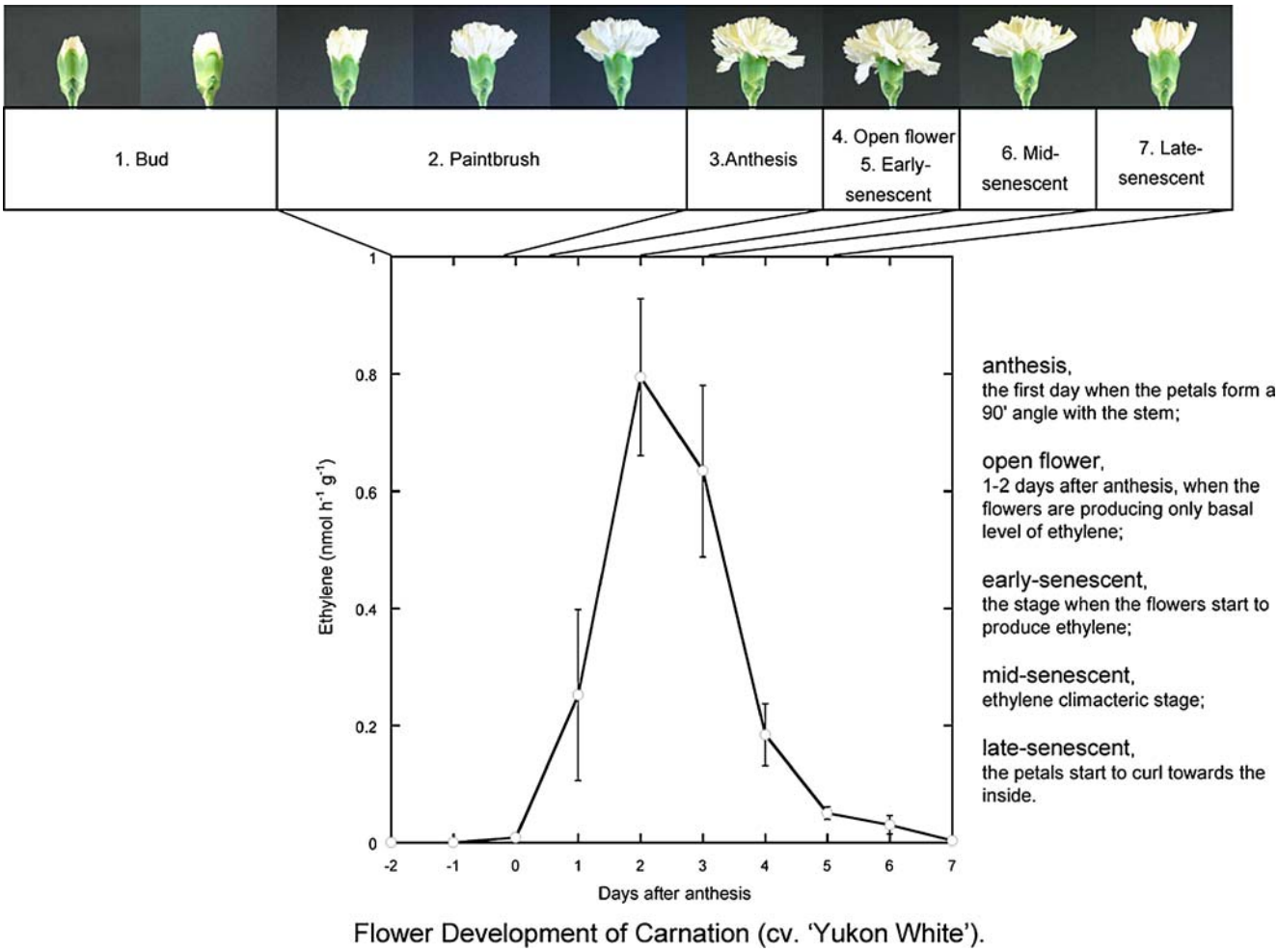


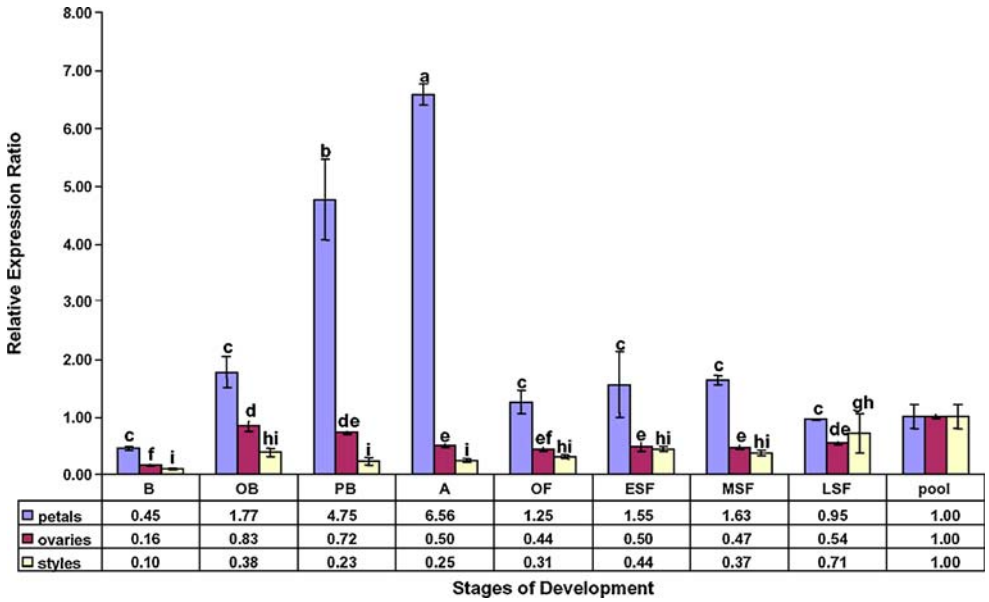

**Fig. 1** Ethylene production of "Yukon White" carnation petals throughout flower development. Carnation flowers were harvested at the following stages of development: bud, paintbrush, anthesis, open flower, early senescent flower, mid-senescent flower, and late senescent flower. *Error bars* represent standard errors of three replicates

of development with a relative accumulation ratio of 0.45 and 6.56, respectively. Between anthesis and open flower stages of development, a major decrease in mRNA abundance was observed, from 6.56 to 1.25. From open flower stage of development through late senescence, transcript levels remained low. Transcript levels of CEBP in both ovaries and styles were lower than the levels observed in the control pool sample throughout development. There was a significant increase in *CEBP* mRNA abundance from bud stage (0.16) to open bud stage of development (0.83) in ovary tissue. Thereafter, mRNA abundance decreased to 0.50 in the anthesis stage of development flowers and remained at low levels through senescence. In styles, *CEBP* mRNA abundance increased significantly from bud (0.10) to open bud (0.38) after which no significant changes were observed.

During ethylene treatment of flowers (Fig. 3), *CEBP* mRNA accumulation decreased in petals from 2.22 at 2 h to 0.32 in the 4-h sample. After this point, *CEBP* transcript levels remained low. In the ovaries, mRNA abundance of *CEBP* decreased dramatically from 1.73 after 2 h of ethylene treatment to 0.55 after 4 h and remained at this level until 12 h into the ethylene treatment. A significant increase was observed at 24 h, returning *CEBP* mRNA levels in ovaries close to initial mRNA abundance levels (1.24). *CEBP* transcript levels in styles increased from an initial value of 0.36 to 1.16 after 1 h of ethylene treatment, followed by a considerable decrease to 0.05 and 0.04, after 4 and 6 h, respectively. *CEBP* mRNA levels returned to initial levels after 12 h of ethylene treatment (0.40).

## CEBP Alignment of a Family of Nuclear-Encoded Chloroplast Protein

We identified over 100 amino acid sequences with some amino acid similarity to CEBP by searching GenBank (http://blast.ncbi.nlm.nih.gov) databases. After filtering through those sequences by excluding partial sequences



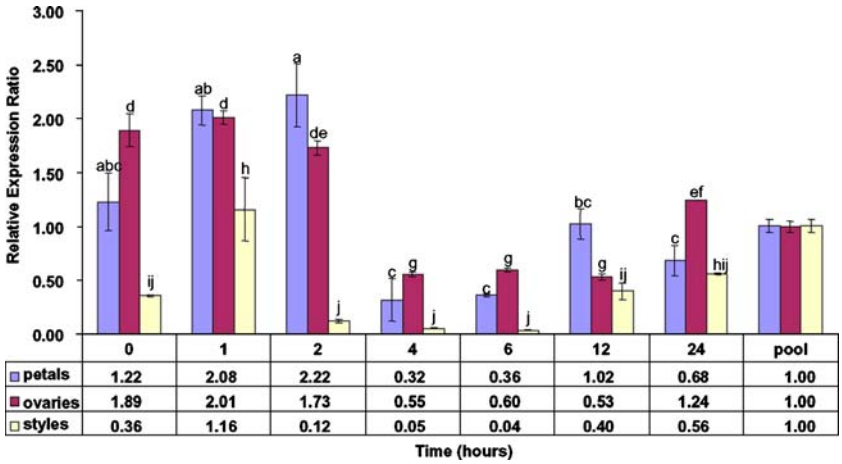

| | B | OB | PB | A | OF | ESF | MSF | LSF | pool |
|---|---|---|---|---|---|---|---|---|---|
| petals | 0.45 | 1.77 | 4.75 | 6.56 | 1.25 | 1.55 | 1.63 | 0.95 | 1.00 |
| ovaries | 0.16 | 0.83 | 0.72 | 0.50 | 0.44 | 0.50 | 0.47 | 0.54 | 1.00 |
| styles | 0.10 | 0.38 | 0.23 | 0.25 | 0.31 | 0.44 | 0.37 | 0.71 | 1.00 |

**Fig. 2** *CEBP* mRNA abundance throughout flower development in petals, ovaries, and styles. Petals, ovaries, and styles were collected from carnation flowers harvested at bud (*B*), open bud (*OB*), paintbrush (*PB*), anthesis (*A*), open flower (*OF*), early senescent flower (*ESF*), mid-senescent flower (*MSF*), and late senescent flower (*LSF*) stages of development. Pool indicates the control sample (equal quantity of mRNA from every sample in development, ethylene, and wounding treatments). *Error bars* represent standard errors of three replicates. *Letters above bars* represent statistical differences at alpha =0.05, according to Tukey's studentized range test. Statistical analysis was done separately for each flower part. *Means with the same letter* are not significantly different

and only including sequences associated with peer-reviewed publications, we continued with 11 sequences that met our criteria. Those 11 sequences were used to align with CEBP sequence in the ClustalW multiple sequence alignment program (http://www.ebi.ac.uk; Thompson et al. 1994; Fig. 4). The sequences contain a putative transit peptide (T), an acidic region (A), two RNA-binding domains (RI and RII), and a putative nuclear localization signal (N). However, *Lilium* GRSF has a shorter sequence, lacking the chloroplast transit peptide and the acidic region.

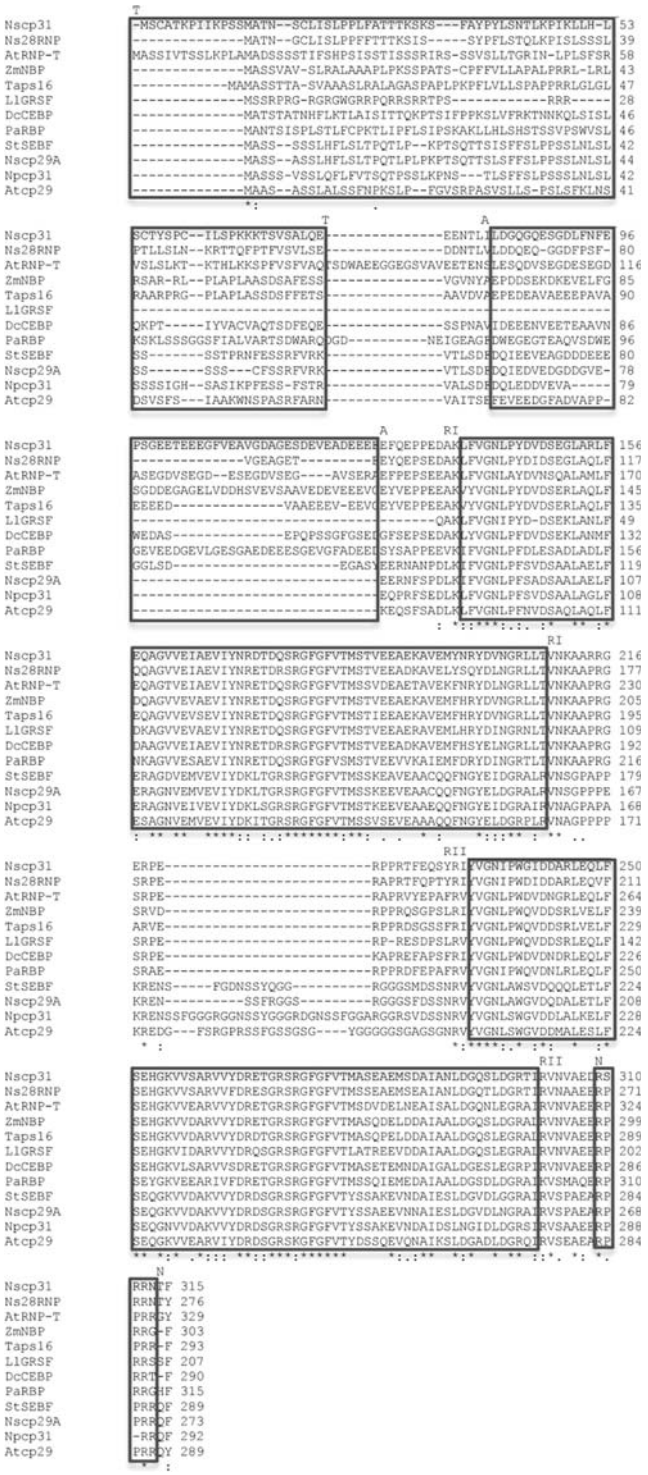
## Subcellular Localization of CEBP

Since CEBP contains putative signals for both plastid and nuclear localization, subcellular localization of CEBP was determined by transient expression of GFP-fused protein in




| | 0 | 1 | 2 | 4 | 6 | 12 | 24 | pool |
|---|---|---|---|---|---|---|---|---|
| petals | 1.22 | 2.08 | 2.22 | 0.32 | 0.36 | 1.02 | 0.68 | 1.00 |
| ovaries | 1.89 | 2.01 | 1.73 | 0.55 | 0.60 | 0.53 | 1.24 | 1.00 |
| styles | 0.36 | 1.16 | 0.12 | 0.05 | 0.04 | 0.40 | 0.56 | 1.00 |

**Fig. 3** *CEBP* mRNA abundance in petals, ovaries, and styles of flowers treated with 10 μL $L^{-1}$ ethylene for 0, 1, 2, 4, 6, 12, and 24 h. Pool indicates the control sample (equal quantity of mRNA from every sample in development and ethylene treatment). *Error bars* represent standard errors of three replicates. *Letters above bars* represent statistical differences at alpha=0.05, according to Tukey's studentized range test. Statistical analysis was done separately for each flower part. *Means with the same letter* are not significantly different




◀ **Fig. 4** Sequence of CEBP and alignment with members of a family of nucleus-encoded chloroplast proteins. The following sequences were chosen for sequence comparison: Nscp31 (*Nicotiana sylvestris*, X57079), Ns28RNP (*Nicotiana sylvestris*, X53933), AtRNP-T (*Arabidopsis thaliana*, X65255), ZmNBP (*Zea mays*, Z11488), Taps16 (*Triticum aestivum*, D38485), LiGRSF (*Lilium longiflorum*, DQ507850), DcCEBP (*Dianthus caryophyllus*, U38483), PaRBP (*Persea americana*, AJ421781), StSEBF (*Solanum tuberosum*, AF389431), Nscp29A (*Nicotiana sylvestris*, Q08935), Npcp31 (*Nicotiana plumbaginifolia, X65117), and Atcp29 (Arabidopsis thaliana, U08467). The putative transit peptide (T), the acidic region (A), the nuclear localization signal (N), and the RNA-binding domains (RI and RII) are boxed*

carnation petals. Both C-terminal and N-terminal GFP fusion constructs were produced and bombarded into the upper and lower parts of the petal tissue. In the upper part of the petals in the anthesis and mid-senescence stage of flower development, GFP signal was observed within structures similar in size and number to chloroplasts (Fig. 5a). The lower part of the petals displayed similar pattern of GFP signal. Since the lower part of the petals contains some green tissue, the GFP signals were merged with the autofluorescence of chloroplasts. The co-localization of the signals demonstrated that CEBP–GFP localizes in chloroplasts. Some chloroplast fluorescence was observed in the upper petals of bud stage of development flowers and merged images confirmed the localization of CEBP–GFP to chloroplast in bud stage of development flowers (data not shown). In contrast, when petals were transformed with the *GFP–CEBP* construct, GFP fluorescence was associated with the nucleus, in both the lower and upper part of the petals (Fig. 6). A similar pattern of GFP green fluorescence was observed in petals in all stages of flower development studied (data not shown).

## Discussion

CEBP is a 32-kDa protein capable of binding to the promoter of *GST1*, an *SR* gene that is activated transcriptionally by ethylene at the onset of the senescence program in carnation petals (Maxson and Woodson 1996). Maxson and Woodson (1996) observed a dramatic decrease in petal *CEBP* mRNA abundance from day 0 (anthesis) to day 6 (senescence). The work presented here examined *CEBP* mRNA levels in much more detail, showing that *CEBP* transcript increased gradually from bud to anthesis stage of development then decreased abruptly from anthesis to open flower stage of development (Fig. 2), an observation that has not been made for any previously characterized carnation *SR* genes (Verlinden et al. 2002). A similar drop in *CEBP* transcript accumulation in petals was observed during the ethylene treatment of flowers, between 2 and 4 h time points, contradicting the conclusion of Maxson and Woodson (1996) that *CEBP* transcript levels are not influenced by ethylene treatment. The limited data set in their study may have led to an erroneous conclusion.

The unique and sudden drop in *CEBP* transcript from anthesis to open flower stage of development leads to the hypothesis that a threshold of unknown origin is reached at this stage of flower development. The only other change

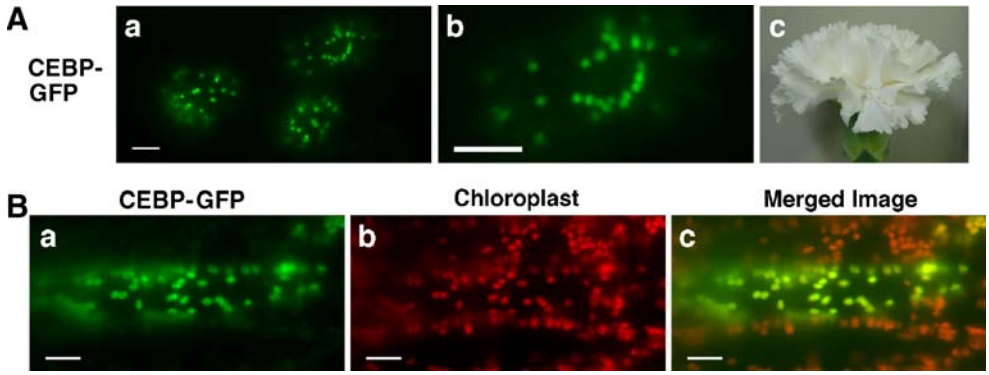



**Fig. 5** Transient transformation of carnation petals from early senescent flower stage of development with *CEBP–GFP* construct. **A** *Upper part* of the petals. *a*, *b* GFP fluorescence. No chloroplast autofluorescence was detected. *c* Carnation flower in early senescent flower stage of development. **B** *Lower part* of the petals. *a* GFP fluorescence. *b* Chloroplast autofluorescence. *c* Merged images from *a* and *b*. *Bars* in each photograph represent 10 μm

reminiscent of the change in *CEBP* mRNA accumulation is the sudden accumulation of *DC-EIL3* during carnation petal development (Iordachescu and Verlinden 2005). Furthermore, CEBP and EILs have been shown to bind very similar promoter regions (Maxson and Woodson 1996; Solano et al. 1998). *DC-EIL3* is a carnation EIL whose transcript level increases during flower development and following ethylene treatment. *DC-EIL3* mRNA started to accumulate at open flower stage of development, the exact same stage of development at which we observed the dramatic decrease in *CEBP* mRNA levels (Iordachescu and Verlinden 2005). Future research will be directed towards understanding the role of CEBP and EILs, at the protein level, and through in vivo studies, in controlling ERF and SR gene expression.

CEBP transient transformation experiments demonstrated that CEBP could localize to either the nucleus or chloroplasts/plastids. After transformation with the *CEBP–GFP* construct, GFP green fluorescence was detected in chloroplasts. Localization to chloroplasts/plastids was most likely the result of the unavailability of the nuclear localization signal to properly direct the fusion protein to the nucleus. The opposite phenomenon was observed when carnation petals were transformed with *GPP–CEBP* construct. In this case, green fluorescence was found only in the nucleus. Presumably, GFP fused to the amino terminus masks the chloroplast transit peptide, resulting in a lack of GFP–CEBP fusion protein localization to the chloroplasts (Tian et al. 2004).

When the upper part of the petals from carnation flowers at anthesis and senescent flower stage of development are

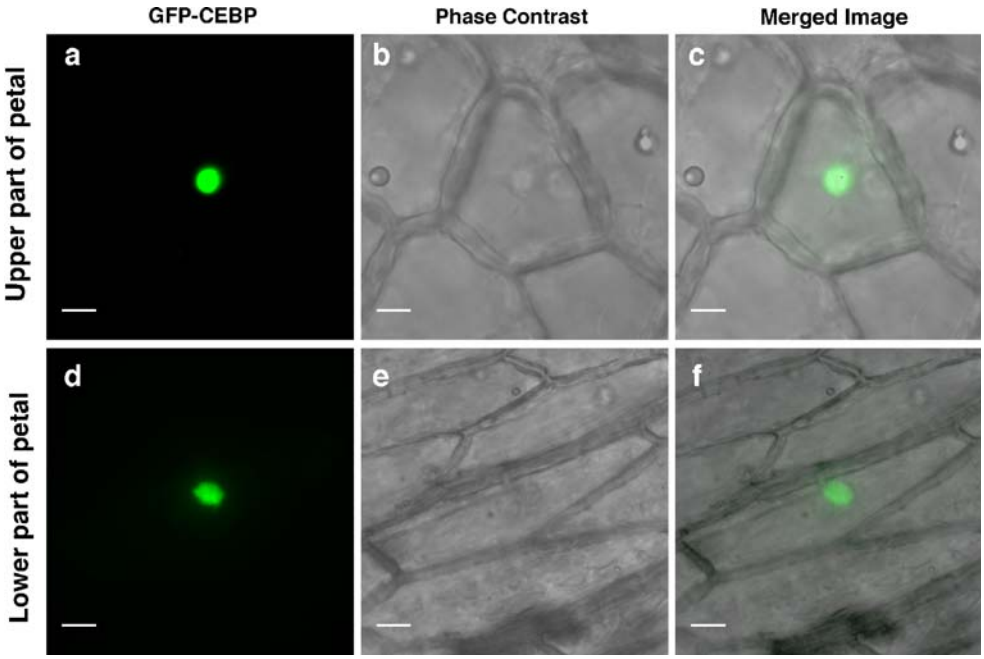



**Fig. 6** Transient transformation of carnation petals from early senescent flower stage of flower development with the *GFP–CEBP* construct. *First column*: GFP fluorescence. *Second column*: phase contrast. *Third column*: merged images from the *first and second column*. The *first row* (**a**–**c**) represents the upper part of the petals. The *second row* (**d**–**f**) represents the lower part of the petals. *Bars* in each photograph represent 10 μm

transformed with the *CEBP–GFP* construct, GFP green fluorescence was observed in cell structures similar to chloroplasts in size and number. However, at this point, no chloroplast autofluorescence was detected. Therefore, no merged pictures could be generated (Fig. 6). Pyke and Page (1998) reported in *Arabidopsis* that as the flowers matured, in the upper part of the petals, chloroplasts redifferentiated into leukoplasts, which were no longer visible with the confocal microscope. However, leukoplasts were visible with an electron microscope. Since at anthesis and mid-senescent stages of flower development, no chloroplasts were detected in the upper petals, we presume that CEBP–GFP can also localize to plastids.

Our results indicate that GFP–CEBP can locate to the nucleus in carnation petal cells. Proteins that have amino acid sequences similar to CEBP have been identified in different species, including spinach, *Arabidopsis*, tobacco, potato, and corn (Schuster and Gruissem 1991; Didier and Klee 1992; Li and Sugiura 1990; Boyle and Brisson 2001; Cook and Walker 1992) and are part of a family of chloroplast-localized nuclear-encoded proteins. Most of these proteins have been shown to be present in chloroplasts, where they have a putative role in splicing/processing and stabilization of chloroplast RNA 3′ ends (Li and Sugiura 1990; Schuster and Gruissem 1991). For some of these proteins, truncated versions missing the N-terminus plastid transit peptide have been observed. These truncated versions were postulated to localize in other subcellular locations besides chloroplasts (Ohta et al. 1995; Didier and Klee 1992; Cheng et al. 1994). Most of the proteins with amino acid sequences similar to CEBP contain the RPRR nuclear localization sequence at the C-terminus. Some of those proteins are thought to operate in the nucleus (Takase et al. 1995; Boyle and Brisson 2001; Haerizadeh et al. 2006). One of these proteins, SEBF, is the only member of the abovementioned group of proteins that has been demonstrated by subcellular partitioning to locate both in the chloroplast and nucleus (Boyle and Brisson 2001). SEBF was shown to function in the nucleus as a transcriptional repressor, by regulating the expression of pathogenesis related *PR-10a* gene (Boyle and Brisson 2001). However, the function of SEBF in the chloroplast remains unclear. Another protein closely related to CEBP, GRSF, is involved in gene silencing (Haerizadeh et al. 2006). Other nuclear-encoded chloroplast proteins have not been studied beyond changes in mRNA abundance, leaving open the possibility that additional members of this family may operate both in the chloroplast and nucleus and be involved in gene silencing.

To summarize, the present study first examined, in detail, *CEBP* mRNA accumulation patterns throughout carnation flower development and following ethylene exposure. Secondly, CEBP transient transformation of carnation petals confirmed nucleus and chloroplasts as a destination for CEBP protein, supporting CEBP function as a transcription factor. Although the results of this work shed some light on the involvement of CEBP as a putative transcription factor in flower development, its precise function remains unclear. Future lines of research will focus on investigating the role of CEBP in flower senescence, including the possibility that CEBP may function as a repressor or silencer of SR gene expression and perhaps flower senescence as a developmental process.

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