SCRI: PIP-CAP NEWSLETTER

Bi- Annual Report
Fall 2023
Volume 1: Issue 1

DNA PROFILING OF TWELVE STRAWBERRY CULTIVARS

NORTH CAROLINA'S FIRST COMPLETELY ENCLOSED INDOOR STRAWBERRY NURSERY

SOIL LESS SUBSTRATE RESEARCH FOR STRAWBERRY PROPAGATION

PIP CAP
PRECISE INDOOR PROPAGATION
COORDINATED AGRICULTURE PROJECT

This work is supported by Specialty Crop Research Initiative (SCRI), Grant no. 2021-51181-35857, Project accession no. 1027418, sponsored by the USDA National Institute of Food and Agriculture.

Mark Hoffmann, project director, shares his thoughts about PIP-CAP.

Chrsitina Ippoliti, PhD student, (left) and Zhongchi Liu, professor, (right) at the California Strawberry Center.

IN THIS ISSUE

A bay with various strawberry cultivars inside Grafted Growers, a fully enclosed, indoor nursery in Raleigh, NC.

Stratified soil-less substrate research at NC State University.

DIRECTOR'S LETTER

Dear friends and colleagues,

"You're only ever really as good as those around you. Your crew [...] accomplish things you never could do alone" (Picard, S3:E4). For once, I could not resist the temptation to use a Star Trek quote for my first official letter as director. The Star Trek franchise has always inspired me. I deeply believe that every "Trekkie" learns invaluable lessons about humility, compassion, peace, equity, teamwork and leadership. Things that are hardly conveyed in other places, when it is more often than not the hero who fights a villain. One lesson I took very much to heart reaches through this newsletter as well as the entire project: People First.

As the 'Enterprise' could not function without the most capable and passionate people on board, the same is true for any research project. This newsletter will be a vehicle not just to convey our research, but - foremost and most importantly - to shed light on all of you: students, post-docs, technicians, PIs, and hopefully growers and industry partners, without whom none of our success stories would be possible.

The Enterprise is the best ship in the fleet because of the crew, who goes beyond and above what's necessary. In this first issue we highlight exactly that work on our 'ship'. And the first and loudest shout out needs to go to Lizeth Vigil, a phenomenally organized, sharp, compassionate and absolutely brilliant person. I feel honored to work with her, and the value she brings to this project can hardly be overstated. Without Lizeth, this ship would not even have left the docks.

But we did leave. And today, we have reached new frontiers: the whole genome sequencing by University of Maryland's Dr. Xi Lou and Dr. Zhongchi Liu, Dr. Brian Jackson's and MS student Brandan Shur's enthusiastic research on soil-less substrate, or Dr. Ricardo Hernandez' engagement to develop an indoor strawberry nursery with Dr. Ibraheem Olasupo and PhD student Michael Palmer. All those projects are united in the brilliance, enthusiasm and passion of all of us. Last, but not least, those attributes also must be given to the most amazing communication team one can wish for: Alexa Artis and Amanda Lewis, who are the reason this newsletter 'beams' us into the new and exciting world of SCRI PIP-CAP.

All that is left to say is 'Engage'. Take us to Volume 1, Issue 1 of the brand new PIP-CAP newsletter.

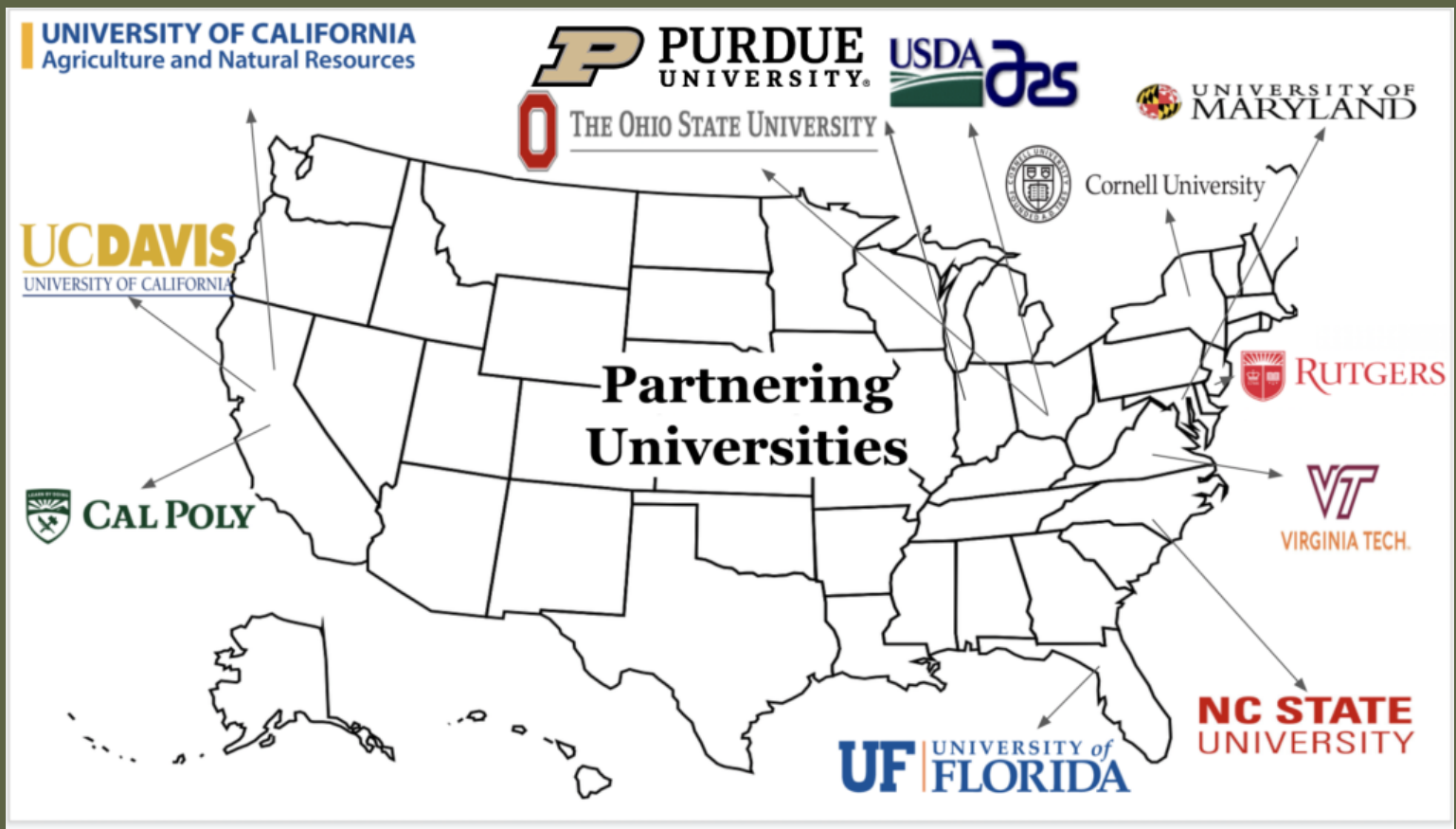
Dr. Mark Hoffmann
Project Director

SPECIALTY CROP RESEARCH INIATIVE

Precise Indoor Propogation - Coordinated Agricultural Project

PIP CAP

The entire PIP-CAP team wants to thank our stakeholders and partners for their continued engagement and support. We started this process in 2017, and since then it has been nothing short of joy and excitement. This is a truly great industry to be part of. Thank you for embracing us, for all your support, and for growing closer together every day.



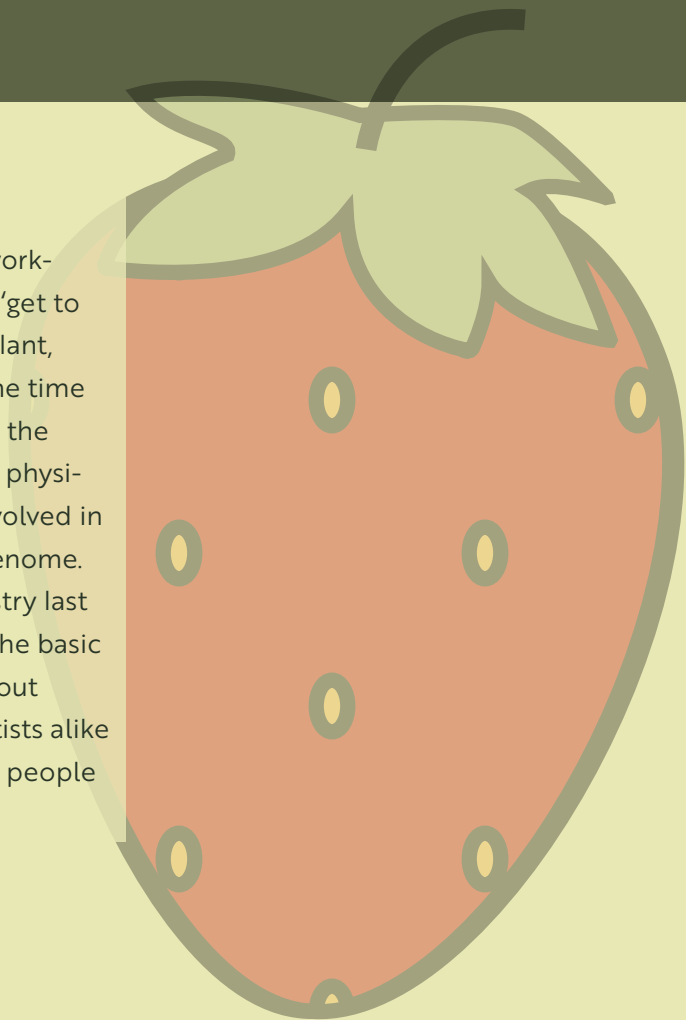
PROJECT OBJECTIVES

1. Characterization of mother plant physiological responses to the environment.
2. Development of environmental protocols for transplant establishment, conditioning and long-term storage.
3. Development of a genetic tool to elucidate the runnering and flowering potential of genotypes, based on phenotypic responses to environmental treatments.
4. Determine expected economic costs/returns to industry of adopting developed techniques, and estimate the economic impact of adoption on the US strawberry supply chain.
5. Translation and integration of new propagation systems with industry partners.
6. Development of services and products, extension and outreach activities to industry and public stakeholders.

By Dr. Mark Hoffmann

The six objectives listed above reveal some, but not all, of the inner workings of this project. What gets me excited is that this we are all about 'get to know each other'. Be it the genetics or physiology of the strawberry plant, or the strawberry nursery industry. We learn every day, and at the same time have the real opportunity to develop meaningful outcomes based on the knowledge we gain. In the past 18 months, we learned so much about physiology of short-day and long-day genetics, we learned about genes involved in flowering and runnering and how their mutations on the octoploid genome. We learned about the strawberry nursery, growing and shipping industry last summer on our field trip. Now, as the project slowly transitions from the basic research into applied phases, we are ready to learn more in depth about plants, genetics and industry, leading to tools that industry and scientists alike will be able to use in the future. Now, let me introduce some of those people who are helping to get us there.

Click here for more details.



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Thanks so much to our investigators, academic advisors and students for working with us on this project!

All of your hard work is greatly appreciated, and we're thrilled to be building a future with cleaner strawberries with you.

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Name	Position and School	Project Team
Rocco Schiavone	Frmr. Technician @ NC State	Translational
Emma Volk	Frmr. Technician @ NC State	Physiology
Ian Mellon	Technician @ NC State	Translation
Caleb Stephenson	Technician @ NC State	Physiology
Lian Duron	MS Student @ Purdue	Physiology
Ava Forysteck	MS Student @ Cornell	Translation
Samson Humphrey	MS Student @ NC State	Physiology
Brandan Shur	MS Student @ NC State	Physiology
Samantha Simard	MS Student @ Cal Poly	Translation
Calyssa Stevenson	MS Student @ NC State	Translation
Baker Aljawasim	PhD Student @ Virginia Tech	Translation
Jung Hoon Han	PhD Student @ NC State	Economics
Chrsitina Ippoliti	PhD Student @ Uni. of Maryland	Genetics
Moein Moosavi-Nezhad	PhD Student @ NC State	Physiology
Michael Palmer	PhD Student @ NC State	Physiology
Yue Shan	PhD Student @ NC State	Economics
Pooja Tripathi	PhD Student @ Ohio State	Physiology
Olga Lialiu	Frmr. Post-Doc @ NC State	Translation
Xi Luo	Post-Doc @ Uni. of Maryland	Genetics
Ibraheem O. Olasupo	Post-Doc @ NC State	Translation/ Physiology
Erin Yafuso	Post-Doc @ USDA	Physiology

IDENTIFYING THE DNA VARIANTS OF 12 STRAWBERRY CULTIVARS

Xi Luo and Zhongchi Liu
Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

Modern strawberry cultivars are genetically complicated. They are octoploids, meaning they have 8 copies of each gene. In comparison, humans are diploids, meaning we only have 2 copies for a gene. These copies of genes are inherited from each parent and interact to determine physical traits (shape, color, height, etc.). We call that 'phenotype'. The specific combination of the copies of genes that determine the 'phenotype', are called 'genotype'.

As diploids, humans are physically very diverse. It's not hard to imagine that there is a lot of 'phenotypic' diversity in octoploid strawberries. Currently, it's unknown how the phenotype of strawberry cultivars is driven by their 'genotype'. We also don't know the specific genetic makeup of most strawberry cultivars. This is important because breeding programs are in desperate need to conserve specific qualities of cultivars (e.g. high yielding), while also improving qualities such as disease resistance. These specific qualities are determined by the genetic makeup, which can now finally be revealed using the latest DNA sequencing technology.

Six short-day and six day-neutral cultivars were studied. The short-day cultivars were Brilliance, Camarosa, Chandler, Fronteras, Radiance, and Ruby June; the day-neutral cultivars were Albion, Cabrillo, Monterey, Moxie, Portola, and Finn. First, we extracted DNA from the leaves of each cultivar and sequenced their genomic DNA via Illumina at 220 million reads per cultivar, which is calculated to be about 145 fold coverage at each locus. Meaning, if the genomic information of each cultivar is like a book, we read the book 145 times to ensure we correctly decode each gene. This way, we can detect rare DNA variants as well as quantitatively determine the genotype at each locus. This resulted in a list of "high impact" DNA variants (i.e. DNA alterations that may destroy the gene), which are likely to impact the phenotype in particular cultivars.

We analyzed the DNA sequences of 14 genes, selected based on their reported effects on runnering or flowering, and identified a number of "high impact" DNA changes. If the entire genome is a book, these 14 genes are key sentences. Specifically for the gene GA20ox4, which encodes a biosynthesis gene for the plant growth promoting hormone Gibberellic Acid (GA) and runnering, we found that 40% of its sequence reads harbor a DNA variant (i.e. a misspelt sentance) in the cultivar Fronteras. In contrast, no misspelling is found in GA20ox4 of Monterey. Our work provides the genetic basis to test if this DNA change in GA20ox4 correlates with different runnering behaviors between the two.

Using DNA sequencing data, we created a comphrensive, high quality, and user-friendly genomic database for 12 modern US strawberry cultivars. This database can be used to pinpoint specific DNA changes in any genes of interest. This database will serve as an important resource for all breeding programs in the US.

The process of the genetics team. We developed whole genomes for 12 strawberry cultivars and are currently making that database available to strawberry breeding programs.

TECHNOLOGY TRANSFER: PIONEERING IS RARELY A LONE PURSUIT

Mark Hoffmann, Michael Palmer, Ibraheem Olasupo, Lizeth Vigil and Ricardo Hernandez
Dept. of Horticultre Science, NC State University, Raleigh, NC

Michael Palmer, PhD, holding a tray of strawberries grown indoors at Grated Growers.

Opening up a new line of thought, a new method of technology or inhabiting a new niche is often associated with one front runner, one special individual. In reality, pioneering is not a solo activity, it often takes a village to move the needle. Keeping this in mind, this project was collaborative from the get-go to achieve one of the main milestones: A fast transition of knowledge and technology into early adopting industry. We'd like to highlight the collaboration between the PIP-CAP team, Grafted Growers LLC and Phlora Inc., as well as the collaboration between PIP-CAP and Plenty Unlimited Inc.

Grafted Growers and Phlora are NC-based ag-biotech start-up companies. Due to their proximity to NCSU, they were a prime partner to set up a fully enclosed indoor strawberry nursery - the first of its kind in NC. But what sounds like a simple task on paper, is a large learning curve for everyone involved, be it modifiying irrigation systems, managing several feet of stolons or a flooded warehouse. We have to thank PhD student Michael Palmer, Post-Doc Dr. Ibraheem Olasupo, and especially Dr. Ricardo Hernandez and the rest of the Grafted Grower's team for their dedication and commitment to overcome such challenges.

Six strawberry cultivars were raised from tissue culture, deep-sequenced for viruses, and established as mother plants (Figure 1). Daughters that derived from those mothers (one generation away from tissue culture) were then rooted at the same facility and shipped for field evaluations to California, Florida and within North Carolina. Each of those steps brought value to our industry partners as well as to the PIP-CAP team. And we only are getting started! In the near future, Dr. Olasupo will begin phenotyping experiments, while the nursery will be scaled up to serve a higher capacity.

Another example of a successful transition of knowledge is our close and consistent collaboration with the Plenty research team in Laramie, Wyoming. What started out as a get-to-know each other has quickly become a monthly meeting between friends, during which we talk - among other things - about strawberries and share information. These conversations have assisted students and industry alike, and helped to develop a mutual environment of trust. Graduate students Samson Humphrey, Moein Moosavi-Nezhad and many more are frequent guests, chatting with the Plenty team about runnering, rooting and research. Four PIP-CAP students visited Plenty for a summer internship program, all of which brought insights back to their home institutions that will improve their research. While you will read more about the internship program in the next issue, two things have become clear: Academia-Industry is a two-way street, and pioneers are never alone.

OPTIMIZING SUBSTRATE USAGE FOR MOTHER PLANT PRODUCTIVITY

Brandan Shur
Dept. of Horticulture, NC State University, Raleigh, NC

Graduate student Brandan Shur and Dr. Brian Jackson at NC State's Horticultural Substrates Laboratory are pioneering the use of soilless substrate stratification in strawberry production to reduce the dependence on traditional substrates, such as coconut coir, peat moss, and perlite. This innovative approach not only has the potential to lower costs, but also to make strawberry mother plant production more efficient by increasing the productivity of the plants.

Soilless stratified substrate..

Substrate stratification is a soilless technique that involves the precise layering or arrangement of various substrate particle sizes or substrate components within a container to optimize plant production. In the context of the PIP-CAP SCRI project, substrate stratification entails carefully structuring alternative and more cost effective substrates, such as wood fiber, in layers beneath a standard substrate mix consisting of perlite, coconut coir, and peat moss. This approach aims to maximize resource efficiency by reducing the overall use of the costly substrates while maintaining the productivity of mother plants.

In this study, 'Albion' strawberry plants were planted into 2.0L pots with five replicates per treatment. Treatments included a control substrate mix that consisted of 50% perlite, 25% coconut coir, and peat moss, with layers of 25%, 50%, and 75% of hammer-milled wood fiber (PTS) made from loblolly pine (Pinus taeda), as well as 100% PTS. This study had a duration of 12 weeks and was grown in a glasshouse on NC State's main campus. Measurements consisted of mother plant and daughter network dry biomass, daughter plant number, stolon number, internode length, total stolon length, mother plant diameter, number of trusses, SPAD chlorophyll content, and weekly pour-thru monitoring of pH and EC. The data that will be shown in this newsletter will be the daughter plant numbers.

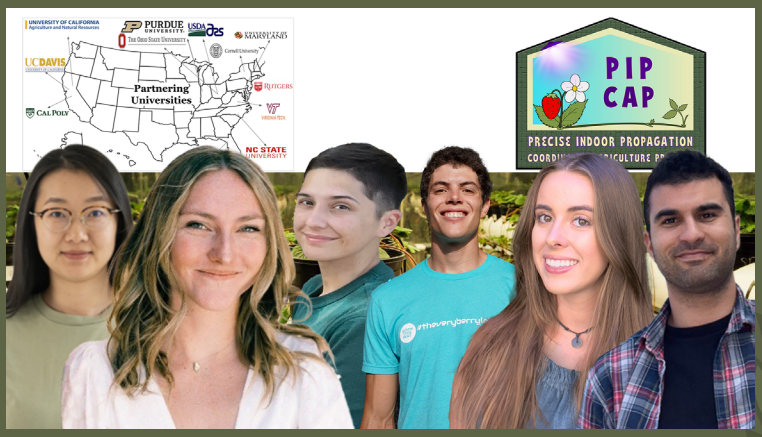
Strawberry plants grown in soilless stratified subrate.

The results of this study reveal a significantly higher number of daughter plants observed in the 25% and 50% PTS layer, as compared to the control substrate. This finding underscores the potential efficacy of our substrate stratification approach, showcasing its potential to enhance the productivity of strawberry mother plants, particularly in layers with reduced substrate components. These results contribute to our ongoing efforts for more sustainable and productive strawberry cultivation methods.

WATCHABLE MATERIAL

SCRI PIP-CAP Internship Series

by the PIP-CAP Communications Team



Six PIP-CAP graduate students and post-docs from four different universities had the opportunity to intern with industry partners during the summer of 2023. Samson Humphrey, Michael Palmer, Moein Moosavi-Nezhad (all NCSU) and Dr. Xi Luo (University of Maryland) spent one week with Plenty Inc. in Laramie, WY. Samantha Simard (CalPoly SLO) spent one at Driscoll's in Watsonville, CA and Ava Forysteck (Cornell University) went to PSI in Watsonville, CA.

How Strawberries are Grown in California

by Gerald Holmes, **Director of the California Strawberry Center, CalPoly SLO**

The CA strawberry industry is one of the largest strawberry industries world-wide. Dr. Gerald Holmes has put together one of the best overviews of how the industry works. This reaches from Nursery operations to growing strawberry fruit in CA. Check out this highly entertaining and educational video.

Strawberry Flower Mapping

by Edward Durner, **Rutgers University**

Dr. Edward Durner from Rutgers breaks down the physiology of a strawberry plant and shares their flower mapping program. The following recording is from the third class of the Southeastern Strawberry School Webinar Series held on April 20th, 2021.

NEXT ISSUE'S OUTLOOK

You can look forward to hearing about these topics next:

- MS Student Profiles
- Post-Doc Profiles
- Student Internship Report
- 2022 Trip to California
- Research highlight: Field Trials
- Research highlight: Strawberry Propagation in Controlled Environments
- & much more!

2022 Trip to California

Members of the project team visited the California strawberry industry, from growers all the way to nurseries in Mcdoel, CA. It was an inspiring and fun experience, and we will report on it in our next newsletter!