Advances in Field-Based High-throughput Phenotyping and Data Management: Grains and Specialty Crops

Summary Report

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TABLE OF CONTENT

1. Introduction

In the U.S., tremendous efforts in agriculture have been focused towards crop improvement through plant breeding programs to develop new cultivars with improved food quality, local adaptation to biotic stress and abiotic stress, and higher yield potential. In the last two decades, advancements in sequencing and molecular technologies have significantly improved plant breeding, and thus crop yield potential. However, the pace of automation for high-throughput plant phenotyping or 'phenomics' is not keeping pace with current plant genomic research. This is particularly true for traditional breeding programs where phenotyping must be done under field conditions. Recently, optical sensing technologies have provided increased opportunities to improve phenotyping capabilities for crop improvement through breeding and genomics in both controlled environment and field conditions, due to its inherent benefits such as high-throughput assessment, efficient use of resources, and unbiased evaluations. However, much of these developments are localized to individual institutes (private and public) with diverse and independent crop- or area-specific approaches. Although local crop validation is important, the knowledge on sensing technologies, engineered systems, and data processing approaches can be applied across multiple crops and cropping systems. Therefore, in an effort to integrate multiple sensor-based phenotyping approaches and progress this new area of research collaboratively to advance the pace and capacity of high-throughput phenotyping, a 2-day conference was organized.

The conference on 'Advances in Field-Based High-throughput Phenotyping and Data Management: Grains and Specialty Crops' was held on 9-10 November, 2015 in Spokane, WA. The specific objectives of the conference were to: (1) bring together a team of researchers (scientists and engineers) working on different aspects of high-throughput phenotyping to identify the current status, gaps, challenges, and potential advancements that can accelerate breeding and genomic research; and (2) take an integrated approach towards developing resource-sharing tools for continuous interactions and knowledge-sharing. Topics discussed during the conference are summarized in Figure 1. The following sections summarize the thoughts based on expert presentations, break-out sessions, panel discussions, and group discussions.

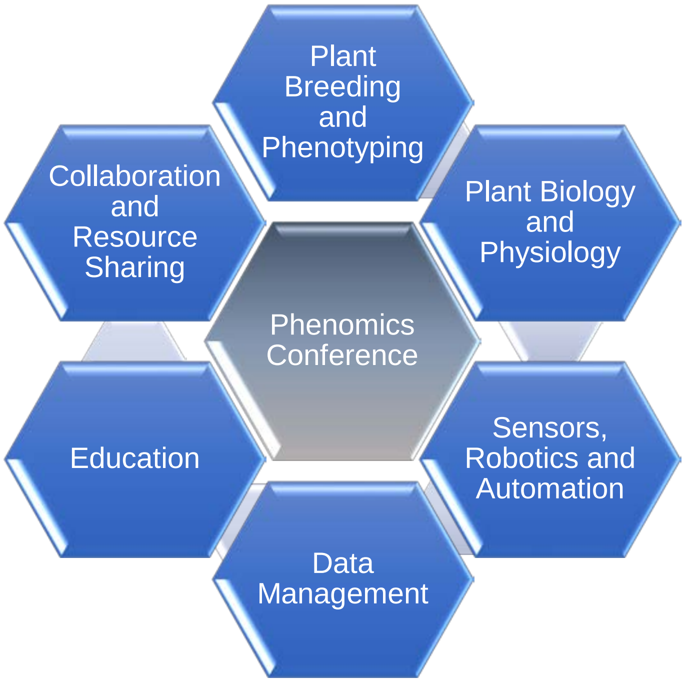

Fig. 1. Topics discussed during the 2-day conference.

2. High-throughput Phenotyping for Crop Improvement and Management: Current Status and Needs

2.1. Crop Phenotyping

Phenotyping is an important aspect of crop improvement. The phenotyping assists in trait assessment for variety selection, assessment of genetic variability to identify desirable traits or eliminate undesirable traits during parent population evaluation, and confident incorporation of genetic tools such as marker-assisted breeding and bioinformatics. The crop improvement process is different for diverse crops such as cereal grains, legumes, tree fruit, and forage crops, although common traits are evaluated depending on the overall goal of the breeding program. For example, assessment of water-use efficiency, evaluating disease or soil-borne pathogen resistance at pre-symptomatic and symptomatic stages, canopy architecture assessment due to its relationship with other critical traits, etc., among others. In general, a common difference between cereal grains/legumes, and horticultural crops such as tree fruits breeding program goals is that the former is leaned towards improving yield potential with abiotic and biotic stress tolerance; whereas, the latter is generally focused towards improving quality, storability, and overall value of the produce. The specific breeding program needs can differ such as field-based automated evaluation of canopy architecture in tree fruits, assessing tuber maturity and specific gravity non-destructively in potatoes, and assessing crop-level measures in grain crops.

Temporal studies in breeding and genomic programs data using sensors can be useful in assessing the dynamic crop responses to environment. However, normalizing the sensor data based on weather conditions, logistics (funds, travel frequency, multiple locations), assessing plant responses due to diurnal variation, and integrating natural soil variability on crop performances remain a challenge.

Some of the common needs in breeding and genomic programs identified during the conference were: development of new sensing tools to evaluate traits that cannot be assessed otherwise, thorough assessment of sensing technologies for crop trait evaluation in a rapid, non-destructive and accurate manner before it could replace standard phenotyping techniques (proof-of-concept studies), high-throughput phenotyping tools to assess roots in-field, tools to assess traits in early growth stages, and high-throughput data processing tools capable of converting sensor data into phenotypes within a short time period for timely crop improvement decisions. The needs for high-throughput phenotyping tools may not be limited to field applications, but tools for assessing produce quality (soluble solids, storability, biochemical composition) in laboratory environment can also be highly beneficial. The need for high-throughput phenotyping tools in controlled environments was especially emphasized for studies involving disease progression (better control of environment) and parent selections. The researchers also indicated for certain phenotypes, the sensor-based rank order may be more critical rather than accurate absolute values for comparing crop performances. Development of sensors to assess products of photosynthesis (sugars, starch, carbon, nitrogen), micronutrients, stoma number and size, which remain a challenge, are desired.

The sensing technologies developed for genomic and breeding programs can also assist other scientists (horticulturist, biologists, plant pathologist, etc.) in their research. Moreover, some of these technologies can be applied in Precision Agriculture towards developing real-time crop production decisions such as monitoring and managing abiotic (heat, water, nutrients) and biotic stress (disease, insect vector) in crops. In addition, other sensing techniques (e.g. technologies for in-tree apple sizing or tree architecture evaluation) can be very beneficial in enhancing the current management practices (such as fitting a thinning model in apple trees).

2.2. Sensing, Robotics, and Automation

Several sensing tools have been applied towards phenomic research, some of the common ones being visible-near infrared spectrometry/imaging, thermal infrared imaging, light detection and ranging (LiDAR) sensor, and X-ray imaging (for root evaluation). Amongst different sensing systems, the visible-near infrared based sensing is the most applied technique. One of the major challenges involves the effect of light quality on spectral information (data) that is utilized to assess phenotype. The light quality can be controlled using artificial lighting, which may affect the phenotype evaluation. Moreover, high quality, high resolution sensory data acquisition can limit the rapidness for data analysis. Similarly, LiDAR can be utilized for sensing crop growth dynamics that can be utilized towards phenotyping; however, some of the potential challenges include the effect of mixed edge, leaf angle, and variable distance between sensor and object, which can affect data quality.

Field-based sensor platforms integrated with sensors can assist in high-throughput field phenotyping. In recent years, several different customized platforms (tractors and sprayers) have been integrated with sensors, in addition to commercial unmanned aerial vehicles (UAVs), to achieve high-throughput phenotyping in field conditions. Some of the challenges in ground platforms involve variable speed at different field terrain conditions, stability of the sensors during motion, limitations for adjusting the sampling rate from multiple sensors (including GPS), and effects of lighting conditions, which can affect the quality of the acquired data and converting sensory data into phenotypes. The application of UAV systems for field phenotyping is limited by sensor payload and other challenges (proximity to airport for legal operation, laws involving licensing for UAV operation, wind conditions, cost-benefit based on associated risks, etc.), although the system offers a simple solution involving transportation from one field location to another, simultaneous plot measurements, and higher field of view (higher rate of data acquisition/high-throughput sensing). The selection of suitable platforms is primarily based on breeding program needs; however, knowledge sharing can assist in establishing standard protocols for field data acquisition. An approach that can potentially be adopted to enhance the application of sensing in field conditions could be adapting field for phenomics rather than traditional approach of adapting phenomics for field, depending on crop of interest without compromising the realistic crop evaluation in field conditions.

Sensor calibration also significantly affects the quality of the data. Real-time calibration in field conditions can enhance the confidence in the sensory data. The traits that remain to be a challenge for field-based assessment given the limitations of the sensor systems include root phenotyping and assessing underground root and tuber produce quality (potatoes, carrots, ginger) non-invasively. Moreover, factors such as crop density needs in certain breeding programs (to avoid tuber bulking and weed growth in potato breeding, to induce competition in alfalfa breeding) to represent real-world scenarios can pose challenges during sensing in field conditions (e.g. plot delineation based on images can be challenging). In addition, data processing challenges involve absence of mathematical algorithms for customized high-throughput analysis and integration of sensors to develop holistic models, especially with increasing complexity of multiple datasets.

The sensing tools have not been fully explored for their potential to assess more physiological, structural, and biochemical traits than those that are currently evaluated. Some of the sensing and automation needs identified were: database of certified/tested sensors in different crops to understand their accuracy, reliability, and limitations, and established relationships between multiple sensing platforms (hand-held, ground, aerial) that could assist researchers towards proper selection of sensing platform and sensors. The sensor development and automation can be addressed in two levels: (i) High-throughput Level: focused on adapting commercial sensors with robotics and automation to enable high-throughput trait evaluation (crop height, vegetation index, plant architecture) that can assist with genomics and crop improvement efforts, and (ii) Discovery Level: focused on developing new sensing tools or adapting sensors from other fields (e.g. biomedical) towards new trait evaluation assisting genomics. *One of the key cultural changes desired to advance phenomics research is multidisciplinary communication between scientists and engineers/technologists as a continuous effort through collaborative research and knowledge-sharing through multidisciplinary meetings and conferences.*

In summary, the phenomic platform development for any crop should involve three focused core areas (Spalding, 2016): Acquisition (raw data production using sensor development, automation, and robotics), Analysis (generation of results using algorithm/program development by converting data into phenotype), and Modeling (creating understanding to relate phenotype to genotype, environmental and influencing other factors). Figure 2 summarizes some of the approaches to assist phenomic research for future.

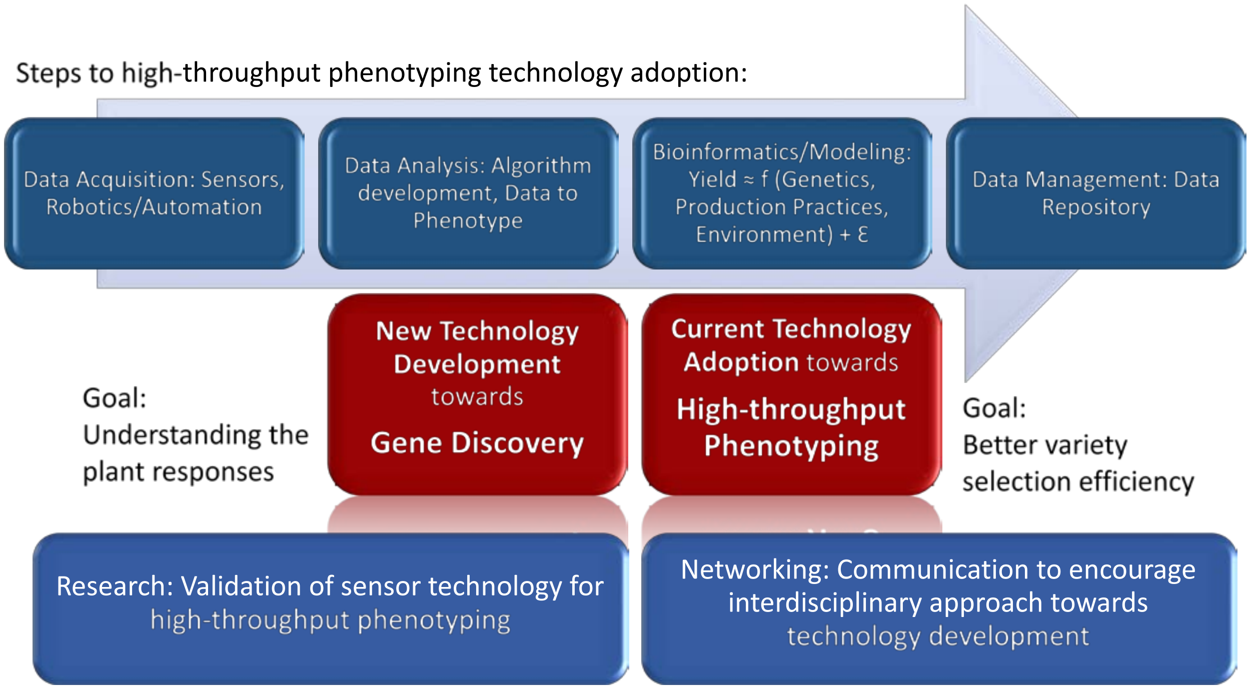

Fig. 2. Phenomic research direction.

3. Educational Needs

Education is a critical component that can greatly enhance the speed at which phenomics tools (sensors, robotics, automations, algorithms, protocols, applications, etc.) can be developed by providing proper training to the next generation researchers. The current educational system does not incorporate multidisciplinary communication, although it is slowly moving towards that direction. Multidisciplinary training is absent even within a science discipline (between biologist, horticulturist, soil scientist, crop scientist, plant pathologist, etc.), let alone between scientists, engineers, and computer scientists. A curriculum change is desired to implement practical multidisciplinary research training. Some of the concepts that were recommended include: (1) setting-up of multidisciplinary 'Institutes' with teams of students having different disciplinary background to encourage collaborative project development, (2) implementing graduate student exchanges (1-3 months or more) to provide a holistic research experience, (3) encouraging 'cultural change' to enable student knowledge sharing and cross-disciplinary communication, and (4) developing focused workshops. One of the major challenges includes availability of funding to allow student exchanges/internships (within and cross disciplinary). Specialized training to researchers working in genomics and breeding programs on the various applications of sensor technologies should be encouraged to allow adoption of sensors into research.

4. Data Management Challenges

Phenomic data management involves three critical components: (i) algorithms and programs to convert the sensory data into phenotypic information, (ii) model development to understand the genotype-phenotype relationships with environmental interactions, and (iii) management of databases to allow resource development and sharing. In regard to the database management, some of the recommended suggestions were as follows: (i) deposition of data into a primary repository as done with human phenomic data, (ii) developing open-source community databases to allow easy access and management of big data for the benefit of all stakeholders (e.g. Genomes to Fields initiative), and (iii) creating complete, accurate meta-data during phenomic data depositions.

In bioinformatics tools development, the challenges that appear during data processing and analysis result from statistical noise due to inappropriate normalization, inappropriate correlation, and harsh thresholding. It should be realized that the standardization of high-throughput phenomic data could be much more challenging than standardization of the genomic data. In the future, emphasis may be given to: (i) development of new community practice for data exchange, (ii) improved techniques for digital representation of the data, and (iii) unbiased assessment of sensor data to eliminate rejection of breeding lines.

5. Platform for Collaborative Research

Private-public partnerships can further enhance the genomic and phenomic advancements that can assist in establishing food security. For example, the concept of better germplasm exchange can benefit all entities of the society, in addition to science and research. There are several phenomic companies that are developing technological tools to assist in high-throughput phenotyping (E.g. LemnaTec, Phenospex, Photon Systems Instruments, Qubit Phenomics, etc.), with a business model to address the phenomic needs for researchers. Some of these companies encourage co-development (system customization, software development for computation) as a process of improving their current product and product utilization.

In regard to developing a phenomics community to encourage collaboration and knowledge-exchange, several platforms within USDA-NIFA and NSF programs, in addition to integration with ongoing efforts by several researchers within U.S. and internationally, are in place. Amongst the platforms discussed were: NIFA Multistate Research Project, NSF Research Coordination Network, Plant Imaging Consortium (http://plantimaging.cast.uark.edu/), and other national and international efforts (e.g. International Plant Phenotyping Network). Although one of the desired outcomes of the conference was development of NIFA Multistate Research Project, some of the limitations recognized were limited funding, limited participation from non-agricultural researchers (computer scientists, engineers) involved in phenomics, and participation generally involves one or few participants within an institute that may not be representative of the regional community involved in phenomics. Funds to cover basic expenses such as conference organization to encourage graduate student participation/communication, website development and maintenance, etc., are also desired. In this regard, NSF Research Coordination Network encourages website and bulletin board development, and networking. An urgent need for multi-disciplinary platform development was identified and encouraged to advance phenomic research.

6. Summary

6.1. Research Needs

Plant geneticists and scientists believe that the current status for the application of high-throughput sensing technologies in crop phenotyping is at '*discovery phase*'; where significant research is needed to further develop this area for technological tools to be adopted as a practical approach towards high-throughput crop phenotyping. It is also indicated that different crops are at different states of readiness for utilizing phenotyping technologies, with much focus given towards cereal/model crops (e.g. Arabidopsis, wheat, maize). An investment in new technologies that can considerably enhance plant geneticists and scientists' research capacities is encouraged. Although the ultimate goal is to develop a low-cost, rapid, and accurate sensing system for crop phenotyping, some compromise based on the sensor abilities for high-throughput phenotyping is acceptable. For example, a low-cost-rapid sensors with lower accuracies in crop phenotyping or rapid-accurate sensors with higher cost for crop assessment. The integration of additional data during sensor data acquisition such as experimental design, soil, and environmental conditions can be useful in data interpretation and analysis. The researchers realize that one of the rapid advancements in high-throughput phenotyping techniques can be implementation of sensor technologies (X-ray sensing, microchips, microsensors) developed for other disciplines (medicine, engineering, space science, etc.) into agriculture.

In summary, future phenomic research can additionally focus on (i) pre-symptomatic assessment of crop responses, (ii) produce end-use quality evaluation (not necessarily field-based assessment), (iii) development of generalized solutions applicable to multiple crops, (iv) development of high-throughput analysis and modeling tools to process phenomic data, (v) community-based efforts to establish test plots (sandbox) to assess and compare multiple sensors, (vi) community-based efforts to create benchmark datasets for computer scientist/data experts to work on to establish data analysis protocols, and (vii) breeding community-based matrix that defines the experimental conditions (plot size, interplant spacing, etc.), phenotypes of interest (simple and complex), and sensing needs to assist technologists towards developing sensing solutions.

6.2. Communication Needs

The scientists and engineers involved in high-throughput crop phenotyping are diverse with committed affiliation and participation associated with their home organization such as American Society of Plant Biologists (ASPB), National Association of Plant Breeders (NAPB), American Society of Agronomy (ASA), Crop Science Society of America (CSSA), Institute of Electrical and Electronics Engineers (IEEE), American Society of Agricultural and Biological Engineers (ASABE), International Society of Precision Agriculture (ISPA), and American Society for Horticultural Science (ASHS), among others. This limits continuous multidisciplinary participation, which is important to advance the field of high-throughput phenotyping and knowledge sharing. *The need and desire for continuous transdisciplinary interaction was greatly emphasized.* Other recommendations include: (1) 'Take an engineer to work day!' concept, where engineers can visit research field sites to understand the needs and practical challenges in field plant research as to build the bridge between disciplines and develop practical solutions, (2) development of activities website to report field data and conferences (to encourage multidisciplinary participation) along with exchange of new concepts, ideas, advancements and publications, and (3) develop an interdisciplinary team to encourage, practice, and implement transdisciplinary communication (plant biologist understanding the capabilities of engineering technologies, and vice versa). The communication between scientists and engineers can be lacking based on their academic/research training, different approaches for problem solving, etc. For instance, the expectations in results (accuracies, correlation, regression) while utilizing sensors for high-throughput phenotyping are different between the two groups (engineers have a higher expectation in regard to sensor accuracies; whereas plant biologist anticipate the practical results based on natural variability in plant responses and environmental conditions). A continuous communication between the two groups will reduce the biases while developing newer technologies during high-throughput plant phenotyping.

7. Conclusion

The conference on ‘Advances in Field-Based High-throughput Phenotyping and Data Management: Grains and Specialty Crops’ involved 69 onsite participants from U.S. with diverse research backgrounds (35% Genetics and breeding, 28% Engineering, 23% Plant biology and physiology, and 14% Bioinformatics). The breeding programs involved multiple cereal grain (wheat, maize, etc.) and specialty (potato, tree fruits, legumes, vegetables, alfalfa, etc.) crops. In general, there were reservations amongst breeders and geneticists on adopting advanced technologies for high-throughput phenotyping in the place of standard methods. However, an excitement and optimism was also observed to utilize sensing approaches for automated/semi-automated phenotyping. The two critical questions that remain to be answered are: *Could phenomics assist in discoveries? Is phenotyping just a tool?* More research can possibly answer these questions in order to utilize sensing tools towards plant research and breeding.

The greatest benefit would arrive “if the high-throughput sensing techniques can be utilized to select good parental varieties that would otherwise be rejected based on standard methods” during plant breeding. This will increase the selection efficiency in field conditions (reducing the rejection rate at each selection stage), thereby providing economic benefits in terms of resources (land space, labor, management, time) and better variety selection. Future research should focus on two phenomic research aspects: (i) Improving the efficiency in which current phenotypes/traits can be measured, and (ii) Measuring new phenotypes/traits that cannot be evaluated otherwise using sensors.

Acknowledgement

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References

Spalding, E. 2016. Towards a more effective, cyber-enabled community of plant phenotype researchers. NSF Software Institutes, Advances in Bioinformatics Grant (OCI - 1216869) funded to investigate the cyberinfrastructure needs of plant phenomic research. More details: http://www.nsf.gov/awardsearch/showAward?AWD_ID=1216869.

APPENDIX I. FINAL AGENDA

Advances in Field-Based High-throughput Phenotyping and Data Management: Grains and Specialty Crops

Red Lion Hotel at the Park
303 W. North River Dr., Spokane WA 99201

9 November, 2015

7.15-8.30 AM Registration *(Prefunction Riverfront AB)*

Conference Opening (Riverfront Ballroom A)

8.30 AM **Program Begins**

8.40 AM **Welcome Address**
Laura Lavine, Ph.D., Washington State University

9.00 AM **What are key research challenges and strategic resource needs for automated field-based phenotyping and data analysis systems—input for the Interagency Working Group on Plant Genomics (IWGPG)**
Jack K. Okamuro, Ph.D., USDA-ARS, Beltsville, VA

Session 1: Plant Breeding and Phenotyping (Riverfront Ballroom A) (Moderator: Edgar Spalding)

9.20 AM **Application of high-throughput phenotyping for varietal selection**
Arron H. Carter, Ph.D., Washington State University

9.40 AM **High throughput phenomics to improve selection efficiency in rootstock breeding**
Gennaro Fazio, Ph.D., USDA-ARS, Geneva, NY

10.00 AM Question and Answers

10.05 AM Break *(Prefunction Riverfront AB)*

10.20 AM **High-throughput phenotyping technologies in cotton and beyond**
Duke Pauli, Ph.D., Cornell University

10.40 AM **Applications of UAVs in potato breeding**
Vidyasagar "Sagar" Sathuvalli, Ph.D., Oregon State University

11.00 AM Question and Answers

Session 2: Sensors and Automation in Field Phenomics (Riverfront Ballroom A) (Moderator: Lee Kalcsits)

11.05 AM **Rapid, in-field, non-destructive sensing systems for plant architecture and internal produce quality in vegetables for high-throughput phenotyping**
David C. Slaughter, Ph.D., University of California, Davis

11.25 AM **5-D LiDAR and its potential to advance phenomics**
Jan U.H. Eitel, Ph.D., University of Idaho

11.50 AM **Advanced sensing for high-throughput phenotyping in grains and specialty crops**

Sindhuja Sankaran, Ph.D., Washington State University

12.10 PM **Proximal and remote sensing for field-based high-throughput phenotyping**
Xu "Kevin" Wang, Ph.D., Kansas State University

12.30 PM Question and Answers

12.35-1.35 PM Lunch *(Riverfront Ballroom B)*
Phenotyping is just a tool
Stefan Schwartz, Phenospex

1.35 PM **Robotics in phenotyping**
Ibrahim Volkan Isler, Ph.D., University of Minnesota

1.50 PM **Preconference survey results**
Sindhuja Sankaran, Ph.D., Washington State University

Session 3: High-throughput phenotyping in breeding programs

2.00-3.15 PM Breakout discussion

Group 1: Riverfront Ballroom A (*Moderator: Jack Okamuro*)
Group 2: Audubon Room (*Moderator: David Slaughter*)
Group 3: Manito Room (*Moderator: Kate Evans)*

3.15 PM Break *(Prefunction Riverfront AB)*

Session 4: Data Management (Riverfront Ballroom A) (Moderator: Sindhuja Sankaran)

3.30 PM **Crop database resources for phenomics data**
Doreen "Dorrie" Main, Ph.D., Washington State University

3.50 PM **iPlant: Infrastructure for large-scale data management and analytics**
Martha Narro, iPlant Collaborative

4.10 PM **Cyber infrastructure for big data from a systems genetics perspective**
Stephen P. Ficklin, Ph.D., Washington State University

4.30 PM **Panel discussion on data management challenges**
Panelists: Dorrie Main, Martha Narro, Stephen Ficklin

5.30 PM Adjourn

6.30-8.00 PM Dinner *(Riverfront Ballroom B)*
Breeding pome fruits for Washington State
Kate Evans, Ph.D., Washington State University
Dinner sponsorship: Monsanto

10 November, 2015

7.00-8.15 AM Breakfast *(Riverfront Ballroom B)*

Session 5: Plant Physiology and Sensing (Riverfront Ballroom A) (Moderator: Gennaro Fazio)

8.30 AM **Phenotyping for calcium related disorders and resistance to abiotic stress in tree fruit**
Lee A. Kalcsits, Ph.D., Washington State University

8.50 AM **Measuring seed traits with automated image analysis**
Edgar P. Spalding, Ph.D., University of Wisconsin, Madison

9.10 AM Question and Answers

9.20-10.30 AM Breakout discussion

Group 1: Riverfront Ballroom A (Moderator: *Edgar Spalding*)
Group 2: Audubon Room (Moderators: *Jack Okamuro; Amit Dhingra, Ph.D., Washington State University*)
Group 3: Manito Room (Moderator: *Lee Kalcsits*)

10.30 AM Break *(Prefunction Riverfront AB)*

Session 6: Education & Private-Public Collaborations (Riverfront Ballroom A)

10.45 AM Panel discussion on education needs
Panelists: Arron Carter; David J. Brown, Ph.D., Washington State University; Gary V. McMurray, Ph.D., Georgia Tech Research Institute

11.15 AM Panel discussion on public-private partnerships
Panelists: Arron Carter, Bob Strand, Gopal Kakani, Ph.D., Oklahoma State University

12.00-1.15 PM Lunch *(Riverfront Ballroom B)*
Field Phenotyping at LemnaTec – The TERRA Reference System
Robert "Bob" Strand, LemnaTec

1.15-2.15 PM Panel discussion on multistate project development
Panelists: Sindhuja Sankaran, Edgar Spalding.

2.15-3.00 PM Summary of breakout sessions
Sindhuja Sankaran

3.00 PM Break *(Prefunction Riverfront AB)*

3.15-4.15 M Reporting and future plans

Funded By: USDA United States Department of Agriculture National Institute of Food and Agriculture

Dinner Sponsor: MONSANTO