An inquiry-based module for exploring student conceptions of plant circadian rhythms, gene expression, and defense against insects

Jeremy L. Hsu (project leader) and Hagop S. Atamian (project co-leader)
Schmid College of Science and Technology
Chapman University

Total amount requested: $6,969.50

This project will develop a 4-5 class curriculum for high school biology students that promotes knowledge of phytohormones and the central dogma. The module, which relies on easily accessible items and requires minimal space, will integrate a hands-on experiment that guides students through replicating research examining circadian entrainment in postharvest cabbage from groceries[1,2]. This work found that plants have cyclical, circadian expression of genes that produce phytohormones, and that such cyclical expression influences herbivory by caterpillars. Such cyclical patterns were found in plants both *in situ* and in postharvest cabbage.

This work provides an ideal platform to shape student conceptions of circadian rhythms, gene expression, and plant herbivory. We will first develop a survey to assess students' previous conceptions about circadian rhythms, and develop and teach a curricular module about circadian gene expression of phytohormones [3]. In the module, students will use light timers to entrain postharvest cabbage to alternating light/dark cycles. Students will similarly entrain caterpillars with these light cycles, and measure herbivory using graph paper. The results should replicate previous work and demonstrate less herbivory when both plant and caterpillar are entrained to the same light/dark cycle, since expression of phytohormones involved in plant defense will be greatest when caterpillars are active. The module will conclude with a discussion of gene regulation and how this influences phytohormones.

Throughout this module, students will think critically about the scientific process, make experimental decisions, and interpret authentic data that they collect. We will teach this module in a partnering high school ourselves before disseminating the curriculum to other high schools. During this process, we will assess the impact of the module on students' self-efficacy, knowledge of gene regulation and phytohormones, and interest in plant biology. We will share our curriculum and results with the broader community through ASPB and related publications.

Goals and objectives: Many high school students hold fundamental misconceptions about plants and genetics [4,5]. For example, students often view plants as static, not recognizing the dynamic interactions between plants and other organisms or the dynamic processes within plants [4,6]. Similarly, students struggle to comprehend the changeable nature of gene expression, particularly in response to environmental stimuli, and the transient nature of mRNA and protein production when DNA remains unchanged throughout these processes [4,7,8]. To counter these misconceptions, we propose a hands-on, inquiry-based module for high school biology that promotes student conceptual understanding of gene expression in plants. This 4-5 class module will have students replicate a series of authentic science experiments using easily accessible supplies (e.g., produce from grocery stores), and challenge students to think critically about the role of environmental factors in shaping gene expression in plants, the impact on phytohormones and phenotype, and how such changes may influence plant defense against insect herbivory.

In addition to designing this module, we will teach and disseminate this module across several partnering high schools. We will then assess the impact of the module on both student understanding of core plant biology concepts as well as on student self-efficacy (their confidence in their ability to do well) and interest in plant biology using pre/post-testing and validated instruments. Given that inquiry-based teaching has been shown to lead to increased scientific skills and interest [9,10], we anticipate that our module will lead to positive outcomes in both content and affect for students.

This integrated, interdisciplinary module will have multiple content learning objectives that span across three of the four ASPB Principles for Teaching Plant Biology. Table I shows each learning objective and its alignment to these principles as well as the high school Next Generation Science Standards (NGSS) for California Public Schools.

Table I. Module learning outcomes and alignment to ASPB Principles and high school NGSS for California Public Schools

Learning objective (LOs) for module: ***Students will be able to…***	**ASPB category**	**ASPB principle**	**NGSS for CA public schools**
LO 1: Explain how changes in mRNA and protein level can lead to phenotypic changes, such as different levels of plant defense against herbivory	Heredity	Central Dogma	HS-LS1-1 *(DNA determines structure of proteins);* HS-LS3-3 (*genetic and environmental factors impact expression)*
LO 2: Recognize that environmental cues, such as light/dark cycles, can influence gene expression			
LO 3: Interpret how varying levels of phytohormones, triggered by changes in environment that influence gene expression, impact plant phenotype	Molecules to Organisms	Phytohormones	HS-LS1-2 *(interacting systems provide specific functions in organisms)*
LO 4: Predict impact on ecosystems if plant defenses against herbivory are changed	Ecosystems	Ecosystem services	HS-LS2-6 *(complex interactions in ecosystem)*

Project details

Project activities and alignment with learning outcomes: The module centers around students replicating a study that found that postharvest cabbage from groceries retain cyclical, circadian expression of genes that produce jasmonate (JA), a phytohormone important in plant defense against herbivory [1,2]. The module will span across 4-5 one-hour class sessions, requires minimal prep time by teachers, and utilizes easily accessible items from the grocery and hardware store. We also anticipate that the experimental setup, consisting of a few small boxes with lamps and cabbage underneath, should fit easily into most classrooms.

In the first class, the instructors will deploy pre-assessments to measure students' baseline levels of knowledge of gene expression in plants as well as students' self-efficacy and interest in plant biology (see last section). Next, students will be given examples of circadian rhythms found in nature, including in plants and animals, and will be challenged to think critically about what could cause such cycles. The instructors will provide guided-inquiry questions on a worksheet to challenge students' preconceived notions and guide them to think about how changes in mRNA and protein levels may impact phenotype (learning objective 1). For instance, one of the questions will ask students to think about where an organism's DNA comes from and if it is likely that an organism changes its DNA every 24 hours. This will continue in the second class, where students will explore examples of differential gene expression leading to changes in phenotype in plants and in humans. Students will be challenged to draw inferences about what likely leads to these changes. For instance, students will be given pictures of a plant during the day and the same plant "sleeping" at night with drooping leaves and challenged to think about what triggers these responses and how they could test their proposed explanations. At the end, the instructors will discuss gene expression as well as how environmental factors, such as light, can influence this expression and thus also impact phenotype (learning objective 2).

In the third class, the instructors will introduce the study system of postharvest cabbage and explain how such plants maintain cycles of gene expression even after harvest. Instructors will introduce the concept of phytohormones and discuss their importance (learning objective 3). This will include a discussion on the role of JA and how it regulates plant defense against herbivory. Students will be challenged to make a prediction about the levels of JA throughout the day and justify their reasoning, which will lead to a discussion on how light influences expression of genes for JA (learning objectives 2 and 3).

Next, the instructors will introduce how cabbage looper (*Trichoplusia ni*) larvae feed on cabbage and explain how such caterpillars also have circadian rhythms that influence their interactions with other organisms. Students will be challenged to think about how they could design an experiment to test the impact of environmental cues on cabbage looper herbivory on cabbage. In particular, instructors will provide information on how both postharvest cabbage and cabbage looper caterpillars can have gene expression be entrained to alternating 12 hour light:dark cycles. Students will work in small groups to make critical predictions when comparing the amount of herbivory of one group where both the cabbage and caterpillars are placed in the same light:dark cycle (Group A; Table II) while a second group is placed in opposite light:dark cycles to ensure that the light entrainment is out of sync (Group B; Table II).

Table II. Experimental design: in group A, the cabbage and caterpillars are entrained to light in sync, while in group B they are entrained out of sync

	Group A: *Light entrainment in sync*		Group B: *Light entrainment out of sync*	
	First 12 hours	*Second 12 hours*	*First 12 hours*	*Second 12 hours*
Postharvest cabbage	Light	Dark	Light	Dark
Caterpillars	Light	Dark	Dark	Light

Students will predict the level of JA expression and herbivory in group A versus group B and sketch out their predictions. Following this, instructors will assist in setting up the experiment by placing 3-cm cutouts of cabbage in cardboard boxes with lamps set to timers. Both cabbage and caterpillars will be entrained to these light conditions for three days.

Three days before the fourth class, students will allow caterpillars to eat the light-entrained cabbage. During this class, students will then measure percent herbivory by placing the cabbage cutouts on graph paper and counting the amount that has been eaten. Students will compare herbivory between the groups, and should discover that there is less herbivory in group A than group B [2]. The module will conclude with a discussion of how these results demonstrate the impact of environmental cues on phytohormone expression (learning objectives 1 and 2), how such differential levels of phytohormones impact herbivory (learning objective 3), and the significance of such environmental cues on ecosystem interactions (learning objective 4).

Target audience, number of participants, and timeline: This module is designed for 11th and 12th grade biology students. During fall 2022, the project leaders will work with biology instructors at Orange High School, which has a long partnership with our university, to teach this module. The project leaders will obtain feedback and analyze assessment data and make necessary modifications. In spring 2023, the project leaders will contact 5 other local high schools and disseminate an instructional guide and supplies for the module. Those teachers will teach the module and provide additional feedback. We estimate that this module will reach approximately 150-180 students (6 classes of 25-30 students). Afterward, we will disseminate our results and all instructional materials and guides through ASPB and publications such as the *Journal of Microbiology and Biology Education*, ensuring our curriculum reaches a broad audience.

Expected outcomes and evaluation: We will develop an instrument aligned with the learning objectives and use a pre/post-test format to assess the impact of our module on student mastery of the learning objectives. In addition , we will modify an existing instrument for measuring interest in plant biology [11] and compare student interest from before and after the module. We will also use validated instruments for measuring self-efficacy [12] in a pre/post format. We expect to see that the module leads to increased knowledge of the learning objectives. In addition, we hypothesize that this module will lead to greater interest in plants, given that the module centers around the dynamic nature of plants, and lead to greater self-efficacy in students given that past inquiry-based experiments have led to greater student self-confidence in their ability to succeed in science [13s]. Finally, we will also obtain feedback from each of the instructors through an instructor survey and use this feedback to modify the curriculum prior to disseminating the results, module, and all instructional materials through ASPB and peer-reviewed publications.

Statement of teaching philosophy: Dr. Hsu and Dr. Atamian, the project leaders, believe strongly in inquiry-based teaching, where students are thinking critically about a question, evaluating different experimental designs and hypotheses, predicting results, and interpreting authentic data. The proposed project is designed as a guided inquiry project, where students are exploring and evaluating data they gather from replicating authentic experiments. This project also draws upon both project leader's expertise in plant biology and molecular genetics, as well as their extensive pedagogical training, experience, and leadership in developing new inquiry-based curriculum and assessment of such curriculum.

Both Drs. Hsu and Atamian are assistant professors at Chapman University. Dr. Hsu is an assistant professor in biology education and Dr. Atamian is an assistant professor of plant molecular biology. First, the two project leaders bring substantial expertise in the disciplines relevant to the proposed curriculum. Dr. Hsu earned his Ph.D. in evolutionary genetics, and he teaches and coordinates the molecular genetics lecture and lab at Chapman. As coordinator, he is responsible for curriculum development and assessment of the lab sequence, which involves a semester-long plant molecular biology project where students are cloning and sequencing a gene from plants. Dr. Atamian earned his Ph.D. in molecular genetics and runs a plant molecular genetics research group at Chapman that focuses on studying plant interactions with the environment. He has expertise in plant circadian rhythms and has published multiple papers in this area, including in *Science*, and has earned recent grants from the US Department of Agriculture (USDA) and the National Science Foundation (NSF).

Both have received extensive pedagogical training and experience. Dr. Hsu was designated a Scientific Teaching Fellow after participating in the 2017 Summer Institutes on Scientific Teaching at the University of California, San Diego, and has also served as a Scientific Teaching Mentor through that program. He has similarly served as a mentor through the NSF-funded Promoting Active Learning and Mentoring (PALM) Network and was recently selected to serve as a mentor for teaching and developing evidence-based curriculum through the NSF-funded Biology Education Intersegmental Collaborative (BEIC). He is a member of the Society for the Advancement of Biology Education Research, where he serves as co-chair of the conference keynote committee, and has published 11 papers on pedagogy and biology education. This includes six first-author, peer-reviewed publications of novel inquiry-based curriculum or assessment of labs and curriculum. He also chairs the university's assessment committee, providing further expertise for him to lead the assessment of the proposed module. Dr. Atamian also has extensive experience with developing and teaching inquiry-based labs, including overseeing and teaching Chapman's genetics lecture and lab course, where he has developed new experiments centered around plant genetics. Both Drs. Atamian and Hsu have also been active with outreach events with high school students, including with students from Orange High School (where this module will be piloted in the fall). Dr. Atamian has also mentored 19 high school students in his lab and has developed and led an outreach program, "Learning Through Harvest", that teaches principles of plant biology and agriculture to high school students with learning disabilities through interactive lessons and field trips. Thus, the two project leaders have extensive experience and expertise in the discipline and in teaching, curriculum design, and assessment to successfully implement this project.

Itemized budget

We request $6,969.50 in total funding for this project. The module relies on inexpensive items that are easily accessible for instructors at grocery and hardware stores (or Amazon), thus allowing the module, once published and disseminated, to be easily replicated by teachers.

Item	**Description**	**Cost**
Organic cabbage for experiments	Estimate of $25 of organic cabbage per school to provide enough for multiple classes x 6 schools	$150
Cabbage looper (*T. ni*) larvae	$32 for 56 larvae per class plus $20 shipping, Frontier Agricultural Sciences (link) x 6 schools	$312
Indoor timers for lights	Estimate of $15 per timer at a hardware store or Amazon; 5 needed per class; x 6 schools	$450
Plant lamps for entrainment of plants and cabbage loopers	Estimate of $25 per lamp from hardware store or Amazon; 6 needed per class; x 6 schools	$900
Miscellaneous consumable supplies	$50 per class x 6 schools, including food for cabbage loopers, graph paper for students, power strips for plant lamps, copies and printouts, etc.	$300
Professional development stipends for teachers	$200 per school x 6 schools. This stipend is requested since teachers at the participating schools will spend additional time to get trained, set up the experiments, and provide feedback.	$1200
ASPB memberships	For Dr. Hsu and Dr. Atamian ($165 each)	$330
Stipend for project leaders	Estimated 25 hours of work for this project per leader x $25/hour, with 11% fringe	$1387.50
Publication costs	Page charges for publishing in *Journal of Microbiology and Biology Education*	$1700
Participant costs	2 $20 gift cards per school to incentivize completion of surveys for assessment (protocol will be approved by Institutional Review Board)	$240
	Total	**$6,969.50**

References

1. Goodspeed et al. *Proceedings of the National Academy of Sciences* **109**, 4674–4677 (2012).
2. Goodspeed *et al. Current Biology* **23**, 1235–1241 (2013).
3. Covington et al. *Genome Biol* **9**, R130 (2008).
4. Wynn et al. *Journal of Microbiology & Biology Education* **18**, 18.1.40 (2017).
5. Mills Shaw et al. *Genetics* **178**, 1157–1168 (2008).
6. Coley et al. *LSE* **11**, 209–215 (2012).
7. McDonald and Gomes. *J of Transformative Leadership & Policy Studies* **3**, 21–34 (2013).
8. Newman et al. *LSE* **20**, ar53 (2021).
9. Beck et al. *LSE* **13**, 444–452 (2014).
10. Smallhorn et al. *Student Success* **6**, 65–72 (2015).
11. Colon, J. *et al. BioScience* **70**, 887–900 (2020).
12. Baldwin et al. *Science Education* **83**, 397–408 (1999).
13. Chiang et al. *Biochemistry and Molecular Biology Education* **49**, 251–261 (2021).