AEIC 2021 Spring Meeting Minutes

P.L. Hunst, AEIC Secretary

Virtual Meeting

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AEIC Spring 2021 Meeting Minutes

April 13-14, 2021

Virtual Meeting

P.L. Hunst (BASF), Secretary

The AEIC Spring 2021 Meeting was held virtually on April 13-14.

AEIC BUSINESS MEETING

Approval of 2020 Fall Meeting Minutes: The minutes were approved by a member vote to approve the minutes as posted on the AEIC website.

Treasurer Report (L. Muschinske): The Treasurer presented the 2021 budget as follows:

ITEM	PROJECTED	ACTUAL
Beginning Balance	38393	38393
2020 dues	6800	4325
Mtg registration	2250	
TOTAL REVENUE	**9050**	**4325**
Expenses		
Scientific paper	2000	
DE Franchise Tax Report	25	25
ANSI/ISO Initiative	2900	
Board Meeting Expenses	300	
Spring Meeting 2020 Expenses	1500	
Website Expenses	500	240
Credit card proc	400	58
Fall Meeting 2021	6000	
Graphic design		
Marketing (brochure)	500	
Subscriptions		
Miscellaneous		
TOTAL	14125	322
BALANCE	31318	40396

A motion was made, seconded and voted positive to approve the Treasurer report.

Membership Update (L. Muschinske): The following table depicts the current membership composition of AEIC:

Category	*Number*	*Projected Dues (Paid)*	*Unpaid*	*Unpaid amount ($)*
Large Companies	7	7000 (3500)	3	1500
Medium Companies	11	4000 (2750)	5	1250
Small Companies	9	1500 (1125)	3	375
Associate Members	3	75 (75)	0	0
Individual Members	2	100 (0)	2	100
TOTAL	**32**	**$12675 (6950)**	**0**	**$3225**

Website Updates (D. Houchins, Romer Labs): Donna H. has taken over for David L. to head up the sub-group. Under "Instructional Materials" section, there will be a clean-up of resources. The "Jobs" section will be removed. The next changes recommended are an update of the FAQS and online resources. The listing of publications will be maintained. The sub-group is looking for additional feedback and suggestions. One question was whether videos could be available on the website. The website has a limitation for file size so it was suggested using another venue such as Google for videos. Matt C. will look into this. One suggestion was also to delete the education page but this is a disconnect with the AEIC mission. Beni K. challenged the group as to whether we are doing our mission as linking to other organizations does not really qualify as doing our mission for education. In the past, AEIC organized workshops with EPA, USDA GIPSA to do training and education. In the future, AEIC may want to consider something for gene editing and detection. AEIC needs more than a website to do education. A request for volunteers was made. The group is now Donna H., Ann S., Ray S., Phil B., Beni K., Farhad G., Luke M., Matt C., Lucy L. who will prepare a proposal for gene editing for the Fall Meeting.

Introduction of 2021 AEIC Vice President (L. Liu): Matt Cheever introduced Kristen Kouba as AEIC VP. Kristen was elected to the position at the end of 2020. Kristen currently works at Corteva. She has been a co-chair of the Protein Working Group.

Fall Meeting 2021 (M. Cheever): The Fall Meeting may be a face-to-face meeting depending on company travel policies. BASF would host the meeting in RTP, NC. Suggested topics were:

- AEIC eduction/training
- Enzymes used in food processing
- mRNA in agriculture
- sustainability
- detection of weed seeds by PCR
- species identification by PCR rather than visual inspection

Composition Working Group (P. Brune, Syngenta): The CWG has been discussing the use of the Dumas method for crude protein instead of the Kjehldahl method. The Dumas method is shorter and more environmentally friendly. There is a lot of information on both methods in the literature but there has been no comparative assessment. The group is planning to put together a funding proposal to do comparative assessment on corn, soybean, canola and cotton to generate more data. In addition to Phil and Mohamed, Nancy G., Carl M. and Keith are working on proposal.

Protein Working Group (K, Kouba, Corteva; T. Geng, Bayer): The Protein WG has five ongoing workstreams.

- Allergen Analysis workstream has published a paper on MS analysis of in vitro digestion products and whether it improves assessment of allergenic potential in the J. of Reg Science.
- The workstream for Mass Spectroscopy for protein quantitation concluded MS is a viable protein technique and have developed a paper to compare with ELISA and show options for quantitating proteins. Not every lab would have MS capability so do not want to eliminate all possibilities.
- The Multiplex Validation workstream meets monthly to discuss their goal to publish guidelines for multiplex analysis.
- The Extraction Efficiency workstream is planning to publish a paper to describe harmonized extraction efficiency method and how this would affect/impact present and future registrations.
- The Intractable Protein group is looking at the technical challenges of these proteins and also at standardizing methods.

NGS WG (Sonia H., Syngenta): Members of the group are Beni K., Sonia H., Min, Farhad G., Huihua F., Matheus B., and Sherry W. The group would like to see the website updated with information on the latest bioanalytical technologies starting with NGS. The group has met twice and discussed draft versions for the website FAQs. A proposasl is to act as the nucleic acid working group to provide information on nucleic acid detection methods and create educational materials.

The Business Meeting was adjourned.

INVITED TALKS

Update on Genome Editing Publication (Ray S., BASF): The book "Application of Sampling and Detection Method in Agricultural Plant Biotechnology" is an international handbook based on a series of workshops carried out for governments in collaboration with ILSI. It provides technical and practical information needed to develop, validate and use detection methods. Chapter 14 is on Detection of Genome Edited Products. The book was begun in 2016 and all chapters were submitted by 2019. 12 of 15 chapters are completed with 3 still under review. Project submitted to Elsevier in March 2021. Chapter 14 on genome-edited product detection was begun in summer of 2020 and completed by end of 2020. It is expected to be completed by April 2021. An invited journal article is also under development for SIVB-Plant to be published in the summer 2021.

USDA Final Rule for Hemp Production (Marielle Weintraug, Pres. US Hemp Authority): Marielle is originally from Eurofins and started working in hemp methods in 2016. She is now the President of the U.S. Hemp Authority. CBD is not yet approved by the US FDA. Congress and some states are moving to make CBD legal in certain products. Thus, there is a patchwork of testing and labeling regulations. CBD product sales have steadily increased from 2014. The FDA and FTC are especially watchful of "egregious claims" which includes a) making unsubstantiated health claims; b) making unsubstantiated guarantees about product conduct in terms of active ingredients; c) making claims on products that turn out to have unacceptable levels of contaminants such as heavy metals. The FTC has announced a crackdown on deceptively marketed CBD products. USA has released its regulations for domestic hemp production which was mandated in the Agriculture Improvement Act of 2018 and appears in 7 CFR Part 990. The USDA AMS provided multiple opportunities for public comment. A total of 5900 comments were received from states, tribes, industry and agricultural organizations. The final rule has key provisions: licensing requirements, recordkeeping requirements for land where hemp produced., procedures for testing the THC concentration, procedures for disposition of non-compliant plants, compliance provisions and procedures for handling violations. The primary observations of the final rule include the harvest window being extended; improved sampling protocols; negligence threshold increased; disposal/ remediation of hot hemp; lab registration with DEA; THC testing and exportation of hemp.

How to Change Public Perceptions of GE Crops by Using the Plants to Fix Agriculture's Biggest Pollution Problem (Stuart Strand, U. of Washington): Nitrous oxide is a greenhouse gas 298 times more potent than carbon dioxide and is the main threat to Earth's ozone layer. Most nitrous oxide comes from agricultural soils. Nitrogen fertilization increases nitrous oxide emissions. There have been attempts to limit nitrous oxide but these have resulted in decreased yields in agriculture, thus, there is a conflict between global warming and feeding the population. In the soil, bacteria use the enzyme nitrous reductase to turn nitrous oxide into harmless nitrogen. Special bacteria can be developed in the lab that have increased nitrous reductase activity, however, these bacteria rapidly die out when introduced into soils. The enzyme could be

introduced into mitochondria of plants (which would act as surrogate bacteria). Mitochondria live in a protected and secure environment within plant cells. Many experiments have demonstrated translocation of genetically engineered proteins into the mitochondria. The mitochondrial proteome can be modified on the nuclear chromosome. Proteins are translocated by targeting a signal sequence on one end of protein. Thus, nitrous oxide reductase could e introduced in the intermembrane space along with helper proteins to add copper to connect electron transport system of plant mitochondria. If nitrous oxide reductase can be engineered into plants, emissions from corn fields of nitrous oxide could be reduced to zero preventing 11 kg nitrous oxide emissions/ha/year. The above ground parts of the plant would remove nitrous oxide from the atmosphere through the stomata. The annual uptake and prevention in corn would be 32 kg nitrous oxide/ha/year. If 15% of global crops were transformed with nitrous oxide reductase, the annual increase of nitrous oxide could be prevented. If 34% of all crops were transformed starting in 2030, atmospheric nitrous oxide could be restored to pre-industrialization levels by 2100. Cost would be low after transformation of plants since the enzyme would be inherited via seed production. Engineering plants with nitrous oxide reductase would provide moral and a legal requirement to plant GM crops.

Novel Quantitative Method for Determination of Genetic Trait Purity (J. Zheng, Indiana Crop Improvement Assn): Indiana Crop Improvement Association (ICIA) was founded in 1900 and is a non-profit located in Lafayette, IN (www.indianacrop.org). It is one of the largest state crop improvement associations and hosts the annual Seed Belt Conference. ICIA provides comprehensive services on a variety of crops and has 1000+ member companies. The R&D program is targeted research with members. In this program, a new method has been developed for sorghum dhurrin free (DF) trait. In sorghum, the toxic cyanogenic glucoside is produced. Purdue University developed a sorghum mutant with DF. A colorimetric assay was developed to identify these plants but it is a labor intensive assay. A high throughput assay based on the SNP change. RT-PC was first attempted but the assay could not distinguish wild-type from DF-free sorghum. The SNP is in a high GC region of DNA. Pyrosequencing, a real-time quantitative DNA sequencing method, was used. The work-flow is:

Samples →extract DNA→conduct PCR→pyrosequencing→plot allele frequency→plot using a regression equation→estimate trait purity

The method works for a large range of seed purity using seed or leaf samples. A patent application has been filed. ICIA has started to provide commercial service for DF trait. The method has broad applications for traits from SNPs, stacks, etc.

InvictDetect Plus: Collaboration between USDA and Private Industry (C. Culkin, Agdia): Agdia was founded in 1981 by Chet Sutula and is still a family-owned company. Agdia focuses on plant pathogen detection, trait identification for GMOs, insect identification, contract assay manufacturing. All research is conducted in-house. Agdia has 40 years of experience with detection technologies. The USDA (Dr. Steven Valles – Florida) reached out to Agdia to assist in development of detection devices for fire ants.

Imported fire ants were introduced to the US from South America in the early 20th century. The red imported fire ant (*Solenopsis invicta*) and the black imported fire ant (*Solenopsis richteri*) are also interbreeding to make another species (*S. invicta x richteri*). Fire ants destroy crops and equipment and out compete native species. Stings are painful and can induce anaphylactic shock. They infest 360 million acres in the US. Eradication efforts have been ineffective. The USDA established a federal quarantine in 1958. Fire ants are spread by humans through the movement of agriculture equipment, grass sod, nursery stock, hay and straw. Regulated items must be inspected by each state. Compliance is by each individual. Shipments are inspected at origin, in transit and at destination. Prompt identification is important to prevent spread to new areas. Imported fire ants are difficult to visually identify from other species. Samples must be sent to an expert which may delay release of goods for up to 2-3 days. USDA wanted a test that is field portable, rapid, easy to use, requires no special equipment, sensitive and specific, uses small ant sample and able to discriminate both species. The USDA developed a monoclonal antibody to ant venom protein 2. The antibody was used for lateral flow test in a kit format (10 strips, tubes/pestles, buffer, volume pipettes). Agdia manufactures kits for commercial sale. It is a 30 min test that uses a 5 ant sample. The kits were validated. A cross-reaction was found between the two species' proteins, however, it is not a problem as an agent will do the same enforcement regardless of the species.

Ambiguous Results – How do you score them? (R. Shillito, D. Syme, BASF): Ambiguous results in PCR are inconsistent or contrasting results from multiple testing replications or estimates of contamination level consistently below the assay limit of detection. An example is a single DNA extraction is tested in multiple PCR reactions with one positive and one negative or multiple DNA extractions from a common sample of ground flour providing a positive and negative result. Another example is the inconsistent or contrasting results in a test for the presence of a disease organism, i.e., a single DNA extraction is tested and gives both positive and negative results in multiple PCR reactions. Ambiguous results are not the result of bad science. They need to be classified (positive, negative, no-call) as a business decision based on the best available science. How to classify ambiguous results are important to a) prevent the use of non-conforming product; b) to prevent the disposal of conforming product. Ambiguous results also occur with protein methods. At the individual lab level, implementation will depend on their internal processes. A pre-determined approach increases efficiency of the process when labs encounter intermediate signals and reduces days to final decision for data. It may also eliminate the need for further testing and provides a clear, consistent rationale for how decisions are made. ISO 24276 states:

- "Results from all test portions shall be consistent. When at least one test portion gives a positive result and at least one gives a negative result, the analysis shall be repeated.
- If at least two repetitions of the procedure (beginning with the nucleic acid extraction) give ambiguous results such as a positive and a negative result, the report should state that the sample is negative at the limit of detection as expressed in ISO 21569 and ISO 21570."

Ambiguous results may be caused by sampling or analytical issues. They may be avoided by planning the experimental design to avoid sampling issues and ensure the correct sample size use for the method LOD. Appropriate reference materials should also be used. A proposal was made that AEIC should have a Working Group on ambiguous results to explore how test sample and test portion in the lab may affect results, to determine how to deal with processed materials. Ultimately, the working group would compose a paper on the topic. Volunteers for the working group: Ray Shillito, Dave Syme, Chelsea Metzler, Donna Houchins, Matheus Benatti, Kalyn Brix, Palmer Orlandi, Tao Geng, Doug Miller, Mary Gadola, Matt Cheever, John Zheng, Angie Umthun, Anna Doornink, Farhad Ghavami, Michael Sussman, Lucy Liu.

UPDATES

AOCS (Scott Bloomer): AOCS Method Development Subcommittee supports methods for proximate composition, nutritional profile, sensory attributes, protein quality, allergenicity and functional properties of developing protein products. The subcommittee chairs are Keshun Liu (USDA) and Catherine Bomont.

AOAC (Palmer Orlandi): AOAC programs include:

- Stakeholder program for infant formula and adult nutritionals
- Cannabis analytical science program
- Food authenticity methods program
  - --3 working groups: non-target testing, targeted testing, molecular applications
  - --also an emergency response guidance which outlines standards/method development principals in emergency situations

New projects include:

- Glutens and food allergens
- Standards for NGS applications
- Standards, methods and validation of non-culturable food-borne pathogens
- Consensus standard for determination of acrylamide to address method gaps in food matrices
- Standard for determination of per- and poly-fluoroalkyl substances (PFAS)
- Standard determination for pyrrolizidine alkaloids in teas and herbal infusions
- Food additive safety: standards for natural colors and flavors

For more information, check the AOAC website under Scientific Solutions.

ISO TC 34/SC 16 (R. Shillito, BASF): Standards were developed by the EU in the early 2000's. A process was initiated to update, however, the EU opposed. The focus is now on developing a broad standard that covers all plants/animals. Sherry Whitt and Huihua Fu are leading this. The sub-sampling document for use of seed calc was finished. Group is working on the meat adulteration standard. Alex Eads is heading up a document on isothermal analysis. Luis Burzio is working on a document for molecular biomarkers for fiber. The microarray detection standard is also being updated. The next meeting of the international committee will be held in September.

AEIC Spring 2021 Attendees:

Name	Organization
Ament, Chris	EFII
Atkinson, Tara	Corteva
Baine, Susan Leslie	EPL Bio Analytical
Basnayake, Veronica	USDA
Bedair, Mohamed	Bayer
Beecher, Brian	USDA AMS FGIS TSD
Benatti, Matheus	Indiana Crop Assn
Bloomer, Scott	AOCS
Bohnker, Laura	EBDI
Brix, Kalyn	SoDak Labs, Inc.
Brune, Phil	Syngenta
Calcaterra, Jennifer	Bayer
Cheever, Matt	BASF
Chou, Yi-Hsiang	Bayer
Culkin, Chris	Agdia
Cummings, Simone	Syngenta
Daher, Mariana	BASF
Doornink, Anna	EBDI
Edmison, Dustin	SGS NA
Fendley, Ann	BASF
Fisher, Ashley	Simplot Plant Sciences
Fu, Huihua	BASF
Fung, James	Indiana Crop Assn
Gadola, Mary	Neogen
Geng, Tao	Bayer
Ghavami, Farhad	EBDI
Gillikin, Nancy	BASF
Goddard, Terry	EnviroLogix
Haudenshield, James	Merieux Nutriscience
Herrero, Sonia	Syngenta
Houchins, Donna	Romer Labs
Huang, Mingya	Bayer
Hunst, Penny	BASF
Johnson, Brenda	EBDI
Kahn, Peter	OMIC USA
Klusmeyer, Tim	Bayer
Kouba, Kristen	Corteva
Leach, Brenton	Indiana Crop Assn

Liu, Zhenjiu	Bayer
Liu, Zi Lucy	Bayer
Maxwell, Carl	Corteva
Metzler, Chelsea	BASF
Milcarek, Justin	Indiana Crop Assn
Miller, Doug	IL Crop Improvement
Muschinske, Luke	Eurofins Microbiology Labs
Novek, Angela	EBDI
Olorunda, John	Merieux Nutriscience
Olson, Tyler	Merieux Nutriscience
Overdorf, Susan	Indiana Crop Assn
Rambow, Dave	Agdia
Rigdon, Shelby	EPL Bio Analytical
Roberts, Jessica	SGS
Secrist, Heather	Food ID
Shillito, Ray	BASF
Shippar, Jeff	Eurofins
Sussman, Michael	USDA
Syme, David	BASF
Temple, Stephen	Forage Genetics
Umthun, Angela	Stine Biotechnology
Von Hendy, Matthew	Green Heron
Wang, Cunxi	Bayer
Wang, Kelin	Bayer
Wang, Rong	Bayer
Wang, Yanfei	Bayer
Wang, YongCheng	Bayer
Warnick, Joe	EPL Bio Analytical
Watkins, Crystal	EPL Bio Analytical
Whitt, Sherry	BASF
Williams, Dalton	EPL Bio Analytical
Williams, Denise	AOCS
Yang, Kong	Eurofins Scientific
Zhang, John	Corteva
Zheng, Jiaojie	Merieux Nutriscience
Zheng, John	Indiana Crop Assn