Article

iCURE (Iterative Course-based Undergraduate Research Experience): A Case-study[S]

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Abstract

Several models suggest ways to expose undergraduates at minority serving institutions or institutions with limited research infrastructures to the iterative process of research. Apprentice-based research experiences allow students to work one-on-one with a research mentor in the hands-on discovery process, but with teaching being a priority for faculty at the aforementioned institutions, financial, spatial, and time limitations for research progress exist. Course-based undergraduate research experiences (CUREs) provide opportunities for a greater number of undergraduates to become familiar with the questions, techniques, and failure involved in research. However, designing projects that a group of students can complete in a semester can be challenging. Inclusive Research Education Communities are intended to promote retention in STEM courses for early college students but have limited benefit for upper-level courses. We sought to create an iterative CURE between fall semester BIOL3900 at the University of North Texas and spring semester CHE397 at Bethel University (Saint Paul, MN) to promote collaboration between unique learning communities. The research goal was to use a tobacco (*Nicotiana benthamiana*) transient expression system as a platform to test gene functions and to engineer valuable bioproducts in plant vegetative tissues. The outcomes of this 2-year integrative module included novel discoveries leading to publications in peer-reviewed journals, cost benefits due to shared resources, continual movement of the project, course-based training for future independent research projects, and improved student attitudes about research.

Keywords: *Plant biochemistry; undergraduate research; lipids; terpenes; CURE*

Introduction

Training our next generation of scientists requires textbook learning, demonstration and practice of laboratory techniques, encouragement to think critically, and exposure to the iterative process of discovery. Most undergraduates are accustomed to weekly laboratory experiments that parallel the classroom topic and because of the quick movement through a textbook, these experiments are "one-off," cookbook-style activities. Students have come to expect this approach based on the current state of high-school science education [1] and can be critical on course evaluations when an instructor deviates from this, saying "lab didn't match what I was learning in lecture." Further, these cookbook experiments falsely lead students to expect that an experiment always "works" or generates results and that a "right answer" exists. The recent pedagogical push to create inquiry-based labs [2] has given students the ownership of experimental design, but even these experiments are single- or two-week activities that do not create a cohesive long-term question for which upper-level students are ready to approach. Embarking on a project where no one knows the outcome can be uncomfortable for undergraduates, but it is this "unknown" that can be exhilarating when scaffolded by the appropriate laboratory environment and community.

Undergraduates in all academic environments face the above problems; however, students at Carnegie R1 public research intensive universities at least have the opportunity to interact with professors conducting fast-paced and impactful research. Even within these institutions, although,

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minorities may not feel supported to seek out research experiences due to lack of previous preparation or competition with their peers. Creating authentic research experiences for undergraduates at 2-year institutions or primarily undergraduate institutions (PUIs) presents an even greater challenge. Faculty at these institutions have large teaching and advising loads and any teaching assistants provided are often students that have just completed the class. Funds and administrative support can also be lacking for the development of authentic research experiences.

These issues are not new. Over the last several decades, the following models have been proposed and implemented to encourage undergraduates to be involved in novel research. Apprentice-based research experiences (AREs) are by far the best method for exposing an undergraduate to the process of scientific discovery. This method pairs a single student with a research mentor for training on laboratory techniques and data analysis. This experience can persist for approximately 1 or 2 years while the student is still an undergraduate and after completing prerequisite courses. These experiences are quite rare, however. At a research-intensive institution, a student securing this experience will likely be at the very top of their class and will have competitively edged-out their peers. Even then, the research mentor will probably be a graduate student or a postdoctoral scientist. An impactful ARE resulting in significant and competitive contribution to the field is rare at a PUI. Faculty members at PUIs have their primary appointment as a course lecturer and lab instructor and may not have a teaching assistant to help with these responsibilities. As a result, the AREs are often part-time and disjointed. With major commitments to teaching, PUI faculty members are strained to find time to apply for research funds to obtain state-of-the-art equipment and reagents to keep up with their R1 counterparts.

The Small World Initiative and SEA-PHAGES programs are inclusive Research Education Communities that link students from multiple institutions with a common scientific problem. These programs are highly collaborative but are geared more for introductory level courses. Because significant centralized information is required, limited topics exist for upper-level classes [3]. Course-based undergraduate research experiences (CUREs) have gained in popularity in recent years and have the ability to involve a large number of students in a research question within the context of a required course for their major. A website exists for networking between faculty involved in the implementation of CUREs in their courses (http://curenet.cns.utexas.edu/). Five core components define a laboratory experience as a CURE: 1) Use of scientific practices; 2) Discovery; 3) Broadly relevant or important work; 4) Collaboration; 5) Iteration [4]. Faculty must be intentional with designing CUREs to incorporate these components as not all have demonstrated a positive effect on student performance [5]. The relevance of a research project is difficult for an undergraduate to sense, perhaps especially when it is initiated by a single professor at a small institution. Further, making significant discoveries in a 16-week semester long course can be challenging when new techniques are being introduced. One such CURE developed for an upper-level biochemistry course that has demonstrated success is BASIL: Biochemistry Authentic Scientific Inquiry Lab (https://basiliuse.blogspot.com/). The curriculum exposes biochemistry students to computer modeling and routine laboratory techniques with an ultimate goal of assigning a function to a protein with a known structure. While the program shares valuable resources with instructors desiring to implement an inquiry-based laboratory experience in their course, it may be difficult to encourage student "buy-in" without direct alignment to the instructor's research interest, or contribution to a real-world issue with which students can identify.

The goal of our integrative module was to create a unique iterative CURE (iCURE) which partnered undergraduates from Bethel University, a Christian liberal arts university set in the suburban Twin Cities area, with undergraduates from the University of North Texas (UNT), a Carnegie R1 public research intensive university that is close to being classified as a minority serving institution—with significant populations of Hispanic and African American students and first-generation college students. A novel project spearheaded by graduate student Yingqi Cai in the laboratory of UNT Regents Professor Kent Chapman was adapted for the fall semester Advanced Research in Life Sciences course (BIOL3900), and then passed to Associate Professor Angela Stoeckman at Bethel University for the Biochemistry II (CHE397) spring semester laboratory course. This collaborative research community has persisted for 2 years. In the intervening summer months, the project was maintained by UNT graduate student Yingqi Cai and undergraduate interns from Lycoming College, a residential liberal arts college in Williamsport, PA (see Fig. 1).

Research Question

To provide the students with an exhilarating and educational experience, we intended to find a project with a significant impact on the scientific field as well as an approach fit for a weekly or twice-weekly class schedule. Plant vegetative tissues, especially leaves, are organs specialized for photosynthesis to supply the remainder of the plant with energy and structural molecules. These tissues represent a large portion of the entire plant biomass, which makes them a prospective platform for production of high-value bioproducts. So far, our iCURE was designed to engineer two types of bioproducts (neutral lipids and terpenes) in vegetative tissues. Neutral lipids, mostly triacylglycerols, are an important source of dietary calories and renewable biofuels [6, 7]. Terpenes are a large group of secondary metabolites with industrial and medicinal applications [8]. Neither neutral lipids nor terpenes accumulate in a large amount in plant vegetative tissues. Neutral lipids are mostly stored in the lipid droplets in oilseeds and terpenes

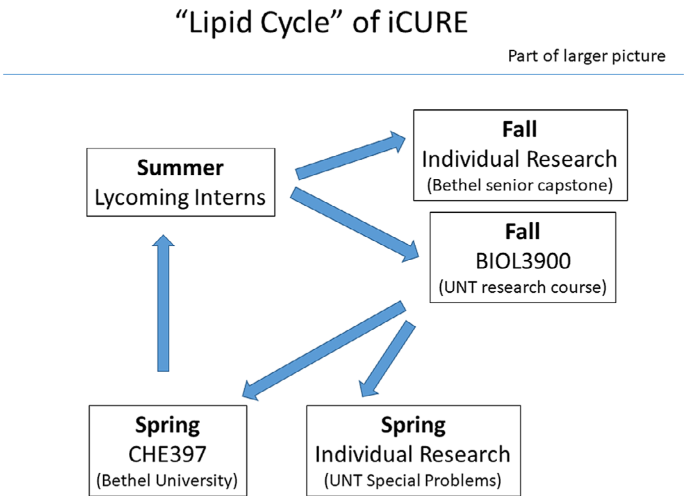

FIG 1 *Diagram illustrating the collaborative nature of this iCURE. Multiple iterations were performed from 2016 to present. [Color figure can be viewed at wileyonlinelibrary.com]*

usually accumulate in specialized plant cells and organs [9–11]. In our iCURE, we tested the functions of two cross-kingdom lipogenic proteins, mouse fat storage-inducing transmembrane protein 2 (FIT2) and diacylglycerol:acyl-CoA acyltransferase 2 (DGAT2), in the accumulation of lipid droplets, neutral lipids, and terpenes in plant vegetative tissues. After the students observed that these cross-kingdom lipogenic proteins could function in plant cells in a manner similar to that in mammalian cells [12, 13], we encouraged the students to evaluate various combinations of these heterologous lipogenic proteins and plant proteins related to lipid and terpene biosynthesis for the most efficient accumulation of neutral lipids and terpenes.

To fit experiments into our class schedules, we established an approach based on the transient expression system in *Nicotiana benthamiana* leaves [14]. This system provides a promising platform to rapidly test gene functions and protein subcellular localizations, as well as evaluate transgene combinations for engineering bioproducts in plant vegetative tissues. We have established a reliable experimental workflow for the students to generate reproducible data in a relatively short time period (Fig. 2).

First, the genes to be tested were cloned and inserted into plant expression vectors, which were then transformed into *Agrobacterium tumefaciens* for plant infection. *Agrobacterium* strains containing genes of interest were infiltrated individually or in combinations into leaves of 4–6 week old *N. benthamiana* plants for transient expression. Five to seven days after infiltration, the infiltrated leaves were collected for quantification of bioproducts using gas chromatography–mass spectrometry (GC/MS) and/or visualization of lipid droplets and fluorescent proteins using a confocal laser scanning microscope. The fall UNT course met twice a week on Tuesdays and Thursdays, and the spring Bethel University laboratory course met weekly on Thursdays. Our rapid analysis approach allowed the students to perform multiple rounds of analysis in one semester and to adjust their experiments based on the data they just collected. Another advantage of our experimental design was that it combined common techniques in molecular biology, cell biology, and biochemistry to solve a research problem with both basic biology implications and biotechnology applications.

Implementation of the iCURE

The BIOL3900 course at UNT was originally developed as part of an HHMI-funded project to enhance undergraduate education. The course was so successful that it and others like it have become part of the regular biology curriculum at UNT, although the courses are no longer funded by external sources. BIOL3900 is capped at 16 students. Most instructional labs at UNT have a capacity of 16–24, so this research course is about the same enrollment as conventional teaching labs at UNT. The major advantage of this course design is that larger numbers of students gain a real research experience, compared to the one-on-one opportunities through "special problems research" courses, where a single principal investigator might mentor one or two students in a semester. This is important in the UNT biology department where there are approximately 2,500 majors, many of which desire a research experience.

Initial presentation of BIOL3900 results by Chapman at the FASEB Lipid Droplet Meeting in the summer of 2016 prompted interest by Stoeckman and expansion of the course program to Bethel University in its current form. Both Chapman and Stoeckman have a long-term research interest in lipid biochemistry, so these topics fit well with their research and educational programs, and make for a natural connection and easy collaboration.

The CHE397 Biochemistry II laboratory course at Bethel University is a required component of the corequisite CHE396 Biochemistry II lecture course. The lecture course typically enrolls 24–30 students each year, with laboratory sections (CHE397) capped at 12 students. The students that enroll in this course are Biology, Chemistry, or Biochemistry/Molecular Biology majors of which the university graduates approximately 50 each year. The majority of these students are juniors and this experience provides students with exposure to biochemistry techniques that inform their decision for a senior capstone research project.

At the beginning of both BIOL3900 and CHE397 courses each semester, the course syllabus and relevant primary literature were made available on the course website or course-management system (Moodle) to provide students with the necessary background knowledge. During the first class period, an introduction of the research problem by oral presentation was given by the course instructor including background overview, research objectives, strategy, and methods. At the beginning of each subsequent laboratory period, a brief

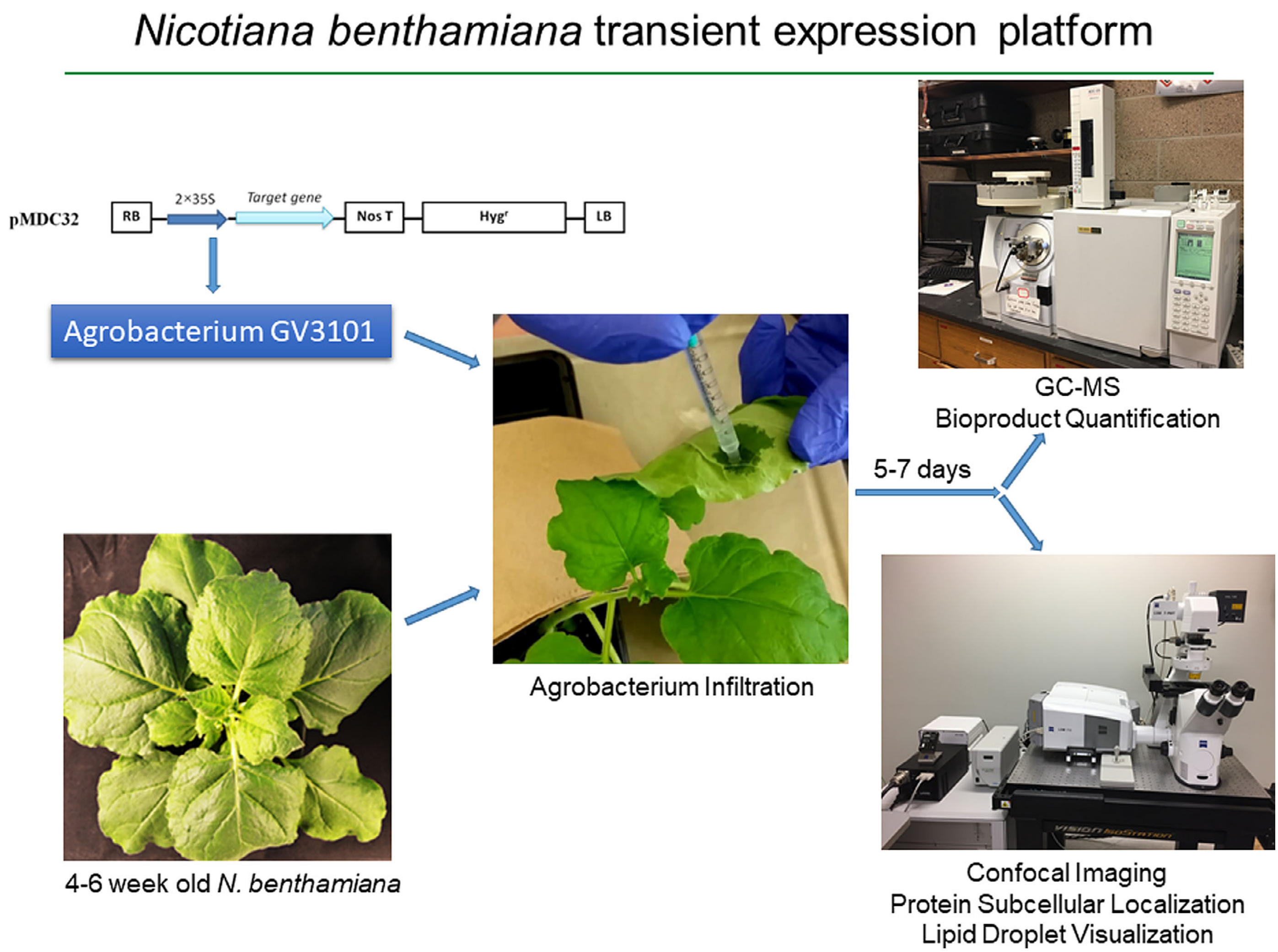

FIG 2 *Experimental flow chart of* N. benthamiana *transient expression system and bioproduct analysis platform.*

lecture helped to organize how the specific experiments of the day would help to solve the research problem.

Students in BIOL3900 at UNT started the project with gene cloning and plant expression vector construction. Then, they tested the gene functions, protein subcellular localizations, and evaluated transgene combinations using the *N. benthamiana* transient expression system (see Supporting Information Table S1 for a sample two-meeting per week experimental schedule). The experimental materials, data and future directions collected by the students were evaluated by research notebook grading, group oral presentations and formal laboratory reports and then shared with the students at Bethel University to help them design their experiments in the following semester. Students in the CHE397 course at Bethel University further optimized transgene combinations for more efficient metabolic engineering (see Supporting Information Table S2 for a sample weekly experimental schedule). Laboratory results from the CHE397 course at Bethel University were assessed by both oral presentations and formal written reports (see Supporting Information for grading rubrics). Summaries of student reports were shared with UNT for another iteration of the iCURE.

Benefits and Outcomes

The benefits of this collaborative iCURE are numerous. First and foremost, the qualitative feedback from students at both institutions was overwhelmingly positive. Course evaluations collected from Bethel students at the end of the semester revealed the following:

> *"I loved that the project gave purpose to the lab. Lab work no longer felt as though I was aimlessly checking things off for my grade – the fact that my work could make a difference brought me the motivation*

necessary to succeed. The overlay of the project taught essential laboratory skills through application and included the important aspect of collaboration by networking with fellow scientists in Texas".

"*Personally, I found the biochemistry lab project to be invigorating. I loved participating in novel research, as the results were a bit of a mystery. I also appreciated learning the process of conducting novel, scientific research and intend to apply the learned techniques in future projects. I also felt our specific topic was valuable as well as applicable to the real world*".

"*Tobacco plants were a simple host to work with and interpreting and comparing results with those from the students in Texas allowed me to see the bigger picture of what our research could accomplish*".

In addition, when UNT students were asked open-ended questions at the end of the semester, the following representative comments were obtained when posed with "what aspects of this class contributed most to your learning?":

"*Because the Advanced Research course with Dr. Chapman is a hypothesis driven research class, it allowed us to utilize many lab techniques learned in previous courses, in addition to new molecular techniques, that gave insight in a more practical way. It also elicited creativity in approaching different methods in collecting useful data.*"

"*It was a hypothesis driven class to which we didn't have known results we were expecting, allowing us to go through real-life research.*"

"*Being able to work with less restrictions than the other lab classes.*"

Further, precourse and postcourse surveys were given to UNT students as part of a national CURE project conducted by David Lopatto and Leslie Jaworski (entire survey results from 2016 and 2017 are available in the Supporting Information). Most notable are the results shown in Fig. 3 which demonstrate significantly higher positive learning gains on 21 items appropriate to the iCURE module when compared to students completing traditional CUREs.

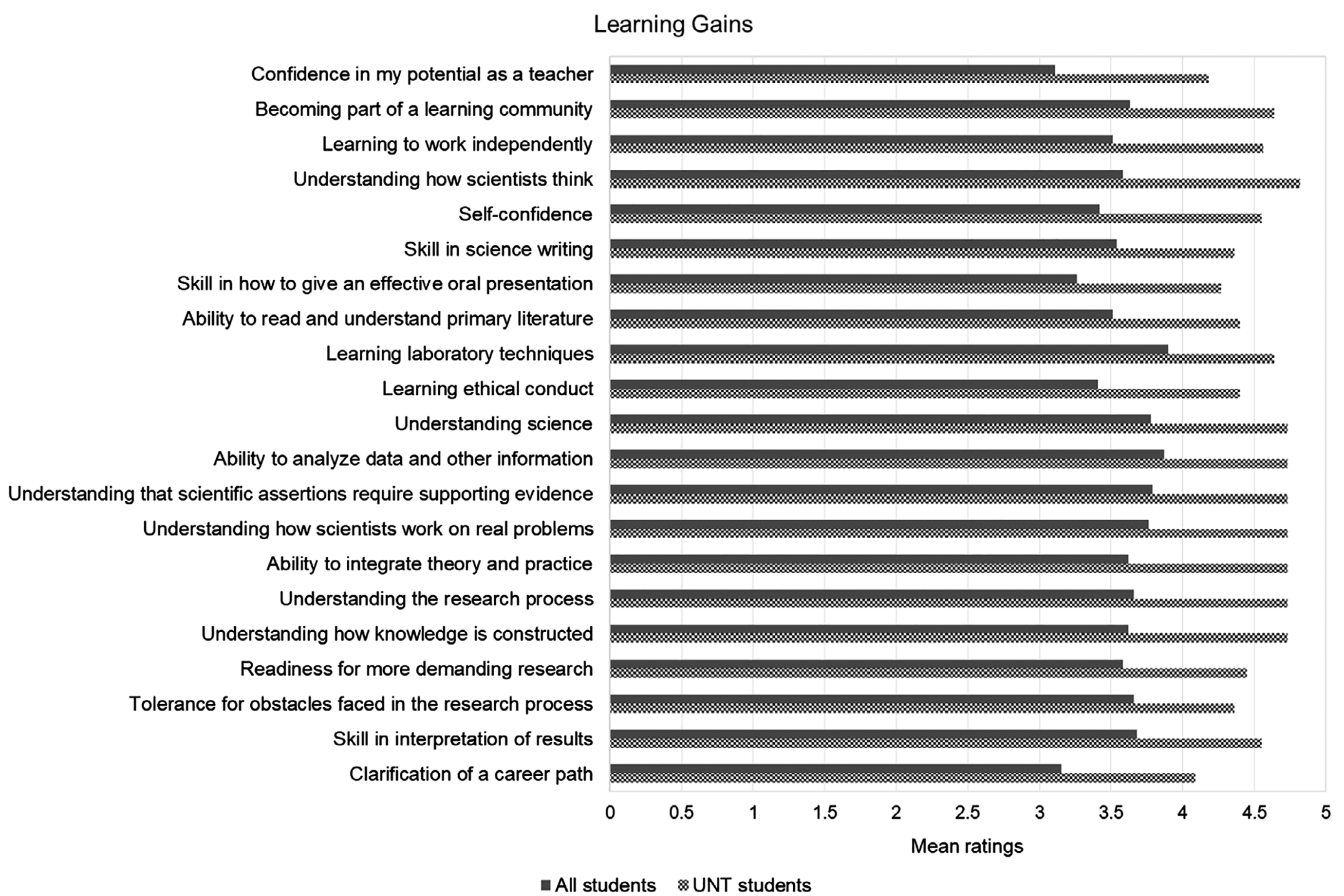

FIG 3 *The data from "all students" includes* n *= 4,432 students nation-wide from when the CURE survey opened on June 1, 2017.*

A second major benefit to this iCURE was its cost effectiveness. *N. benthamiana* plants are easy to grow, maintain, and propagate, and manipulations can be done on the benchtop. Animal facilities or biosafety cabinets often used in cell biology and biochemistry laboratories make participation costly. Plasmids, glycerol stocks of *Agrobacterium* strains, and GC/MS or TLC standards were easily shared and shipped between institutions.

Next, the ability to keep the research moving from one semester to the next allows a project to be competitive. Our iCURE resulted in two research papers published in high-profile peer-reviewed journals [13, 15]. The paper published in *Plant Biotechnology Journal* was featured on its cover in July 2017. These publications revealed the functional roles of a mouse lipid-binding protein FIT2 in lipid droplet biogenesis and coaccumulation of neutral lipids and sesquiterpenes, which suggested a novel strategy of using mammalian lipogenic proteins to promote production of high-value hydrophobic bioproducts in plant vegetative tissues. Authorship decisions in publications that result from iCUREs may be made on a case-by-case basis. Two authors in the first publication listed [13] were students in the BIOL3900 class at UNT who continued as individual researchers. Their subsequent matriculation as graduate students merited additional recognition. Other student researchers from both UNT and Bethel University are mentioned in the Acknowledgement sections of the publications.

Finally, emerging from these research experiences in their coursework allows students to jump into an independent research project, without having to schedule one-on-one time to be taught laboratory techniques. In fact, after completing the Bethel University Biochemistry II course in Spring 2018, two students conducted a project in the following semester to produce other types of sesquiterpenes (α-humulene and β-caryophyllene) in *N. benthamiana* leaves. Taking techniques already learned in this course, they overexpressed *Arabidopsis* terpene synthase TPS21 along with avian farnesyl diphosphate synthase (FDPS) in *N. benthamiana* leaves, which led to accumulation of non-native sesquiterpenes (Fig. 4). The accumulation of α-humulene and β-caryophyllene was enhanced when the students coexpressed the mouse lipogenic protein DGAT2 with TPS21 and FDPS (Fig. 4). These results further advanced our previous study [15] by suggesting that our strategy of using lipogenic proteins to promote the accumulation of a particular type of terpene (capsidiol) could be generalized for engineering various terpenes in plant vegetative tissues.

Future Goals

A major goal for the advancement of the iCURE model of student learning is to standardize the way the two courses are assessed. As this project was a case study, we expect to build on information gained and develop not only a precourse and postcourse survey that is unique to this type of module, but also consistent rubrics for assessment of written and oral presentations and record-keeping in research notebooks. Analysis of these standard measures will then be useful in comparing learning gains between students in the different institutional environments.

Related to goals for the research question, the topics for these courses continue to evolve as results are obtained. Consequently, specific future goals will be determined not only by experimental data, but by student ideas. For example, in this last iteration of the module, students at Bethel University were presented with the results from our most recent research publication "Mouse lipogenic proteins promote the coaccumulation of triacylglycerols and sesquiterpenes in plant cells" [15]. Biochemistry students were then asked to "propose" a different nonpolar molecule which may potentially be incorporated with

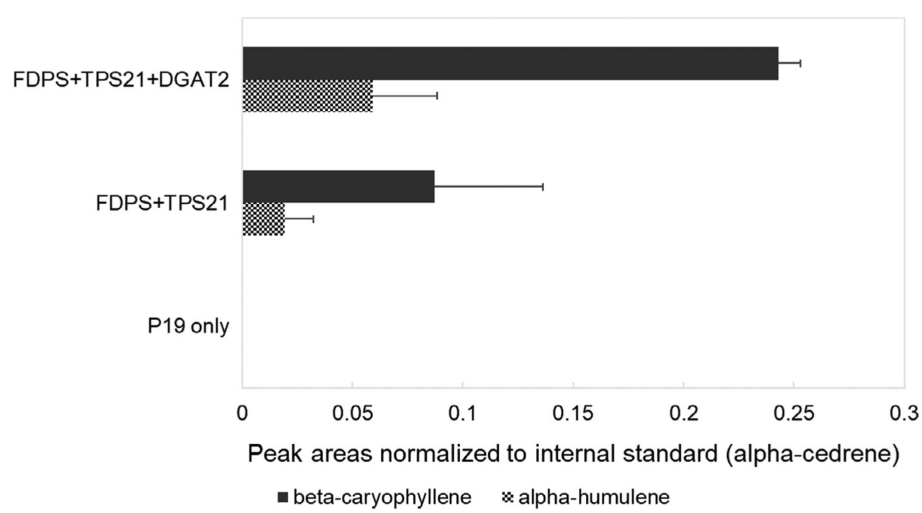

FIG 4 *α-Humulene and β-caryophyllene content in infiltrated* N. benthamiana *leaves. Averages and SEMs are plotted from three biological replicates.*

triacylglycerols in lipid droplets. Student ideas are currently being collected and assessed for experimental feasibility.

As mentioned in the introduction, five criteria are currently defining a laboratory experience as a CURE. We would like to investigate the specific components of our iCURE that elevated student satisfaction above the traditional CURE (see Fig. 3). Further, while students at both UNT and Bethel were aware of each other's contributions, a more personal connection would be valuable in future iterations of this module. Oral presentations at UNT in the fall semester could be recorded and watched by Bethel students at the beginning of the semester and vice versa. In addition, students finishing up their work in the spring semester at Bethel University could write notes to UNT students about what to expect in the fall or research tips learned along the way. In our current societal state where fear-of-missing-out is pervasive, students are motivated by seeing what their peers are doing and achieving.

Materials and Methods

Precourse and Postcourse Surveys

Students at UNT were asked to sign a consent form on the first day of class to allow information about their participation in the course to be gathered and shared. Also on the first day of class, student perceptions of research were captured in a precourse survey, and this was compared with a postclass survey and with student responses collected nationally as part of an HHMI project conducted by David Lopatto and Leslie Jaworski. From their materials:

> "*The CURE survey offers a comparison of learning benefits between course experiences and undergraduate research experiences. The pre-course survey collects student data based upon demographic questions, reasons for taking the course, level of experience on various course elements, science attitudes and learning style. The post-course survey parallels the pre-course survey and includes additional questions that focus on student estimates of learning gains in specified course elements, estimates of learning benefits that parallel questions in the summer undergraduate research experience (SURE) surveys, overall evaluation of the experience, and science attitudes.*"

We are grateful to these educators for their considerable efforts. The implementation of this plant biochemistry project at Bethel University was a new undertaking and student consent for the precourse CURE survey was not obtained in advance of the project, therefore only qualitative feedback is included here.

Experimental Protocols

N. benthamiana plants were grown by the course instructors in a greenhouse or growth chamber at 28 °C with a 14/10 hour light/dark cycle 4 weeks prior to the start of the class. Plant expression constructs were generated at UNT as described previously [13, 15] and shared with Bethel University. The *Agrobacterium* (GV3101) used for infiltration was inoculated by course instructors 1 day before infiltration. Lipid droplet visualization and protein subcellular localization were performed at UNT using a Zeiss confocal laser scanning microscope (LSM 710). Analysis of fatty acids and terpenes was conducted at both UNT and Bethel University using GC/MS equipped with Agilent GC 7890A/MSD 5975C system and HP-5MS capillary column (UNT) or Shimadzu GC-2010/QP2010S and Restek Rxi®-5MS capillary column (Bethel). See Supporting Information and the class webpages (https://guides.library.unt.edu/biol39002017; https://guides.library.unt.edu/biol39002016) for detailed protocols.

Acknowledgments

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