Gene Note

Cloning of a cDNA encoding EIN3-like protein (DC-EIL1) and decrease in its mRNA level during senescence in carnation flower tissues

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Abstract

A cDNA clone encoding a putative EIN3-like protein (DC-EIL1) was obtained from total RNA isolated from senescing carnation (Dianthus caryophyllus L.) petals using RT-PCR and RACE techniques. The cDNA (2382 bp) contained an open reading frame of 1986 bp corresponding to 662 amino acids. The amino acid sequence of the N-terminal half of the protein, ranging from 80–300 amino acid residues, had 84% identity with that of the corresponding regions of Arabidopsis EIN3 and tobacco TEIL, although the overall identity was 49% and 52%, respectively. Northern blot analysis revealed that the amount of mRNA corresponding to DC-EIL1 decreased in flower tissues, especially in petals, during natural senescence and senescence induced by exogenously applied ethylene or ABA.

Key words: Carnation, EIN3-like protein, ethylene signalling, flower senescence.

Since the isolation of an ethylene receptor gene ETR1 (Chang et al., 1993), several genes encoding components involved in the ethylene signalling pathway have been identified in Arabidopsis to date (Johnson and Ecker, 1998; Kieber, 1997). These genes include those for other ethylene receptors, CTR1, EIN2 and EIN3 (ETHYLENE-INSENSITIVE3) and its homologues. EIN3 and its homologues, EIL1-3, are located at the most downstream position of the signalling pathway and act as a transcription-activation factor in Arabidopsis (Chao et al., 1997). Most recently, TEIL (tobacco EIN3-like) was identified and functionally characterized as an EIN3-like protein in tobacco plants (Kosugi and Ohashi, 2000).

Ethylene plays a crucial role in the senescence of carnation flowers. During natural- and pollination-induced senescence of the flowers, ethylene is first produced in the pistil and the evolved ethylene acts on petals and induces the expression of genes for ACC (1-aminocyclopropane-1-carboxylate) synthase, ACC oxidase and cysteine proteinase, resulting in the auto-catalytic ethylene production from the petals and wilting of the petals (ten Have and Woltering, 1997; Jones and Woodson, 1999; Shibuya et al., 2000). Also, exogenous ethylene applied to carnation flowers, which have not yet started ethylene production, induces autocatalytic ethylene production in petals, resulting in wilting of the petals (Borochov and Woodson, 1989; Wang and Woodson, 1989; ten Have and Woltering, 1997; Shibuya et al., 2000). Thus, carnation petals are a useful material for the study of ethylene perception and its signalling during flower senescence. Genes have been identified for DC-ERS2 (Accession No. AF034770), DC-CTR1 (Accession No. AF261147) and DC-CTR2 (Accession No. AF261148) and DC-EIL1, which are counterparts in carnation of the respective genes in Arabidopsis. Changes in the amount of mRNAs have recently been analysed for ethylene receptors, DC-ERS2, DC-ERS1 (Chang et al., 1997) and DC-ETR1 (Nagata et al., 2000), in relation to opening and senescence of carnation flowers (K Shibuya et al., unpublished results). A cDNA for carnation EIN3-like gene, DC-EIL1, and changes in its mRNA levels in flower tissues during senescence is described here.

A partial-length cDNA (470 bp) was amplified by PCR with total RNA obtained from senescing carnation (Dianthus caryophyllus L. cv. Nora) petals and primers derived from Arabidopsis EIN3. The sequence of primers were 5′-CAA-GATGGGATCTTTGAAGTATAT-GTTGAA-3′ (corresponding to the amino acid residues 96–104 of EIN3 protein) for the upstream primer and 5′-AABCACGCGACTTCTCCAGCCT-TCTT-3′ (corresponding to the amino acid residues 244–251 of EIN3 protein) for the downstream primer (Chao et al., 1997). The PCR product was cloned into pBluescript II (Stratagene) for sequencing. Using the nucleotide sequence, sequence specific primers were designed. Then, the upstream cDNA of 377 bp was obtained by 5′-RACE (rapid amplification of cDNA ends; Frohman et al., 1988) and the downstream cDNA of 1672 bp by 3′-RACE with the sequence specific primers. The three partial cDNAs were reconstituted to make a composite cDNA. Finally, a full-length cDNA of 2382 bp was amplified with primers derived from both ends of the composite cDNA and the total RNA as a template and designated as DC-EIL1 (Accession No. AF261654).

The cDNA is 2382 bp long and contains a 1986 bp open reading frame, a 39 bp 5′-flanking sequence and a 357 bp 3′-flanking sequence with a polyA tract. The predicted protein consisted of 662 amino acids and had a calculated molecular mass of 74.2 kDa. The N-terminal half of the deduced protein had higher similarity to the corresponding regions of EIN3 in Arabidopsis and TEIL in tobacco than its C-terminal half to the corresponding regions of the latter two (Fig. 1). In particular, the amino acid sequence of the N-terminal half of the protein, ranging from 80–300 amino acid residues, had 84% identity with that of the corresponding regions of Arabidopsis EIN3 and tobacco TEIL, although the overall identity was 49% and 52%, respectively. The DNA-binding domain is localized in the N-terminal half of TEIL (Kosugi and Ohashi, 2000). DC-EIL1 had a highly acidic region at the N terminus, a proline-rich region.

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and small clusters of basic amino acids in the N-terminal half, as observed with EIN3 (Chao et al., 1997). Moreover, the DC-EIL1 had a single mono-amino acid repeat composed of five glutamine residues in the C-terminal half of amino acid residues 613–617. Chao et al. suggested that these motifs act as possible transcriptional activation domains in EIN3/EILs (Chao et al., 1997). DC-EIL1 had a lysine residue at position 246, which is conserved in EIN3 and TEIL. In Arabidopsis, this lysine residue is responsible for the ein3-3 mutation, which renders the mutant insensitivity to ethylene (Chao et al., 1997). Changes in the DC-EIL1 mRNA level were monitored by Northern blot analysis with a probe for DC-EIL1 mRNA and mRNAs isolated from petals, ovaries and styles of carnation flowers, which underwent natural senescence or senescence induced by exogenously-applied ethylene or ABA (Fig. 2). During natural senescence of the flower, ethylene production started to increase 5 d after the full opening of the flower (day 0) and peaked on day 6. In carnation flowers treated with exogenous ethylene on day 0, autocatalytic ethylene production started 4 h after the application of ethylene, and increased thereafter up to 24 h. At 24 h, the flower petals had wilted. ABA treatment advanced the onset of ethylene production by 2 d, and ethylene production reached a maximum on day 4, resulting in petal wilting. In agreement with the increase in ethylene production, the mRNA levels for ACC synthase and ACC oxidase in the petals and ovaries were increased. In Fig. 2, however, only the results for ACC oxidase mRNA in petals are shown.

The probe for DC-EIL1 mRNA was constructed by amplification by PCR with appropriate primers of a cDNA fragment of 470 bp, corresponding to the positions 328–797 of the DC-EIL1 cDNA, followed by labelling with 32P. The probe detected one mRNA of about 2.8 kbp. However, this did not always rule out the possible hybridization with mRNAs for unidentified EILs, if any, other than DC-EIL1 in carnation
plants since the probe was derived from the conserved coding region of DC-EII1 cDNA.

The amount of DC-EII1 mRNA decreased in petals during natural senescence of the flower, except for the amount on day 4 that was higher than that of the other time points from day 2 to day 6 (Fig. 2A). In ovaries and styles the mRNA level did not decrease during senescence, rather it increased slightly in the former at the later stage of senescence. Ethylene decreased the level of mRNA in both petals and styles; the decrease was evident 8 h or 12 h, respectively, after the start of ethylene treatment (Fig. 2B). ABA treatment increased transiently the level of mRNA in petals, the maximum level being attained on day 2, and decreased it at a later stage of senescence (days 4 and 5) (Fig. 2C). ABA also decreased the level of mRNA in ovaries (day 4). By contrast, a small amount of mRNA was accumulated after treatment with ethylene or ABA in styles. The accumulation of mRNA in styles was not investigated further. The DC-EII1 mRNA was present in leaves and stems in addition to flower tissues of carnation plants at the full-opening stage (Fig. 2D). The mRNA level was higher in the ovary and stem tissues than in the other tissues.

The present findings indicated that the level of DC-EII1 mRNA decreased in flower tissues, especially in petals, of carnation flowers during natural senescence or senescence stimulated by exogenous ethylene or ABA. Since ethylene production increased during natural senescence and after application of ABA, and since exogenous ethylene lowered the mRNA level, it is highly probable that ethylene is responsible for the decrease in mRNA. Thus, the present observation is in marked contrast to the previous findings that ethylene did not affect the level of EIN3 mRNA in Arabidopsis (Chao et al., 1997) and that of TEIL in tobacco (Kosugi and Ohashi, 2000). However, an alternative interpretation is that some unidentified senescence factor(s) other than ethylene might cause the decrease in the level of DC-EII1 mRNA.

In a separate experiment, a decrease was found in the amount of mRNAs for ethylene receptors, DC-ERS2 and DC-ETRI, in petals of carnation flowers during senescence (K Shibuya et al., unpublished results). Since ethylene receptors are a negative regulator of ethylene response, the decrease in the amount of mRNAs and, in turn, proteins, of ethylene receptors in carnation petal tissues would increase the sensitivity to ethylene of the tissues, resulting in the enhanced ethylene response in the tissues. By contrast, since EIN3 and its homologues are shown to act as a positive regulator of ethylene response (Chao et al., 1997), it is thought that the decrease in the mRNA levels of DC-EII1 and, in turn, proteins would decrease the ethylene response of the petal tissues. Taken together, the concomitant decrease in mRNA levels for ethylene receptors and DC-EII1 appears to cause a multiplex regulation, i.e. coarse and fine tuning, of ethylene response in senescing carnation petals.

References


