Feed the Future Innovation Lab for Collaborative Research on Grain Legumes
LEGUME INNOVATION LAB

2015 ANNUAL TECHNICAL PROGRESS REPORT
(October 1, 2014 – September 30, 2015))

Project Code and Title:

Improving Photosynthesis in Grain Legumes with New Plant Phenotyping Technologies

Lead U.S. Principal Investigator (PI) and affiliated Lead U.S. University:

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Kennedy Muimui, ZARI, Zambia
Wayne Loescher, MSU
James Kelly, MSU
Tim Close, U.C. Riverside
Phil Roberts, U.C. Riverside
Maren Friesen, MSU, Plant Biology

Other Direct Participants and Collaborators

Greg Austic (MSU)
Dan TerAvest (MSU)
Robert Zegarac (MSU)
Donghee Hoh (MSU)
Wayne Loescher (MSU)
Isaac Osei-Bonsu (MSU)
Isaac Dramadri (MSU)
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I. Abstract of research achievements and impacts

(A succinct narrative of technical progress of project, including key research and capacity strengthening achievements and outcomes, during the corresponding performance and report period--1200 character limit)

To avert food shortages and feed its growing population, there is critical need for increasing the productivity of grain legumes in Zambia, which ranks 164 out of 184 countries in the Human Poverty Index. Grain legumes are important crops in Zambia constituting both critical sources of protein and income. Bean production is constrained by its low inherent photosynthetic efficiency which is highly sensitive to abiotic and biotic stresses, including diseases, pests, low soil fertility, heat and drought.

To achieve major gains in yield, we need to improve both the robustness and the efficiency of photosynthesis. This is a complex problem requiring the combined application of advanced genomics and high throughput phenotyping approaches. We will take a critical step in this direction by establishing a base of phenotyping technologies and advanced genetics and genomics approaches to identify quantitative trait loci (QTLs) that condition more efficient and robust photosynthesis and productivity in cowpea and common beans. We will also test the ability of a newly developed research platform, PhotosynQ, to enable researchers and farmers to conduct plant phenotyping experiments, analyze data and share results, and thus allow improvements in breeding and management on local to global scales.

Our approach is to harness two new phenotyping technologies, the Dynamic Environmental Phenotyping Imager (DEPI) and the PhotosynQ platform, a field-deployable network of handheld sensors (MultiSpeQ) and associated on-line communication and analysis tools.

II. Project Problem Statement and Justification

(Present a concise problem statement and justification for the research and capacity strengthening activities carried out in this project. Please do not provide a cut-and-pasted version of the project problem statement from the Technical Application. -2000 character limit)

The goals of the proposed research are to assess the possibilities of 1) accelerating breeding efforts to improve grain legumes using two innovative technologies for high-resolution, high-throughput phenotyping and 2) integrating these tools into a region-led, multi-national effort to improve grain legumes for agricultural production in Africa. The proposed project addresses several challenges that currently limit the application of these techniques for phenotype-driven plant screening, selection and engineering for agriculture in Africa, including the cost of the instrumentation, the availability of networks to share and analyze results and computational tools to usefully interpret phenotypic measurements in terms of genetic variations in yield and robustness. Advances in Internet communications, rapid prototyping and manufacturing, basic and applied science (including genetics, genomics, biological spectroscopy and data mining) are providing opportunities for professional and citizen scientists everywhere to “leapfrog” old technological impediments and take leading roles in improving local crops. Furthermore, a dramatic drop in price and increase in accuracy of sensors means that tools to measure soil, seed, and plant health do not have to be prohibitively expensive for anyone, anywhere.

III. Technical Research Progress

(Describe the research activities (research methods, studies conducted, analyses completed, and significant findings) completed under each objective during the FY 2015 reporting period. Present sufficient detail so that the reviewers will understand and have confidence that the research was carried out in a manner that meets high scientific standards. Discuss briefly primary results, findings or technologies developed that give evidence of technical progress toward objectives.

If certain research activities described in the FY 2015 Workplan were not completed, these must be identified and an acceptable explanation provided in the field (VI) "Explanation for Changes." If implementation of selected research activities will be delayed until the coming Fiscal Year (FY 2016), please also indicate this and again provide a brief reason for the workplan change.)

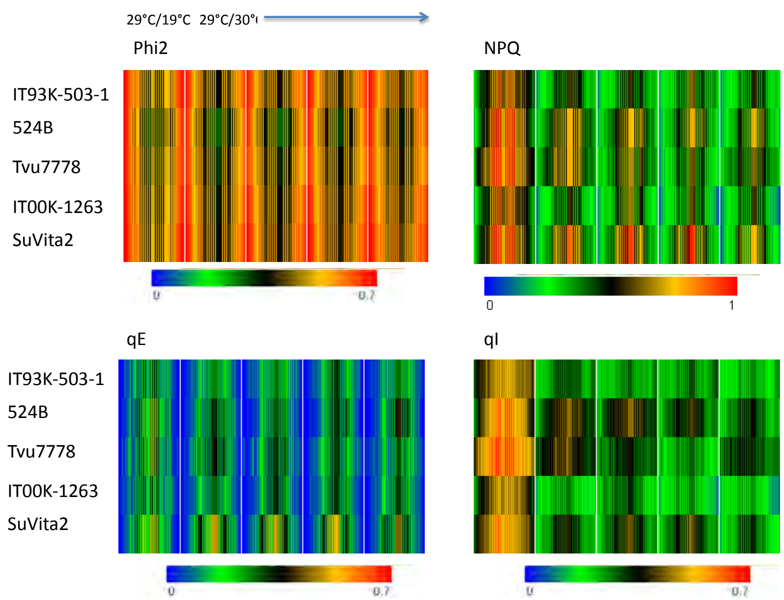

Figure 1. Differential effects of nighttime temperatures on photosynthetic parameters in five cowpea accessions. Shown are a series of heat maps taken over a five-day period depicting the responses of four photosynthetic parameters, the quantum efficiency of photosystem II (Phi2), nonphotochemical exciton quenching (NPQ), 'energy dependent' NPQ (qE) and long-term or photoinhibitory NPQ (qI). On the first day, plants were exposed to 29°C during the day and 19 °C the night. On the second day, this regime was changed so that the nighttime temperature was increased to 31°C.

Objective 1:

Objective 1) Probing photosynthetic responses in RIL and GWAS lines.

This objective aimed to determine if DEPI could reveal phenotypic differences in cowpeas and common bean RIL and GWAS lines that could potentially be mapped. In the 2015 work period we tested identified conditions that result in substantial photosynthetic phenotypic differences between selected cowpea and common bean parent lines. Because of changes in the start date, we focused mostly on cowpeas. We found clear differences in responses of photosynthesis to fluctuating light, daytime temperature and nighttime temperature (Figure 1). All three effects are potentially important for plant productivity. It is becoming increasingly clear that responses to rapid fluctuations in environmental conditions, especially light, are critical for efficiency and robustness of photosynthesis.

Interestingly, we saw large phenotypic variations with lower rather than higher daytime temperatures; this phenoptype may be important for breeding plants that perform well in cooler climates. Finally, effects of nighttime temperatures are very interesting, and perhaps most immediately applicable, given the known effects of high nighttime temperatures on flower viability and yield.

These results set us up for more detailed experiments, specifically to determine if we can identify QTLs that condition these response. Thus, in the 2015-2016 work year, we will focus on building rapid, high throughput methodology for mapping QTLs associated with these properties using the DEPI platform. The key question we will ask is: how can we reliably (with high statistical power) probe photosynthetic responses in RIL and GWAS lines?

Objective 2: Increase the capacity, effectiveness and sustainability of agriculture research institutions which serve the bean and cowpea sectors in the target FTF countries by establishing an African-USA community of networked scientists, extension agents, students and growers to address field-level research and production questions.

A major goal of this aim is to test the feasibility of using PhotosynQ to enhance local efforts to improve grain legume productivity. To achieve this, the project will integrate our HC collaborators at each stage, enable them to train and lead collaborators in both US and HC sites, and test the utility of the platform in the HC.

In 2015, two graduate students—Isaac Dramadri (from Uganda, currently in the Kelly lab at MSU), and Kelvin Kamfwa (from Zambia, Uganda currently in the Kelly lab at MSU), were trained in the operation, theory and use of the PhotosynQ platform for local field application. They then used these devices to perform field experiments, the results of which are now being processed. We have also improved the reliability, calibration and appropriate methodologies for the field experiments in greenhouses and fields at MSU.

A second advance in this objective was the validation and testing of the PhotosynQ platform. Graduate student Isaac Osei-Bonsu performed key experiments that established that the PhotosynQ MultispeQ device was able to rapidly measure photosynthetic responses that are relevant to field conditions (Figure 12). He also showed that the two platforms (DEPI and PhotosynQ) produce comparable results and thus that we can probe photosynthesis under both controlled laboratory and true field conditions. These results are part of a manuscript on PhotosynQ that will be submitted soon.

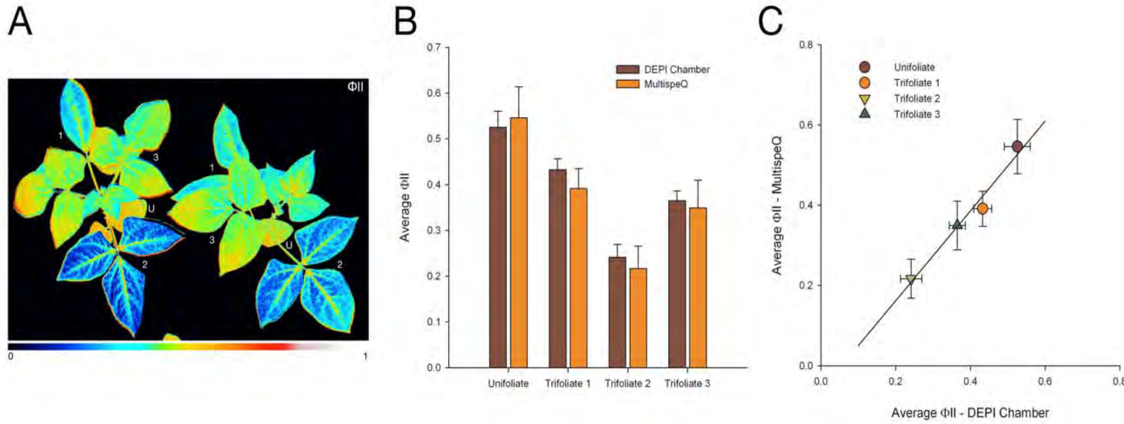

Figure 2: Comparing PSII yield (ΦII) measurements performed on cowpeas in a DEPI Chamber and using the MultispeQ, measuring the light intensity in the chamber and replicating it inside the MultispeQ using the red actinic LED. The measurements using the MultispeQ were taken on the same leaf used to determine ΦII from the image collected in the DEPI Chamber. (A) Example false color image of cowpea recorded in a DEPI chamber. The coloration represents the measured ΦII values as indicated in the color gradient below. U – Unifoliate, 1-3 – Trifoliate. (B) Averaged ΦII values from three biological replicates for the Unifoliate and the first three Trifoliates, comparing the DEPI Chamber and the MultispeQ. (C) Individual ΦII measurements recorded with both instruments. The line represents the linear fit ($R^{2=}$ 0.9614).

Most exciting, we have initiated an analysis of data from both LIL-funded and other projects using PhotosynQ in an effort to determine which parameters and approaches may be useful indicators of the productivity and robustness of photosynthesis. By comparing the results from multiple MultispeQ field trials in both Africa and U.S., we were able to develop methods that give early estimates of crop yield and the onset of diseases, which we think can be applied in Africa to crop management, determining the genetic bases of performance phenotypes, and directing breeding efforts. It is important to note that the commonly used phenotyping measurements (SPAD, photosystem II photochemical efficiency) by themselves were poor predictors of yield or disease. However, MultispeQ provides rapid measurements of multiple (both traditional and novel) phenotypic parameters, and multi-variant analysis algorithms, including contributions from environmental and plant multiple phenotyping parameters, showed strong correlations with eventual yield. An example data set is shown in Figure 3, depicting the correlation models for seed yield for a trial using four sunflower varieties. MultispeQ data were taken during flowering. The “standard” photosynthetic parameter Phi2 (R2=0.15, top panel) showed only weak correlations, whereas an algorithm using five parameters showed good correlation (R2=0.66, middle panel). When variety was included in the model, the correlation was further improved (R2=0.88, bottom panel). This result is important because it indicates the possibility of distinguishing between variety and field conditions impacts on yield. Qualitatively similar results were obtained for pigeon pea

(Malawi) and wheat (MSU).

In addition, trials on Soybean sudden death syndrome (Michigan) suggest that PhotosynQ parameters may be used as early indicators of disease.

Based on these results, we will accelerate and expand our proposed work.

In 2016, our major focus will be on moving the platform to the fields in both the US and HC. Specifically, Kelvin Kamfwa will initiate his part of the project at University of Zambia, involving four masters or Ph.D. students.

Training and initiation of field research at UC Riverside and NDSU. We are expecting a new student, Isaac Osei-Bonsu, a Legume Scholars Program, from Ghana to join the project. Isaac will initially work on the with the UC Riverside or NDSU groups to help train and interpret data from common bean and cowpea field trials set up with our collaborators in NDSU and UC Riverside. The student team will be immediately supervised by Greg Austic, Dan TerAvest and Jeffrey Cruz (USA, Kramer lab).

The goals for 2015-2016 year:

5) Transferring 15 PhotosynQ MultispeQ units to Zambia and initiating first field trials (Sept 2015-Jan. 2016).
6) Training of four students in Zambia in the use of PhotosynQ platform (Target Date: Jan. 2016).
7) Testing of field measurement protocols in Zambia (Target Date: Jan., 2016)
8) Development of rapid cowpea and common bean phenotyping protocols in DEPI chambers (Target Date: Feb., 2016)
9) Initial feasibility study of cowpea lines for QTL mapping in DEPI (Target Date: March, 2016)
10) Initial feasibility study of cowpea lines for QTL mapping in UC Riverside (Target Date: Sept. 2016)
11) Initial feasibility study of common bean lines for QTL mapping at NDSU (Target Date: Sept. 2016)
12) Detailed study of selected cowpea or bean lines for phenotypes under simulated environmental conditions (Target date: Oct, 2016)
13) Use DEPI results from outcome 12 to determine which sets of lines are most promising for QTL mapping (Target date: March-April, 2016)
14) Assessment of field performance of PhotosynQ platform in Zambia (Target date: Oct, 2016).

IV. Major Achievements

1) First proofs of concept for QTL mapping of photosynthetic properties in cowpea and common bean in controlled simulated environments;
2) Validation of DEPI and PhotosynQ platforms;
3) Initial results that suggest PhotosynQ measurements can produce actionable results from field trials;

4) Training several graduate students

V. Research Capacity Strengthening
(*Describe here how collaborative research activities supported by the project over the past year have contributed to the strengthening of institutional capacity to carryout multidisciplinary research on grain legumes and solve the problems facing the legume sectors in their home countries and regions. Appropriate capacity strengthening items to present in this section include research equipment (>$5,000) procured, laboratory and analytical facilities developed, participation in professional meetings or other networking activities, etc.*

Please also describe in this section the activities completed and equipment procured utilizing FY 2014 and 2015 "Supplemental Institutional Capacity Strengthening" funds provided to host country institutions in the respective project and expended in FY 2015.

We have made progress in several areas of research capacity building.

First, as described above, LIL support was used to test and validate the beta prototypes of the PhotosynQ MultispeQ devices. Important lessons about the manufacturing and calibration process, which were incorporated in the improved "locked beta" units that were distributed in Africa. This knowledge was contributed to the new version of the instrument, will be used to produce the next version of the MultispeQ due out in April 2016.

A total of 10 beta devices, together with computer and other support, have been produced for delivery to Zambia for the 2016 growing season. These will be received by Dr. Kelvin Kamfwa when he returns to Zambia in early 2016.

Several LIL participants have been trained in the use of the PhotosynQ platform, including co-P.I. Kelvin Kamfwa, graduate students DongHee Hoh, Isaac Osei-Bonsu and Jesse Traub, as well as collaborators Tom Close (U.C. Riverside), Phillip Roberts (U.C. Riverside) and Phil McLean (NDSU).

PhotosynQ Lending Library: Based on results from this and other project, we established a PhotosynQ Lending Library program, allowing partners to have access to a library of instruments and training. This allowed us to use relatively few devices to collect a large amount of data. This library has resulted in a large number of new users, both in Africa and in the US, and has services our collaborators in California and North Dakota.

PhotosynQ Mobile Phenotyping groups: Based on our experiences in several countries, we developed and implemented a new platform wherein crews of qualified, trained data collectors can travel to experimental stations and farms and collect large amounts of high quality data in short periods. This effort has been very successful, increasing the number of projects, the number of data points taken and the quality of the results. It also help collaborators analyze, and interpret their results. As a result of this success, we have 'cloned' this program at several locations, including the US. In addition, our Mobil Phenotyping Group leader in Malawi is working to establish his own independent company to phenotype plants across Malawi.

Expanded Network of Researchers and Projects in Malawi. Because of the success of our initial trials, the establishment of the Mobile Phenotyping Groups and very high interest in the technology, we were also able to expand our network of scientists to include 4 researchers from Malawi's Department of Agricultural Research Services (DARS). These four scientists are situated at 3 different research stations across Malawi: Chitedze Research Station, Kasinthula Research Station, and Bvumbwe Research Station. In conjunction with these scientists we were able to successfully conduct data collection on 12 projects both on research stations and on smallholder farms (See table below). These projects were very diverse in their goals and potential impacts to researchers and, ultimately, smallholder farmers in Malawi. These projects range from plant breeding/variety selection of various crops (maize, common beans, pigeonpea, sunflower, sweet potato, and quinoa) to cropping systems studies that focus on the effects of tillage, crop rotation, residue, fertilizer rates, and irrigation schemes on crop production of maize, cowpea, and sweet potato.

VI. Human Resource and Institution Capacity Development

(This section is a compilation of project short term and degree training activities completed by the project during the performance period. This is intended to be independent of research capacity strengthening activities described in the previous section.

1. Short-Term Training

i. Purpose of Training: Instruct users and collaborator in the use of the PhotosynQ platform and MultispeQ device.
ii. Type of Training: both on- and off-site training as well as internet-based instruction, one-on-one with PhotosynQ developers and users.
iii. Countries Benefiting: Uganda, U.S.A., Zambia
iv. Location and dates of training
v. Number receiving training (by gender): 1 F, 3 M
vi. Home institution(s): Several LIL participants have been trained in the use of the PhotosynQ platform, including
   a. Graduate student, now co-P.I. Kelvin Kamfwa (MSU, U. Zambia),
   b. Graduate student DongHee Hoh (MSU)
   c. Graduate student Isaac Osei-Bonsu (MSU, Ghana)
   d. Graduate student Isaac Dramadri (MSU, Uganda)
   e. Graduate student Jesse Traub
   f. Lab members from collaborators in labs of
      i. Tom Close (U.C. Riverside)

ii. Phillip Roberts (U.C. Riverside)
iii. Phil McLean (NDSU).
iv. James Kelley (MSU)
v. Wayne Loescher (MSU)

vii. Institution providing training or mechanism: MSU, U. Zambia

2. **Degree Training**
(Provide the following information for each person in degree training with full or partial financial support from the project.)

i. Name of trainee: Isaac Osei-Bonsu
ii. Country of Citizenship: Ghana
iii. Gender: M
iv. Host Country Institution Benefitting from Training: U. Zambia
v. Institution providing training: MSU
vi. Supervising CRSP PI: Kramer
vii. Degree Program: Ph.D.
viii. Field or Discipline: Plant Biology
ix. Research Project Title (if applicable): QTL Mapping of photosynthetic properties in grain legumes
x. Start Date: 9/1/2015
xi. Projected Completion Date: 2019
xii. Is trainee a USAID Participant Trainee and registered on TraiNet? (?)
xiii. Training status (Active, completed, pending, discontinued or delayed): Active

VII. Achievement of Gender Equity Goals

(Describe progress in achieving gender equity goals set for the project during the performance period.)

VIII. Achievement and Progress Along the Impact Pathway

(At the project planning and workplan development stage, PIs had developed an Impact Pathway identifying major outputs of your research, users of these outputs, vision of success and steps towards achieving that vision of success. In this Impact Pathway worksheets, your project had also identified strategies and action plan your team will undertake over the next 4.5 years to translate the outputs into outcomes. Provide an update on your achievement of these strategies identified in the Impact Pathway so as to gauge your team's efforts and progress towards achieving the 'vision of success'. Discuss any constraints encountered and steps taken to mitigate them.)

Outputs:

We proposed to "Provide advanced scientific instrumentation for developing countries: The project will produce 20 MultispeQ instruments, 16 of which will be delivered to labs in Zambia and Uganda. Qualitatively, these instruments will immediately allow researchers in Africa to perform cutting edge research, enabling them to perform the work described in the proposal. In addition, we expect the capabilities of the instruments to enable researchers in HCs to initiate new research projects. “

In our first partial year of funding, we have exceeded our goals. The beta devices have been produced, tested and refined; a suit of experimental protocols have been developed; the field trials on grain legumes have been performed both in the USA and in Africa; sufficient units are available for the next year’s work in Zambia, and key members of the teams have been trained in the us of the platform; and early results from these trials shows promise for direct applications for stress detection, crop management, and breeding.

We are thus well set up to approach the next year’s goals, which address the remaining metrics.

IX. Explanation for Changes *(Identify and justify all changes or inability to complete research and training activities in the FY 2015 project Workplan. If specific activities have been delayed, indicate when they will be carried out in the future and confirm that sufficient funds have been encumbered to support these activities in the future. Please be reminded that delayed activities will need to be reported in future annual project technical progress reports.)*

We have made more progress than anticipated, and thus our next year goals are accelerated. As detailed in the 2016 Work Plan, we will expand the deployment of the PhotosynQ platform to more users and field trials, and to assess the utility of DEPI to map photosynthetic responses to at least two environmental challenges.

X. Self-Evaluation and Lessons-Learned

(Discuss challenges, failures and certainly successes in implementing the respective collaborative research and capacity strengthening project during FY 2015. Provide also a self-evaluation on the strength of the collaborative relationship among the team of U.S. and Host Country scientists participating in the project and suggestions for improving the effectiveness and productivity of the project. Share lessons-learned and recommendations that might be beneficial to other projects and the Management Office for administration of the Legume Innovation Lab.)

We are particularly excited by the early results from our field trials, which appear to show direct utility for the PhotosynQ measurements in crop management and plant breeding. We are also gratified by the interest in the community in using the platform.

One major goal that we hope to tackle in 2016 is the engagement of women students, researchers, extension agents and farmers. The platforms we are deploying offer the possibility of bringing in more diversity, especially with the expected expansion of our goals. We do have specific goals to support women students in Zambia. However, reaching the broader impact we hope for will take some effort and require expertise beyond that of our group. Thus, and we need and hope to directly engage experts and other gender-equality efforts in this area.

XI. Scholarly Accomplishments

(Identify all publications, thesis completed, presentations, professional recognition, awards, patents and Plant Variety Protection Certificates that give evidence of scholarly accomplishments by both U.S. and Host Country scientists during the performance period. If reprints/copies are available of publications, please mail electronic to the Management Office.)

Two manuscripts citing support from LIL are in preparation.

XII. Data Management

(In compliance with USAID standard provisions, please list all data sets generated by the LIL project that have been submitted to publically accessible data bases for archiving along with their location.)

N/A

ANNEXES:

Annex 1. Tables, Figures and Photos Cited in the Report

Annex 2. Literature Cited *(List of all literature cited in the body of the technical progress report.)*