Expression of DC8 is associated with, but not dependent on embryogenesis

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Abstract

DC8 is a late embryogenesis-abundant (LEA) protein gene isolated from carrot (Daucus carota). Deletion analysis of the DC8 promoter was performed to determine the sequences required for ABA and seed-specific regulation of DC8 transcription. To investigate the mechanism of DC8 expression during seed development, chimeric gene constructs containing DC8 promoter fragments fused to a promoterless beta-glucuronidase gene (DC8:GUS) were introduced into carrot, tobacco (Nicotiana tabacum) and Arabidopsis thaliana plants. Seed-specific DC8 expression patterns was conserved among the three plant species. However, differences among the species in the patterns of DC8 expression in the embryo and endosperm that correlated with differences in the rates of embryo and endosperm growth were found. Lack of correspondence between DC8 activation and embryo development among the seeds of the three species suggests that DC8 expression, which is associated with seed maturation, is not coupled to the embryo development program. The presence of DC8 activity in carrot callus and endosperm is consistent with the notion that DC8 expression is independant of embryo morphogenesis. A similar DC8 activity time-course during callus induction and seed development suggests that explantation and 2,4-D treatment initiates a course of events similar to that in the carrot ovule. After fertilization, two pathways one leading to embryo development and another to seed maturation are initiated, but they are not closely linked. As a result we find DC8, part of the maturation program, being activated at different embryonic stages in different plant species.

Introduction

After double fertilization, seed development begins with endosperm and embryo growth and differentiation. During subsequent seed maturation the seed storage proteins, starch, and lipids accumulate before the seed undergoes desiccation and dormancy [34]. It is characteristic of late embryo development for both the endosperm and mature embryo to express seed storage reserve genes [13]. Many other hydrophillic proteins with unknown functions that share similar sequence repeats also accumulate in mature embryos and endosperm. They are called late embryogenesis-abundant (LEA) proteins and are thought to be involved in seed maturation, for example, protecting cells during seed desiccation [7, 21].

Abscisic acid, ABA, plays a major role in seed
maturation and regulates the expression of the LEA genes [8, 10, 11, 24, 27, 30]. Cis-elements and transcription factors mediating ABA-inducible LEA gene expression have been identified [16, 29, 38]. Some ABA-inducible LEA genes are inducible only in cells formed in the seed [14, 32], while others appear to lack seed specificity [30, 38]. Carrot LEA genes that encode hydrophilic proteins, such as DC8, are expressed in carrot zygotic embryo and endosperm tissues, in somatic embryos, and in callus [2, 4, 9, 46]. The ABA treatment causes large increases in levels of mRNA and protein in cultured carrot cells, but ABA cannot induce DC8 expression in mature leaf or root tissues [14, 18]. Other carrot LEA genes, such as DC24, and the carrot oleosin protein gene, DC59, have similar expression patterns [17, 19].

Because LEA mRNA and protein abundance was correlated with late embryonic development, LEA gene expression was thought to be regulated by the embryonic developmental program and LEA gene activities have been used as stage-specific markers to characterize embryo-lethal mutants [43, 44]. Therefore, it was puzzling to find DC8 expression in early embryogenesis and in non-embryo tissues such as callus and endosperm [14]. On the other hand, since both the embryo and the endosperm desiccate during seed maturation, we might expect the expression of genes involved in desiccation protection in both of these tissues.

To investigate LEA gene regulation, we performed a detailed analysis of the temporal and spatial pattern of DC8 expression. To address the issue of early DC8 expression, we compared DC8 expression in carrot to its expression in species with different rates of seed development. We found that DC8 expression is more closely correlated with growth rates than with the embryo’s developmental stage. This result, coupled with DC8 expression in the endosperm and embryogenic callus, suggests that the seed maturation program, as represented by LEA gene expression, is activated at the same time as embryogenesis, but is not linked with specific embryonic stages.

Materials and methods

Plant materials and culture conditions

Carrot (Daucus carota L. cv. Juwarot) seedlings were germinated on moist filter paper, transplanted into soil and grown to maturity in a greenhouse maintained at 24 °C, 16 h light. Tobacco (Nicotiana tabacum cv. Wisconsin) and Arabidopsis thaliana seedlings were germinated on agar plates containing (2/5 MS medium [31] transplanted into soil and grown to maturity in a greenhouse maintained at either 24 °C, 16 h light for tobacco, or 21 °C, 9 h light for Arabidopsis. Carrot plants were induced to bolt by vernalizing at 4 °C for 45 days. Before anthesis, the umbel was covered with a paper bag. Pollen grains from an older umbel were dusted onto newly emerging pistils in the umbel which were then covered again.

Transgenic carrots were constructed as described by Goupil et al. [14]. Arabidopsis plants were transformed with the DC8(505):GUS construct according to procedures described by Valvekens et al. [42]. DC8(2600):GUS transgenic tobacco was generated according to Horsch et al. [20].

Carrot tissue cultures were initiated by culturing petiole segments on B5 medium supplemented with 1.0 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) at 24 °C [12]. To initiate a suspension culture, two-week-old callus was transferred into liquid B5 medium supplemented with 1.0 mg/l 2,4-D. To initiate the development of somatic embryos, 10- to 15-day-old suspension cultures were diluted into B5 medium without 2,4-D [39].

Arabidopsis calli were initiated from root segments and cultured at 24 °C on callus-inducing medium (CIM, Gamborg's B5 medium supplemented with 0.5 mg/l 2,4-D and 0.05 mg/l kinetin) [5, 12]. Tobacco callus was initiated from leaves and cultured on MS medium supplemented with 1.0 mg/l 2,4-D.

GUS activity assays

Mature leaves of transgenic carrot, tobacco, and Arabidopsis were collected from greenhouse-