ABA and Oxygen Crosstalk during Seed Development
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ACCOMPLISHMENTS

Test Plant Selection

Differentiating of RCBc and RCBn: The rapid cycling B. rapa (RCBr) and B. napus (RCBn) germplasm was initially considered as our test plants because their compact size, short life cycle, and have served as model plants for a series of experiments on seed development in unusual environments (e.g. in Space). However, further evaluation deemed that they are not feasible for the purposes of this project because of a) their small seeds (ca. 2.5 mg) seed for RCBr, making some biochemical assays and in situ procedures more challenging.

Canola (Brassica napus L. cv Westar) germplasm: It was obtained from Paul Williams at the Crucifer Genetics Cooperative and had been used in the original green seed studies in the 1970s ff. However, this batch of seeds was over 18 years old and had low vigor. Through a couple of growth cycles, the seeds were restored to their vigor (Figure 2). Aliquots of the newly harvested seeds have been provided back to the Crucifer Genetics Cooperative (Madison, WI) and are being used in our experiments. It produces mature seed with average weight 3.9 mg/seed and more than 2 dozen seeds/silique.

Experimental Systems

Three systems were evaluated, their merits and pitfalls are summarized in Table 1.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Merits</th>
<th>Pitfalls</th>
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<tbody>
<tr>
<td>in situ siliques on the plant</td>
<td>Least disturbance</td>
<td>Difficult to apply elicitors (e.g. O2, ABA, and ABA catabolism inhibitor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uncertain about the level of the elicitor the seeds are exposed to.</td>
</tr>
<tr>
<td>in vitro pod culture</td>
<td></td>
<td>Convenien to apply elicitors (O2, ABA, ABA inhibitor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tested for RCBr</td>
</tr>
<tr>
<td>in vitro pod-free seed</td>
<td></td>
<td>Direct exposure to elicitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Convenien to harvest samples post treatments.</td>
</tr>
</tbody>
</table>

Gaseous microenvironment in siliques

GData in Table 2 demonstrated that the gaseous environment around the seed is more oxygenated in siliques experienced non-lethal freezing than control siliques.

Table 2. Gas Composition in Siliques (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO2 (%)</th>
<th>O2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark at 5°C</td>
<td>3.1 ± 0.8</td>
<td>20.1 ± 0.6</td>
</tr>
<tr>
<td>Dark at 22°C</td>
<td>2.0 ± 0.2</td>
<td>18.7 ± 0.4</td>
</tr>
</tbody>
</table>

ABA and its metabolite Determination

In order to provide direct evidence for the O2 and ABA involvement in green seed formation, we worked to implement a rugged and robust analytical procedure for the determination of ABA and its main catabolites in the experimental specimen. A method based on GC/MS analysis of plant extracts upon partial purification and derivatization and the use of stable-isotope labeled authentic compounds is currently under validation.

Figure 3. GC/MS chromatogram of (a) standard material ABA and (b) ABA spiked plant extract. ABA was able to be separated from complex sample matrix.

Figure 2. Canola plants grown in a walk-in controlled environmental chamber at KSC (co-PD\'s facility)

Hypothesis

Two chemical and biological processes underlying the green seed formation following a non-lethal freezing have been proposed previously:

- Freezing → rapid desiccation and/or potential ice crystal formation → reduced or lost oxygen (e.g. POD) activity → inability to support chloroplyt degradation
- Freezing → ABA levels declined precipitously in the embryos → inhibit normal chloroplyt catabolism.

It was observed in Dr. Musgrave's lab that a) seed development is sensitive to O2 concentration and in O2-limited under normal conditions; b) an acute chilling episode (± 5 °C) resulted in an increase in oxygen tension as well as carbon dioxide in seedpods.

An alternative hypothesis:

Freezing → transient rise in pod O2, (the actual stress) → accelerate ABA catabolism → inhibit normal chloroplyt catabolism.

Long term goal

To understand how ABA and oxygen interact to control seed maturation within the unique microenvironment of the developing seed, in a well-defined model system; to new strategies to address the green seed problem in canola.

Specific Objectives

- Elucidate freezing induced changes in gaseous environment within siliques.
- Investigate the impact of the seed microenvironment (particularly, O2 level) on green seed formation and ABA metabolism.
- Probe the roles of O2, ABA and its catabolites in chloroplyt degradation.

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