ABA and Oxygen Crosstalk during Seed Development

Mary E. Musgrave and Lanfang H. Levine[2]

[1)] Department of Plant Science and Landscape Architecture, University of Connecticut, Storrs, CT 06269

[2)] Enterprise Advisory Services, Inc., ESC-Team QNA, Kennedy Space Center, FL 32899

INTRODUCTION

Green Seed Problem

The normal course of seed development in many oil seeds (such as soybean, canola, and flax) begins with a green, photosynthetic embryo and ends with a mature embryo that is essentially devoid of chlorophyll. In canola, several environmental factors in combination with agronomic practices can affect the ability of seed to rid itself of chlorophyll. The two most common are frost and extreme hot dry weather at or near swathing, that can lead to green seeds instead of yellow seeds at harvest (Figure 1). The green color is expensive to be removed from resulting oil through processing steps that significantly reduces oil yield. Canola is a major crop in Canada (16.04 million acres in 2008), providing 18% of farm revenues. An early frost at a critical seed developmental stages (e.g. between 60 to 65% seed moisture) frequently provokes green seed formation in canola. It is estimated an annual loss of $100M in farm revenue due to green seed incidence in North America.

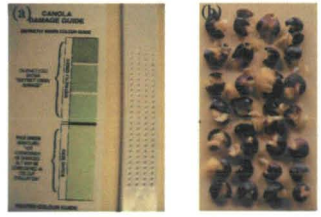

Figure 1. (a) Canola seed color guide used for the assessment of green seeds; (b) crushed sound mature seeds.

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 enzyme(e.g. PaO) activity → inability to support chlorophyll degradation
- ◆Freezing →ABA levels declined precipitously in the embryo → inhibit normal chlorophyll catabolism.

It was observed in Dr. Musgrave's lab that a) seed development is sensitive to O_2 concentration and is O_2-limited under normal conditions; b) an acute chilling episode (- 5 ^{0}C) resulted in an increase in oxygen tension as well as carbon dioxide in seedpods.

An alternative hypothesis:
Freezing → transient rise in pod O_2 (the actual stress) → accelerate ABA catabolism→ inhibit normal chlorophyll a 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, O_2 level) on green seed formation and ABA metabolism.
- ◆ Probe the roles of O_2, ABA and its catabolites in chlorophyll degradation.

ACCOMPLISHMENTS

Specific focus in first year:
Evaluate germplasm and methodologies that could be appropriate for investigating the role played by oxygen in the seed environment.

Test Plant Selection

Deselecting of RCBr 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 10 years old and had low vigor. Through a couple of growth cycles, the seeds were restored to their vigor (Figure 2). Aliquots of the freshly 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.

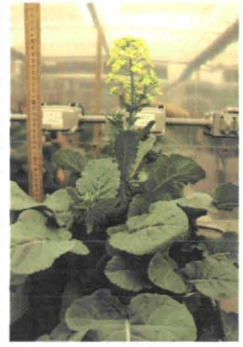

Figure 2. Canola plants grown in a walk-in controlled environmental chamber at KSC (co-PD's facility)

Experimental Systems

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

Table 1. Merits and Pitfalls of Three Experimental Systems

Experimental System	Merits	Pitfalls
in situ silique on the plant 	• Least disturbance	• Difficult to apply elicitors (e.g. O_2, ABA, and ABA catabolism inhibitor). • Uncertain about the level of the elicitor the seeds are exposed to.
in vitro pod culture 	• Convenient to apply elicitors (O_2, ABA, ABA inhibitor) • Tested for RCBr	• Uncertain about the level of the elicitor the seeds are exposed to. • Potential ethylene build-up in the culture vessel.
in vitro pod-free seed 	• Direct exposure to elicitors. • Convenient to harvest samples post treatments. • Tested in studies examining the effect of O_2 availability on seed dormancy.	• Seeds separated from their maternal tissues and natural microenvironment. • Developmental course of immature seeds may be altered by not only the treatment variables.

Gaseous microenvironment in siliques

- ❑Data in Table 2 demonstrated that the gaseous environment around the seed is more oxygenated in siliques experienced non-lethal freezing than control siliques.
- ❑External oxygen concentration required to simulate such internal gaseous environment is estimated to be between 60 and 100%.

Table 2. Gas Composition in Siliques (%)

Treatment	CO_2 (%)	O_2 (%)
Dark at -5 ^{0}C	3.1 ± 0.8	20.1 ± 0.6
Dark at 22 ^{0}C	2.0 ± 0.2	18.7 ± 0.4

ABA and its metabolite Determination

In order to provide direct evidence for the O_2 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.

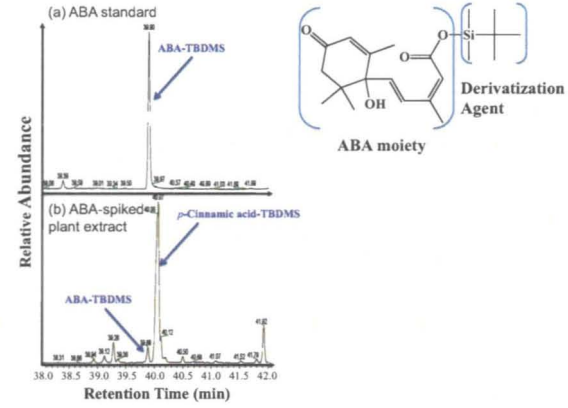

Figure 3. GC/MS chromatogram of (a) standard material ABA and (b) ABA spiked plant extract. ABA was able to be separated from complex sample matrix.

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