

Title Ethylene signaling during flower development and senescence in carnations (*Dianthus caryophyllus* L.)

Author Mihaela Iordachescu and Sven Verlinden

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Abstract

The plant hormone ethylene plays an important role in numerous plant growth and developmental processes, including flower development and senescence. Once perceived by ethylene receptors, the ethylene signal is transduced through a series of components until it reaches its ultimate targets (e.g. senescence-related (*SR*) genes). EIN3, a transcription factor and positive regulator of the ethylene signaling pathway, most likely affects these *SR* genes. Using a combination of approaches, three *EIN3-like* (*EIL*) cDNAs, *DC-EIL1/2* (AY728191), *DC-EIL3* (AY728192) and *DC-EIL4* (AY728193), were isolated from carnation (*Dianthus caryophyllus* L.) petals. The cloned cDNAs share a high amino acid identity among each other and with previously cloned EILs. *DC-EILs* transcript analysis performed on vegetative and flower tissues (petals, ovaries and styles) during growth, development, and senescence (natural and ethylene-induced) indicated that the mRNA accumulation of the *DC-EIL* family of genes in carnation is regulated developmentally and by ethylene. Especially *DC-EIL3* mRNA showed considerable accumulation upon ethylene exposure, during flower development, and upon pollination in petals and styles. Interestingly, decreasing levels of *DC-EIL3* mRNA were found in wounded leaves and ovaries of senescing flowers whenever ethylene levels increased. Flowers treated with sucrose showed a two-day delay in the accumulation of *DC-EIL3* transcripts when compared to control flowers. These observations suggest an important role for *DC-EIL3* during growth and development. Changes in *DC-EIL1/2* and *DC-EIL4* mRNA levels during flower development, and upon ethylene exposure and pollination were very similar, but less dramatic than changes in *DC-EIL3* transcript levels. mRNA levels of the *DC-EILs* in styles of pollinated flowers showed a clear relationship with ethylene production after pollination. The characterization of the *EIN3-like* genes in carnation indicated in the present study showed transcriptional regulation not previously observed for *EILs* in other plant species.

CEBP, a nuclear encoded chloroplast protein and putative repressor of ethylene signaling, most likely regulates the transcription of *SR* genes containing an Ethylene Response Element (ERE) by preventing transcription until certain developmental conditions are met. The characterization of changes in

CEBP mRNA levels in flowers (petals, ovaries and styles) during flower development and senescence (natural and ethylene induced), as well as in leaves following wounding, indicated that *CEBP* is down-regulated developmentally and by ethylene. Interestingly, during flower development and senescence (both natural and ethylene-induced), *DC-EIL3* transcript started to accumulate at the same point *CEBP* transcript levels decreased. *CEBP* transcript levels decreased dramatically after anthesis, as opposed to the gradual decrease throughout development of the petal chlorophyll content and chloroplast number. Furthermore, transient transformation of carnation petals by particle bombardment with *GFP* -tagged *CEBP* indicates that *CEBP* can be localized to both chloroplasts and nuclei.

Based on the above-mentioned results, as well as previous findings, a novel mechanism that regulate ethylene signaling regulation was proposed. In this model, early in petal development, when chloroplasts are numerous, chloroplast-located *CEBP* plays a role in processing and/or stabilizing chloroplast RNA, whereas nucleus-located *CEBP* acts as a repressor of *SR* genes. When developmental changes initiate chloroplast degradation as part of developmental processes associated with aging, *CEBP* levels decrease, possibly through a negative feedback loop. Lower levels of *CEBP* allow the promoters of the *SR* genes to become available for activation by *DC-EIL3*, initiating the first steps of the petal senescence process.