Title Ethylene regulates programmed cell death (PCD) associated with petal senescence in carnation flowers

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Abstract

Carnation (*Dianthus caryophyllus*) flowers are highly sensitive to ethylene. The characteristics of programmed cell death (PCD) and the involvement of ethylene on PCD associated with petal senescence in carnation (*Dianthus caryophyllus* cv. Barbara) flowers were investigated. The number of "nuclei with decreased DNA", the changes in nuclear morphology and DNA degradation were used as markers of PCD in petals. The number of nuclei with decreased DNA in the petals, as determined by flow cytometry, increased with the petal wilting, which was accompanied by an increase in ethylene production. Microscopic observation of the nuclei showed chromatin fragmentation within the nuclei, suggesting that nuclear fragmentation did not occur in the petals. Agarose gel electrophoresis revealed the progression of DNA degradation in the petals. Exposure to ethylene accelerated the onset of chromatin fragmentation and DNA degradation. The application of aminoethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis, and silver thiosulphate complex (STS), an inhibitor of ethylene action, delayed petal senescence and suppressed progression of PCD. In particular, chromatin fragmentation in the petals was rarely observed in STS treatment. Continuous treatment with cycloheximide (CHI), an inhibitor of protein synthesis, suppressed progression of PCD and markedly delayed flower senescence as did AVG and STS. CHI treatment suppressed the climacteric increase in ethylene production in the flower. Expression of an ACC synthase gene, *DC-ACS1*, and an ACC oxidase gene, *DC-ACO1*, in the petals was also suppressed by CHI treatment. These findings suggest that PCD-associated with petal senescence in carnation is highly regulated by ethylene.