MOLECULAR AND PHYSIOLOGICAL DIFFERENTIATION OF POLLEN IN-VITRO

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1. INTRODUCTION

It is unknown how the programs underlying plant developmental processes manifest themselves in terms of ordered gene expression (1). An important developmental process in plants is pollen grain development (gametogenesis). The pollen grain is the progenitor of the sperm cells and the vehicle for their delivery to the embryo sac (2). The pollen grains are produced in the anther which consists of four chambers or pollen sacs. In a young anther, the pollen sac encloses a homogeneous group of diploid cells (microspore mother cells). These microspore mother cells undergo meiosis resulting in clusters of four haploid microspores (tetrad). Each microspore passes through the first haploid mitosis resulting into a pollen grain composed of a large vegetative and a small generative cell, the latter situated in the middle of this grain (the mid-binucleate stage). The further development of this young and infertile pollen into fertile pollen i.e. the development of the male gametophyte, is a process which can be followed in-vitro. The in-vitro development of young tobacco pollen into mature and fertile tobacco pollen proceeds in a rather simple medium with glutamine and sucrose (3). What we observe in the in-vitro situation is that the wall surrounding the pollen becomes thickened and that the pollen starts with the accumulation of starch immediately after initiation of the in-vitro culture. Within three days of in-vitro culture at a temperature of 28°C the gametophytes have accumulated an abundant amount of starch granules. At any moment during pollen development in-vitro the pollen can be tested for in-situ pollination, in-vitro germination (formation of a pollen tube), and subjected to labeling experiments without the interfering presence of the anther tissue. We wanted to study gene expression underlying the developmental process of gametogenesis in-vitro. Therefore, the system was first compared with in-vivo development.
2. COMPARISON OF IN-VITRO AND IN-VIVO POLLEN DEVELOPMENT

2.1. Germination, seed set and time of pollen development

Pollen development in-vivo finally results in fertile pollen i.e. pollen capable of fertilization. The pollen matured in our in-vitro system at least got to have that potential. So first we compared pollen developed in-vivo and in-vitro for their ability to germinate (to form a pollen tube) and to set seed. There is a peak in the percentage of grains germinating in-vitro after three days of in-vitro maturation (see Table 1.). This percentage is comparable with the germination percentage of in-vivo developed pollen. Moreover, three days of in-vitro culture produce pollen which shows an optimum in the number of produced seeds after pollination of emasculated flowers. Preliminary results indicate that the number of seeds from pollen developed for three days in-vitro equals that of pollen developed in-vitro. Furthermore, there is seed set from pollen developed either for one or two days in-vitro (see Table 1.). However, this pollen not only produces reduced numbers of seeds but the time necessary for seed set is extended (M.M.A. van Herpen, in preparation). The period of time necessary for the in-vitro development of fertile pollen equals that of in-vivo development i.e. 3 days. However, pollen developed in-vitro for four days is no longer able to germinate or to set seed indicating that the process of gametogenesis in-vitro can not be fully compared with the process in-vivo in which the pollen remains fertile over a long period of time.

Table 1. Germination and seed set ability of pollen during development in-vitro starting from the mid-binucleate stage (0 days). Germination and seed set ability of pollen developed in-vivo serve as a control.

<table>
<thead>
<tr>
<th>Development</th>
<th>0d</th>
<th>1d</th>
<th>2d</th>
<th>3d</th>
<th>4d</th>
<th>In-vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination in-vitro (%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>±75</td>
<td>1</td>
<td>±80</td>
</tr>
<tr>
<td>Seed set</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

2.2. Gene expression

Further similarities between pollen development in-vivo and in-vitro became apparent when gene expression was studied with the help of a granule-bound starch synthetase probe (M.M.A. van Herpen, in preparation) and two other pollen cDNA clones prepared from mRNA from mature tobacco pollen developed in-vivo (K.A.P. Weterings, in preparation). No differences in expression of these genes