INTRACELLULAR LOCALIZATION OF CALMODULIN ON EMBRYONIC AXES OF CICER ARIETINUM L.

Josefina Hernández-Nistal, Juan J. Aldasaro, Dolores Rodriguez, Josefa Babiano and Gregorio Nicolás

Plant Physiology Department, Biology Faculty
University of Salamanca, Salamanca, Spain

INTRODUCTION

Calcium plays an important role in the regulation of plant metabolism and in mediating the adaptation of plants to environmental changes. Some of these functions are mediated through its binding to calcium binding regulatory proteins, the most important among these is calmodulin (CaM). We want to know if CaM has a role in the early stages of seed germination. So we have estimated its content in isolated subcellular fractions and in sections of 36 and 72 hours old embryonic axes germinated in different media: H₂O-25°C, H₂O-30°C and ABA-25°C, in order to prove if temperature and germination inhibitors have any effect on CaM levels.

MATERIALS AND METHODS

Seeds were germinated in H₂O (25-30°C) or ABA 25μM-25°C during 36-72h. The starting material was the embryonic axes. To obtain protoplast, mitochondrial and microsomal fractions Muto's (1982) method was followed. The purification of the nuclear sap, chromatin and nuclear membranes enriched fractions, as Matsumoto et al. (1984). To isolate cell wall proteins, LiCl was used (Huber and Nevins, 1980). The localization of CaM on the embryonic axes was done on 1.5 or 3 mm long radicle segments, both in 36 and 72h old. CaM was assayed by radioimmunoassay using 125I RIA kit (Amersham, England).

RESULTS

Table 1. Percentage CaM/total protein in cellular fractions of 36h old embryonic axes.

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<tbody>
<tr>
<td>H₂O-25°C</td>
<td>1.24</td>
<td>0.4</td>
<td>0.4</td>
<td>2.5</td>
<td>0.5</td>
<td>2.4</td>
<td>1.80</td>
<td>2.4</td>
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<tr>
<td>H₂O-30°C</td>
<td>2.31</td>
<td>0.6</td>
<td>0.1</td>
<td>9.3</td>
<td>1.1</td>
<td>0.9</td>
<td>0.86</td>
<td>0.04</td>
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<tr>
<td>ABA-25°C</td>
<td>0.32</td>
<td>1.3</td>
<td>0.8</td>
<td>4.1</td>
<td>1.7</td>
<td>0.4</td>
<td>0.44</td>
<td>0.1</td>
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DISCUSSION

CaM level is greater in the intact embryonic axes from seeds germinated at 30°C than in those germinated at 25°C. ABA reduces significantly the amount of CaM in the embryonic axes as it does in radish seeds (Cocucci, 1984). In the cell wall, the main Ca++ store in plants, the ratio CaM/total protein was the largest in H$_2$O-30°C. Among the subcellular fractions, the mitochondria has more CaM than protoplast and microsomal fraction, as Muto (1982) reported in wheat leaf cell, but in the cytosol, it appears at a low level, probably due to non-specific binding of cytosolic CaM to membranes during the experimental procedures. ABA increases CaM levels in cytosol, protoplast and microsomal fractions. The known regulatory role of Ca++ in mitosis and cytokinesis of plant cell are consistent with a nuclear locale for CaM. In the fraction purified from nuclei the percentage CaM/total protein in H$_2$O-25°C is always the largest one, mainly in nuclear membranes and in chromatin. Matsumoto et al., (1984) reported a CaM-like activity associated with chromatin of pea buds.

The involvement of Ca++ and CaM in the activation of growth in other plant systems and in membrane functions suggests that the Ca-CaM complex could be implicated in the early germination phases. To prove it CaM levels were related to radicular growth ability as suggested by its relation to segments of 36h old embryonic axes. Figure 1 shows that the largest percentage is in the Ia segment, the apical one, where the cells are going to undergo mitosis. In the Ib segment, where the cells are in elongation, the level of CaM is diminished. These results agree with those of Hausser et al. (1984) who visualized increased CaM in the tip of growing algae, root hairs and pollen tubes.

In order to complete this result, the percentage CaM/total protein was analyzed in 72h old segments. Ia was also the highest value, but lesser than in 36h. In Ib segments the level has decreased considerably. However, the V segment (the bud), which had a very low level of CaM in the 36h old embryonic axes, now has a higher rate. We can conclude that a high CaM level allows cell elongation and division, and in this way could promote germination as suggested by Cocucci (1984).