DNA, RNA AND PROTEIN CONTENT OF TISSUE DURING GROWTH
AND EMBRYOGENESIS IN WILD-CARROT SUSPENSION CULTURES

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SUMMARY

A highly selected population of cells (clumps from 63 to 125 μm in diameter), obtained
by screening 14-day-old stock suspension cultures of wild carrot (Daucus carota L.), was
used to initiate cultures in this study. Time-course changes in DNA, RNA and protein
were followed when these cultures were grown in the presence or absence of 2.25 μM 2,4-
dichlorophenoxyacetic acid (2,4-D). The data show that growth of these cultures, particu-
larly in the early part of the growth curve, is different from that in most other studies re-
ported on suspension cultures initiated without screening. The gross compositional analy-
sis shows that this difference stems from the very high RNA:DNA and protein:DNA ratios
of the cellular material used as the inoculum in this study. The presence of 2,4-D in the me-
dium promoted total RNA and protein levels. Correlations were sought between the ap-
pearance of embryos in the absence of exogenous 2,4-D and gross compositional differ-
ences developing in cultures grown in the presence and absence of 2,4-D. The handling of
cultures during inoculation appeared to have led to a substantial loss of DNA. This had,
however, little effect on dry weight or protein content of the tissue.

Key words: Daucus carota; 2,4-D; embryo number; DNA; RNA; protein.

INTRODUCTION

Carrot (Daucus carota L.) cell cultures have proven to be exceptionally useful in studies of the
fine structural and biochemical events accom-
panying adventive embryogenesis (1–6). In our
investigations concerning the effect of nutrition on
the production of somatic embryos in carrot cells
(7–9), we have routinely used tissue, screened to
give material from 63 to 125 μm in diameter, here-
in referred to as proembryonic masses (PEMS).
When PEMS are grown at an appropriate density
in a medium without an external supply of auxin,
the progress of growth and embryo formation can be conveniently followed and the data so obtained
subjected to quantitative analysis (9). Thus
PEMS constitute a selected and rather homoge-
neous population of cells offering excellent starting
material for biochemical studies in suspension
culture.

The present work describes the time-course of
growth and embryogenesis when the PEMS were
allowed to grow in suspension culture. Accumula-
tion of DNA, RNA and protein was followed
through the growth cycle of PEMS in the presence
and absence of an external supply of auxin.

MATERIALS AND METHODS

Culture conditions. The origin and the cultural
practices employed to maintain the stock cultures
have been described (4, 10). The culture medium
described by Wetherell (11) was modified by addi-
tion of 10 mM myoinositol. Proembryonic masses
(PEMS), 63 to 125 μm in diameter, were prepared
from 14-day-old cultures as described previously
(8). Packed tissue volume (PTV) was estimated as
described previously (8).

All cultures were initiated in 500-ml Erlen-
meyer flasks containing 100 ml medium and 100
μl PTV freshly prepared PEMS. When present in
the medium, 2,4-D was provided at 2.25 μM. The
flasks were agitated on a gyratory shaker at 100
cycles per min in darkness at 25°C.

Two sets of experiments were performed. In
one set, all cultures included 2,4-D. Cultures ini-
tiated by the use of PEMS as the inoculum are re-
ferred to as cultures A. After 9 days of growth,
when cultures A were in exponential phase (see Results), two flasks were pooled and 5-ml portions used to initiate another set of 100-ml cultures, hereafter referred to as cultures B. Still another set of cultures were similarly initiated after 15 days of growth when cultures A had reached stationary phase (see Results); these are called cultures C. For each inoculation, the flask was flamed and shaken vigorously, and 5 ml inoculum transferred with a sterile pipette to 95 ml fresh medium. Dry-weight determinations and other measurements reported for cultures B and C at 0 time were done on suspension of cells from cultures A after all the new cultures had been inoculated. In the second set of experiments, all cultures were initiated with PEMS. Half the cultures included 2,4-D which are referred to as +D cultures; the remaining half were not provided with 2,4-D and are accordingly referred to as −D cultures. Thus +D cultures are similar to cultures A.

Cell extraction and growth measurements. At suitable intervals, two or three flasks from each culture were treated as follows. From each flask of −D cultures, duplicate 1-ml aliquots were taken just before harvesting for estimation of embryo number and distribution of stages as described previously (8, 9). The tissue from each flask was harvested by centrifugation at 400 x g for 10 min to give independent samples. The tissue was treated with boiling methanol for 1 min. After cooling, each suspension of tissue in methanol was homogenized with a VirTis Homogenizer operated at full speed (45,000 rpm) for 4 min. Each sample was subjected to ultrasound from a "Biosonic" ultrasonic generator operated at 80% power for 1 min. The samples were stored at −20 °C until all samples from an experiment were collected. The samples were extracted simultaneously at 4°C with the solvent series recommended by Holdgate and Goodwin (12). The resulting white residues were air-dried and then dried overnight in vacuo over KOH. The residue from each sample was weighed to give the "extracted dry weight." Weighted portions (3 to 6 mg) of each sample were extracted with 1.0 ml 0.3 N KOH at 37°C for 16 to 18 hr. After centrifugation at 1000 x g for 10 min, the supernates were removed and the residues re-extracted with 1.0 ml 0.3 N KOH at 37°C for 2 hr. After centrifugation at 1000 × g, the residues were washed twice with 0.5 ml portions of 0.3 N KOH. The extracts and washings were combined and made to 3.4 ml. Aliquots (2 × 0.2 ml) were removed for protein determination. The remainder of each KOH extract was cooled to 4°C and 1.5 ml cold 1.3 N perchloric acid (PCA) containing 1 mM MgCl₂ was added. Solutions were left overnight at 4°C to allow hydrolysis of RNA. Following centrifugation at 1000 × g for 10 min, the pellets were washed twice with 2.5 ml 0.5 N PCA. The supernates and washings were combined and adjusted to 10 ml with 0.5 N PCA (RNA fraction). The residual pellets were hydrolyzed in 1 ml 10% (w/v) PCA at 70°C for 20 min, cooled and centrifuged at 1000 × g to give the supernate DNA fraction.

Protein in duplicate 0.2-ml samples of the KOH extracts was determined by a microbiuret method (13) using bovine serum albumin as a standard. RNA was measured on duplicate 0.2-ml samples of the RNA fraction by the cupric ion catalyzed orcinol reaction (14). DNA in duplicate 0.4-ml samples of the DNA fraction was determined by the diphenylamine reaction (15). Highly polymerized yeast RNA and calf thymus DNA (Sigma Chemical Co.), treated in parallel with the tissue extracts, were used as standards for the nucleic-acid estimations.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>TOTAL NUMBER AND DISTRIBUTION OF EMBRYOS AT VARIOUS TIMES IN WILD-CARROT CELL SUSPENSION CULTURES GROWING IN THE ABSENCE OF 2,4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Period</td>
<td>Embryos</td>
</tr>
<tr>
<td>Days</td>
<td>Total/ml culture</td>
</tr>
<tr>
<td>0.3</td>
<td>None recognized</td>
</tr>
<tr>
<td>6</td>
<td>1030±104</td>
</tr>
<tr>
<td>8</td>
<td>1021±102</td>
</tr>
<tr>
<td>9</td>
<td>1281±204</td>
</tr>
<tr>
<td>10</td>
<td>1310±120</td>
</tr>
<tr>
<td>12</td>
<td>1920±251</td>
</tr>
<tr>
<td>14</td>
<td>1566±278</td>
</tr>
</tbody>
</table>

* Duplicate 1-ml aliquots were taken from three replicate cultures. Values are the mean ± SD of six determinations.