Research Experiences for Urban Science Teachers

Transcriptomic Analysis of *Camelina sativa*: a Comparison of the Effects of Light on Seed Germination Through Integrated Experiential Learning in Biotechnology

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RET Program Overview

Providing authentic experiences for STEM students can be challenging for teachers who have been removed from a laboratory setting for an extended period of time or have not had extensive laboratory training as part of their education. The Center for Science and the Schools (CSATS) provides a summer research experience geared to immerse teachers in research experiences in industry or laboratory settings. This poster reflects a survey of the biotechnology research conducted in the Central PA Biofuels laboratory located at Penn State Harrisburg in Middletown, PA.

Background: Transcriptomic Analysis of *Camelina sativa*

Camelina sativa, also known as false flax, is an oil seed crop that has gained particular interest in recent years due to its high level of Omega-3 fatty acids and seed oil content of 29 - 41% (*Camelina*, 2008). Seed germination is the critical first step to producing a hearty crop and can be studied at the observable phenotypic and molecular level. The overall aim of the project is to identify certain genes that are up-regulated or down-regulated in *Camelina sativa* when exposed to particular germination treatments. This poster provides an overview of newly acquired techniques required to complete the transcriptomic analysis of *Camelina sativa* var. Suneson.

Figure 1. *Camelina sativa*.

Seed sterilization and Aseptic Tissue Culture

In vitro seed germination requires the proper use of aseptic technique using the laminar flow hood and seed sterilization prior to culture (Refer to **Figure 2**). Approximately 150 seeds were sterilized by washing with 2 drops of TWEEN 20 detergent, 70% ethanol and 10% bleach. In sterile conditions, autoclaved filter paper was placed in sterile plastic petri dishes with 3 ml of sterile water (Refer to **Figure 3**). 20 *Camelina* seeds were placed on each plate. Triplicate plates were created for each treatment, light and dark, for a total of 6 plates per experiment. The number of seeds in stage 4 germination (Refer to **Figure 4**) were counted for each plate at 24 and 48 hours.

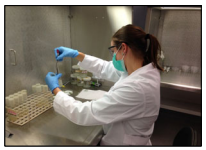


Figure 2. RET teacher practices sterile technique during rose micropropagation.

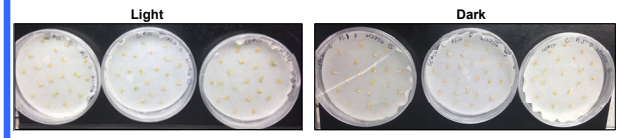
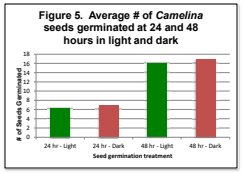


Figure 3. 48 hour seed germination of Light and Dark treatments.

Figure 4. Microscopy picture of Stage 4 seed germination.



RNA extraction of 48 hour seedlings

RNA was extracted from the *Camelina sativa* seedlings that had been cultured *in vitro* for 48 hours using the Sigma-Aldrich's Spectrum Plant Total RNA Kit. The *Camelina* seedlings were homogenized in liquid nitrogen for approximately 45 minutes. The tissue was suspended in a lysis buffer/2-ME solution and filtered with a filter column. The lysate containing RNA was then bound to a binding column. The column was washed 3 times followed by 4 water elutions of the extracted RNA. Optical density (OD) readings of the RNA elutions were obtained using Thermo Scientific's Nanodrop 2000 to determine the purity and concentration (ng/µl) of the RNA (Refer to **Table 1**; **Figure 6**). This information determined the RNA samples used for cDNA synthesis. A 260/280 ratio for pure RNA Is ~2.0.

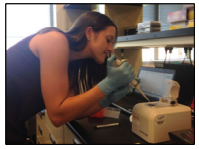


Figure 6. RET teacher places a sample of total RNA onto a Nanodrop2000 to collect optical density readings.

Table 1. Total RNA optical density readings of *Camelina sativa* germinated in light and dark

Sample	Concentration (ng/µl)	260/280	260/230
Light	818.0	2.16	2.37
Dark	748.4	2.16	2.38

cDNA synthesis

Based on purity and concentration, cDNA was synthesized from elution 1 of the extracted RNA for both the light and dark treatments. A 3X cDNA synthesis reagent master mix was created with reverse transcriptase and buffer, dNTPs, random primers, and RNase inhibitor. The master mix was then added to a PCR tube with nuclease free water and .2 µg of RNA. The amount of RNA used for the reaction was calculated using the RNA optical density readings (Refer to **Table 1**). The optical density readings were taken for the cDNA to ensure standard concentrations of cDNA for subsequent qRT-PCR analysis (Refer to **Table 2**). A 260/280 ratio for pure cDNA is ~ 1.8.

Table 2. cDNA optical density readings of *Camelina sativa* germinated in light and dark

Sample	Concentration (ng/µl)	260/280	260/230
Light	2390.4	1.79	2.27
Dark	2390.0	1.78	2.27

RT-PCR and qRT-PCR Analysis

Table 2. Gene information for stress-related genes in *Camelina sativa*.

Gene Abbreviation	Gene Name	Function	Ta(°C)
SIFF-B	Salinity-induced factor for fertility	Regulation of fertility	56.0
Helicase B	Helicase	Maintenance of repression 1, putative	56.0
PDF 1.2	Plant defensin 1.2	Plant defense against fungal pathogens	57.0
PAL1	Phenylalanine ammonia-lyase	Defense response, response to UV-B, and response to oxidative stress	57.0
TPR	Translocated promoter region	Involved in mRNA export from the nucleus in plants	58.1
SIFVG-B	Salinity-induced factor for vegetative growth	Involved in vegetative growth	59.9
PLSO4	Pyridoxal kinase salt overly sensitive 4	Development of root hairs, salt stress signaling pathway	61.0
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Catalyzes the 6th step of glycolysis in glucose metabolism	61.0

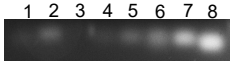


Figure 7. RT-PCR gel electrophoresis showing 7 stress-related genes in seeds from the control light treatment. 1 – SIFFB, 2 – Helicase B, 3 – PDF 1.2, 4 – PAL1, 5 – TPR, 6 – SIFBG-B, 7 – PLSO4, 8 - GAPDH

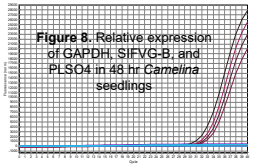
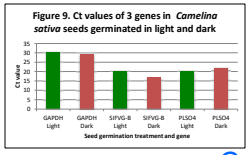


Figure 8. Relative expression of GAPDH, SIFVG-B, and PLSO4 in 48 hr *Camelina* seedlings



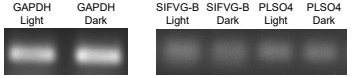


Figure 10. qRT-PCR gel electrophoresis showing the relative expression of 1 housekeeping gene and 2 stress-related genes in light and dark.

Conclusions

Light and dark are important factors in seed germination, but only have a slight difference for germination rates in the current preliminary data. Considering the transcriptomics aspect of the research, good quality RNA was effectively extracted from the 48 hr *Camelina* seedlings to make high quality cDNA. In the qRT-PCR reaction, an equal concentration of expression was achieved in both treatments for GAPDH, the housekeeping gene. Without establishing an equal expression of GAPDH, it is difficult to substantiate conclusions about the levels of expression for other genes. SIFVG-B, a vegetative growth gene, is expressed similarly in seeds germinated both in light and dark. PLSO4, a gene involved in the development of root hairs, also does not have a difference in the level of expression, as shown by the results of the qRT-PCR. These genes may not be affected by the presence of light. Further investigation will be done to test the effects of hormones and other stress factors on seed germination at the molecular level once phenotypic differences are observed.

Acknowledgements

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References

Camelina. (2008). Retrieved July 25, 2016, from http://extension.oregonstate.edu/gilliam/sites/default/files/Camelina_em8953-e.pdf

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