ISU Crop Bioengineering Consortium: Activities and Strategies

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The ISU Crop Bioengineering Consortium (CBC) was organized to address the urgent, grand challenge to provide sufficient food, feed, biofuels and biorenewable chemicals for the world's burgeoning population, through basic and applied research, to enable the bioengineering of valuable traits in a variety of crops. Importantly, the novel technologies employed can produce bioengineered crops that contain no transgenes and thus may face less-stringent regulation than classic genetically modified organisms (GMOs).

By 2050, the demand for staple food crops alone will require yield increases of nearly 80%. In addition, dwindling petroleum supplies, higher energy prices, dependence on energy imports, and the environmental consequences of fossil fuel use mandate development of renewable energy sources (including biofuels) and biorenewable chemicals. We need to increase food and biomass-crop productivity via both increased yield and expansion onto marginal lands. Historical yield increases have succeeded via improved management and intensive breeding, but we have begun to fall short of demand. Paradoxically, current crop varieties leverage only a fraction of the genetic potential available through natural and bioengineered alleles. Moreover, the discovery rate of potentially useful genes now clearly outstrips crop-testing capabilities. Therefore, technologies, as described here, that complement traditional management and breeding but dramatically accelerate production and testing of improved crops, are in critical demand.

Crop productivity is far from its yield potential due to losses from environmental and biological challenges, including water and nutrient limitations, non-optimal temperatures, diseases, pests, and weed competition. Many potentially ameliorating traits, such as increased photosynthesis or tolerance of environmental and biological challenges, have been recalcitrant to modern plant-improvement techniques. The CBC will try to overcome this intractability by establishing an innovative platform for the facile identification and incorporation of these beneficial traits based largely on new genome-editing technology. The transformative, novel genome-editing approaches known collectively as new breeding technologies (NBTs), developed in part through the pioneering efforts of ISU scientists including the CBC's Bing Yang[1], can make critical plant traits accessible to modern genetic tools and accelerate the production of improved crops. Just as improved plant breeding and crop management spawned the Green Revolution in the 1960s, so too could this new technology transform crop improvement in this generation.

Current crop-improvement efforts predominately emphasize transgenic technologies, which face public and regulatory challenges that dramatically increase both the time to market and the costs to obtain approval. Such hurdles are likely to increase for transgenics, so crop biotechnology companies are seeking alternative, non-transgenic approaches for the rapid engineering of crop traits. Because NBTs can generate small deletions or single-nucleotide changes in specific genes, and the tools that deliver them can be removed from an end product through traditional breeding, these innovative technologies provide an opportunity to make valuable genome changes without "leaving the tools at the worksite." Thus, NBTs offer a game-changing, non-transgenic approach to crop bioengineering. Because of the better regulatory prospects for NBTs, these technologies will have tremendous appeal, especially where transgenic products face strong public resistance. Nonetheless, the long-term sustainability of NBTs as the preferred approach to generate novel crop germplasm will depend on externalities to the technology itself, including the perceptions of interested and affected stakeholders through the technology's effect on regulation as well as anticipated demand.

Establishing the ISU Crop Bioengineering Consortium

The CBC was established July 1, 2013, with funding from the ISU Presidential Initiative for Interdisciplinary Research (PIIR). The CBC, which comprises 25 faculty (Table 1), 21 from ISU and 4 from other institutions, capitalizes on core strengths of ISU, including a long history of innovation in the area of plant-genome engineering, outstanding plant-transformation capabilities and world-class excellence in plant genetics and genomics. Establishment of the CBC promises to move ISU plant scientists in collaboration with selected researchers at other institutions to the next level through effective multi-disciplinary collaboration.

[1]See pp. 53–59.

Table 1. Outline of the CBC platform, illustrating the bidirectional interactions among the core research teams (gene/trait discovery, NBT/transformation, trait evaluation/integration, and regulatory, economic, environmental and societal impacts), the new germplasm developed and the beneficiaries of the germplasm.

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Becraft, Philip. Professor, Dept. of Genetics, Development and Cell Biology, ISU.
Bhattacharyya, Madan. Associate Professor, Dept. of Agronomy, ISU.
Hayes, Dermot. Professor and Pioneer Chair in Agribusiness, Dept. of Economics, ISU.
Howell, Stephen. Professor, Dept. of Genetics, Development, and Cell Biology, ISU.
Lamkey, Kendall. Professor and Pioneer Distinguished Chair in Maize Breeding, Dept. of Agronomy, ISU.
Lawrence, Carolyn. Associate Professor, Dept. of Genetics, Development, & Cell Biology, ISU.
Lubberstedt, Thomas. Professor and K.J. Frey Chair, Dept. of Agronomy, ISU.
Salas-Fernandez, Maria. Assistant Professor, Dept. of Agronomy, ISU.
Schnable, Patrick. Baker Professor of Agronomy, Dept. of Agronomy, ISU.
Vollbrecht, Erik, Associate Professor, Dept. of Genetics, Development, & Cell Biology, ISU.
Whitham, Steve. Professor, Dept. of Plant Pathology & Microbiology, ISU.
Wolf, Clark. Professor, Dept. of Philosophy & Religious Studies, ISU.
Wolt, Jeffrey. Professor, Dept. of Agronomy, ISU.
Wright, David. Associate Scientist, Dept. of Genetics, Development, & Cell Biology, ISU.
Yin, Yanhai. Associate Professor, Dept. of Genetics, Development, & Cell Biology, ISU.
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Brendel, Volker. Prof. of Biology and Computer Sci., Dept. Biol. & School of Informatics and Computing, Indiana U.
Huber, Steven. USDA-ARS Plant Physiologist and Professor, Depts. of Plant Biology & Crop Sciences, Univ. of Illinois.
Ladunga, Istvan. Professor, Dept. of Statistics, University of Nebraska-Lincoln.
Weeks, Donald. Maxcy Professor of Agriculture & Natural Resources, Dept. of Bioch., Univ. of Nebraska-Lincoln.

Therefore, the mission of the CBC is to:

> *deploy innovative, transformative genome-engineering technologies that identify, validate, and rapidly, but precisely, integrate strategically important traits and underlying genes into key crop plants.*

In practical terms, this means that the CBC will:

- Develop innovative, new technologies to enable and improve crop-genome engineering.
- Employ NBT genome-engineering approaches to enable basic research in plant biology, utilizing key crop plants, including maize, soybean, rice and sorghum, to facilitate identification of potentially beneficial genes and traits.
- Employ NBT genome-engineering approaches to incorporate and integrate potentially beneficial traits in important crop plants targeted by the CBC, maize, soybean, rice and sorghum, generating modified, improved lines (null segregants) containing no transgenes.
- Understand the regulatory, economic and societal implications of NBTs.

The core of the CBC comprises innovative genome-editing methods, including TALEN- (transcription activator-like effector nuclease) and CRISPR- (clustered regularly interspaced short palindromic repeats) based technologies, in building a platform for the identification and validation of strategically important plant genes/traits and for the subsequent rapid and precise integration of promising traits into important crop plants. These innovative technologies will accelerate both fundamental research that identifies genes controlling critical traits, and engineering of desired gene modifications that tests traits in crop plants. ISU has a catalog of attractive gene targets at various stages of verification, established capabilities for NBT-based genome editing, and the ability to transform target crops, all of which form the CBC framework. As illustrated in Figure 1, the CBC is establishing a platform comprising: active gene discovery and validation; incorporation of target gene modifications into crop plants using NBT approaches and novel delivery methods; trait verification and integration; and evaluation of regulatory, economic, environmental and societal impacts of the technology and the resulting traits.

Research at ISU and elsewhere has identified a surfeit of gene targets for crop improvement, including those influencing yield, photosynthesis, stress tolerance, and disease and pest resistance. The time is ripe to launch a public-sector infrastructure for rapid, precise crop bioengineering; ISU is ideally suited to lead this effort, and the CBC will serve this need. ISU has played key roles in development of the TALEN technology, one of the key approaches at the core of this endeavor, and it boasts international leadership in plant biology and genetics, plant transformation, plant breeding, crop production, crop-genome informatics, risk assessment, and agricultural economics. In addition to launching technology platforms, the CBC will establish four complementary, basic research foci to be addressed by the teams indicated in Figure 1:

- Core technology development and implementation;
- Gene/trait discovery;

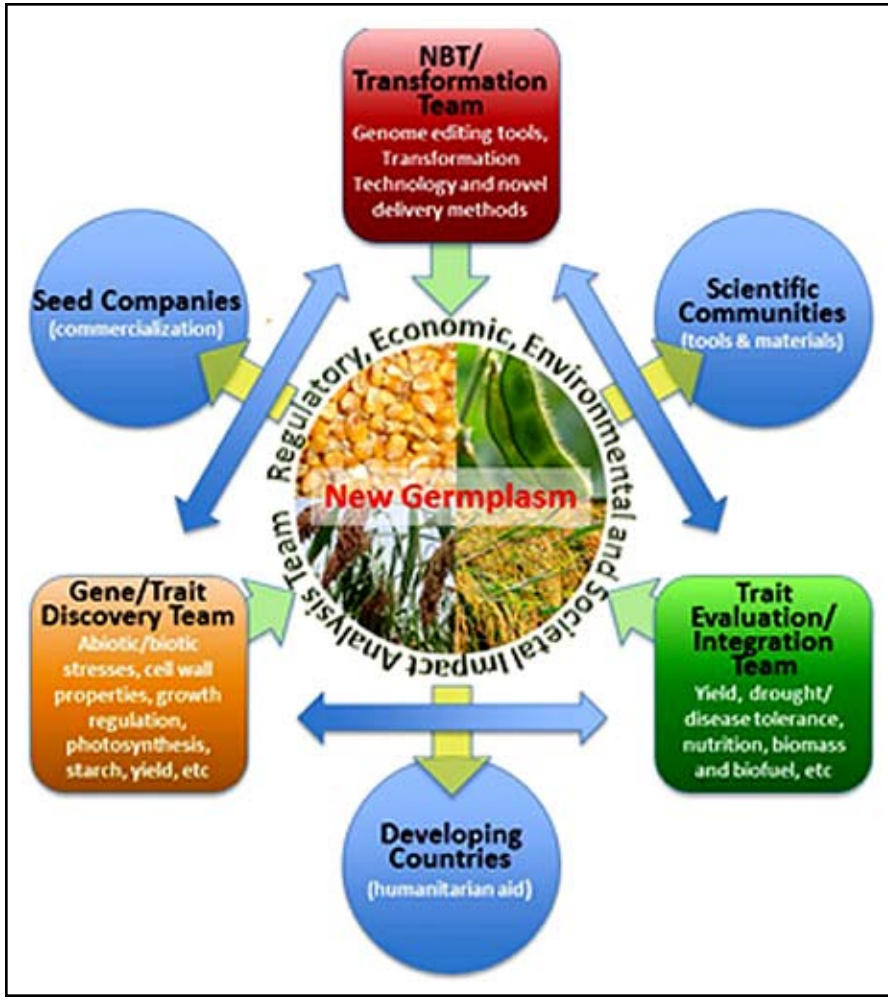

Figure 1. Outline of the CBC platform, illustrating the bidirectional interactions among the core research teams (gene/trait discovery, NBT/transformation, trait evaluation/integration, and regulatory, economic, environmental and societal impacts), the new germplasm developed and the beneficiaries of the germplasm.

- Trait verification and integration; and
- Regulatory, economic, environmental, and societal impacts.

Core-Technology Development and Implementation

The initial core enabling technology of the CBC was the highly innovative TALEN technique, based on the TAL effectors (TALEs) secreted by plant pathogenic *Xanthomonas* bacteria (Christian *et al.*, 2010; Li *et al.*, 2011; Voytas *et al.* 2011; Yang *et al.*, 2011; Bonus

et al., 2012; Joung and Sander, 2013; Wright *et al.*, 2014). TALEs bind to specific plant-DNA sequences and modify gene expression. TALE proteins use a simple recognition "code" to bind specific, target DNA in their host plant. Within the central region of the TALE protein are several repeats, each recognizing one of the four DNA nucleotides. This simple code can be used to engineer designer TALENs, which are fusion proteins comprising custom TALE DNA-binding domains and the DNA-cleavage domain of the endonuclease FokI, that have specificities for preselected DNA sequences. TALENs produce DNA double-strand breaks that lead to mutagenic insertions/deletions at the gene target sites or, in the presence of a donor DNA template, integration of new DNA at the gene-target sites by homologous recombination.

In addition to improving TALEN technology and demonstrating its application in targeted crop plants (see below), the CBC also has expanded its capabilities for genome engineering by incorporating the newer CRISPR/CAS9 technology and demonstrating its effectiveness in crop plants targeted by the CBC. The prokaryotic CRISPR/Cas9 system (Cong *et al.*, 2013; Mali *et al.*, 2013), in its simplest form, consists of only two genes: one encoding Cas9 and one encoding a "guide" RNA that enables Cas9 to identify and cleave a "target" DNA sequence. As with TALEN-based DNA breaks, Cas9-based breaks lead to either insertions/deletions at the target sites or homologous-recombination-based integration of sequences from a template. CRISPR/Cas9 has been functionally demonstrated in a number of eukaryotes, but had not been demonstrated in photosynthetic organisms, so the CBC adapted this new system for use in plants (Jiang *et al.*, 2013, 2014; Zhou *et al.*, 2014).

The CBC also is pursuing other novel, enabling technologies, including breakthrough protein-delivery technologies and software development, as well as developing and incorporating high-throughput processes across the whole pipeline from construct design and construction to identification of edited plants. Direct delivery of proteins into plant tissues has recently been demonstrated by CBC members (Martin-Ortigosa *et al.*, 2012, 2014). This innovative approach should enable delivery of genome-editing proteins rather than having to integrate transgenes encoding the editing proteins. This technology will completely bypass DNA (transgene) integration into the plant genome, while generating precisely modified and truly "non-transgenic" plants. It will also shorten the time from lab to field testing by avoiding the need to remove the editing transgene DNA from the engineered plants.

The ability to engineer genomes is extremely powerful, but the ability to engineer genomes in a high-throughput platform, to rapidly make large numbers of edits in large numbers of plants, greatly amplifies the power of the technology. The CBC is developing high-throughput processes for all stages of the genome-engineering pathway, beginning with development of software for the prediction of CRISPR-editing targets for any gene in a variety of genomes, including the ability to target one or more specific members of a highly similar gene family. This software is currently undergoing beta-testing by CBC members, but will be made publicly available on the CBC website (cropbioengineering.iastate.edu/). Efforts also are underway to improve the throughput for transformation and screening using a variety of process-automation approaches.

Enabling Basic Plant-Biology Research

A major strength of the CBC is the enabling of exploratory basic research to reveal the function of key plant genes, thus identifying potential targets for modification. Numerous, potentially beneficial candidate genes have been identified already by CBC members, and vast numbers of additional candidates can be identified from analyses of transcriptional profiling, proteomics, and genomics data. However, verification of any candidate gene function requires direct gene manipulation, as by using genome-editing technology to precisely inactivate or alter the expression of the candidate gene to reveal any resulting mutant phenotype.

The CBC has invested much of the past year demonstrating the efficacy of genome-editing technology in the target crop plants. Initial work using TALEN technology for genome editing in plants focused on rice (Li *et al.*, 2011, 2012, 2013). Proof-of-concept demonstration of TALEN function in maize and soybean has been successful, including the demonstration of including inheritance and segregation of edited genes in maize (Char *et al.*, 2015). Demonstration of genome editing has been expanded to include the CRISPR platform, which has proven highly efficient in rice (Kitaokaa *et al.*, 2014; Zhou *et al.*, 2014) and maize (CBC, unpublished).

Gene/Trait Discovery and Incorporation of Beneficial Traits

Once identified, desirable gene modifications can be rapidly, precisely and efficiently integrated into cultivars using genome-editing technology. For example, the use of TALEN technology by the Yang[2] lab to modify the regulatory region of a specific rice gene verified that the targeted regulatory sequences facilitated pathogenicity of the rice-blight pathogen; furthermore, disruption of this region conferred resistance to the rice-blight pathogen (Li *et al.*, 2012). We have disrupted a variety of specific genes in maize based on hypotheses that their disruption may, in some cases, increase growth or, in other cases, make beneficial changes in the properties of starch. Similarly, we hypothesized that disruption or modification of specific soybean genes involved in carbon assimilation will result in increased carbon assimilation and yield. We are testing this hypothesis using genome-editing technology to disrupt or modify the genes in question. Because these gene manipulations in maize and soybean are predicted to improve plant growth, yield or starch characteristics, verification of the hypothesis could lead to the integration of a valuable trait into these key crops.

Verification of predicted traits or phenotypes resulting from targeted genome modification is an essential part of the CBC mission, as is verifying that the integrated genome modifications are inherited in a simple, predictable manner. Because of the high precision of genome-editing technologies, once a predictable genomic location is altered, confirming the inheritance pattern of the modified locus is straightforward. Self-pollination of a heterozygous initial transformant or of a non-transgenic, but edited, plant will generate a segregating, second-generation population in which inheritance patterns can be verified

[1]See pp. 53–59.

by genotyping multiple individuals and any editing transgene locus can be eliminated. Moreover, phenotyping of the trait of interest in a segregating population will establish a genotype-phenotype relationship, if it exists, and confirm or refute the predicted phenotypic benefit.

The strategy developed by the CBC for commercialization of beneficial traits is based on partnering with the crop-biotechnology industry. The intellectual property landscape for genome-editing technologies is not clear at present, but as an academic entity, the CBC enjoys relative freedom of operation for academic research. Companies operating in the crop-biotechnology industry are likely to be licensed and pursuing use of NBT approaches for crop improvement. Crop-biotechnology companies are undoubtedly using genome-editing technologies to generate gene modifications predicted to produce potentially beneficial traits. However, many potentially beneficial traits either will not be predicted or will be judged as too risky for pursuit. Therefore, demonstrated and verified beneficial traits are expected to be of interest for further investigation and potential commercialization by industrial partners.

Regulatory, Economic, Environmental, and Societal Impacts

The possibility that plants modified by genome-editing technologies, *i.e.* using NBTs, may qualify as non-GMO is one of the most intriguing aspects of this new approach to crop improvement. If NBT approaches carry a dramatically lower regulatory burden, their advantages in bringing traits to market could be extraordinary. Public concerns regarding bioengineered crops and products have been acutely focused on the transgenic technologies used to produce them. Because NBT approaches result in non-transgenic products, it is plausible that they may carry less perceived and actual risk, and that regulatory concerns will be minimal.

The regulatory, economic, environmental and societal impacts team is charged with addressing the anticipated market potential, and economic sustainability of improved varieties developed using NBT approaches. This team also will examine (1) whether the public perceives NBT products to be *risky* either to human interests or to the environment, and (2) whether existing regulatory measures will apply to crop varieties produced with NBT approaches. This research examines existing regulatory regimes, as well as evaluates the reasons used to justify and defend them. Association with the CBC facilitates study of TALEN- and CRISPR-derived products including comparative risk analysis, study of perceived risk and the basis for perceived risk, and review of existing regulations.

The CBC is already a significant participant in the discussion of NBT regulatory issues, having hosted an international workshop, *Science and Opportunities in Using Site-Directed Mutagenesis for Plant and Animal Improvement*, November 4–5, 2013, and was invited to help plan NABC 26, *New DNA-Editing Approaches: Methods, Applications and Policy for Agriculture*, hosted by Cornell University and the Boyce Thompson Institute.

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DR. SPALDING joined Iowa State University in 1984 as an assistant professor in the Department of Botany. He served as chair of the Interdepartmental Plant Physiology program at ISU from 1992 until 2000. In July 2003, he assumed the position of chair of the newly formed Department of Genetics, Development and Cell Biology, and served as chair of the Department of GDCB until July 2011, when he became interim associate dean for research and graduate studies in the College of Liberal Arts and Sciences, and formally accepted the position of associate dean in January 2013. Spalding also became the director of the ISU Crop Bioengineering Consortium in July 2013.